

Plant Functional Traits of *Aconitum spicatum* and *Aconitum naviculare* Along the Elevation Gradients in Central Nepal



Dissertation Submitted for the Partial fulfillment of the Requirement for
the Master's Degree in Science, Central Department of Botany,
Institute of Science and Technology, Tribhuvan University

Submitted by

Sangita Bashyal

Exam Roll No.: Bot800/076

T.U. Regd.No.: 5-2-49-150-2015

Batch: 2076/078

Ecology and Resource Management Unit

Central Department of Botany

Institute of Science and Technology

Tribhuvan University, Kirtipur, Kathmandu, Nepal

June 2023

DECLARATION

I, Sangita Bashyal, hereby declare that this dissertation entitled “**Plant Functional Traits of *Aconitum spicatum* and *Aconitum naviculare* Along the Elevation Gradients in Central Nepal**” is my original work, and all other sources of the information used are properly acknowledged. I have not submitted it or any of its parts to any other universities for any academic award.



.....

Sangita Bashyal

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date: 26th June, 2022

LETTER OF RECOMMENDATION

This is to certify that the dissertation work entitled “**Plant Functional Traits of *Aconitum spicatum* and *Aconitum naviculare* Along the Elevation Gradients in Central Nepal**” has been completed by Ms. Sangita Bashyal under our supervision. This entire work was accomplished based on the candidate’s original research work. To the best of our knowledge, the work has not been submitted to any other academic degree. It is hereby recommended for acceptance of this dissertation as part of the requirement of a Master’s Degree in Botany at the Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal



Supervisor

Dr. Bharat Babu Shrestha

Professor

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal



Co-Supervisor

Dr. Uttam Babu Shrestha

Director

Global Institute for Interdisciplinary Studies

(GIIS)

Kathmandu, Nepal

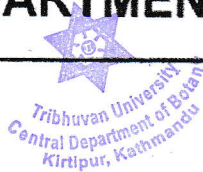
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TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY

Ref No:

Kirtipur, Kathmandu
NEPAL



LETTER OF APPROVAL

The M.Sc. dissertation entitled “**Plant Functional Traits of *Aconitum spicatum* and *Aconitum naviculare* Along the Elevation Gradients in Central Nepal**” submitted at the Central Department of Botany, Tribhuvan University by Ms. Sangita Bashyal has been accepted for the partial fulfillment of her Master’s Degree in Botany (Ecology and Resource Management).

EXAMINATION COMMITTEE

External Examiner

Dr. Som Prasad Paudyal

Associate Professor

Tri-Chandra Multiple Campus

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Internal Examiner

Dr. Yadav Uprety

Associate Professor

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Co-Supervisor

Dr. Uttam Shrestha

Global Institute for Interdisciplinary

Studies (GIIS)

Kathmandu, Nepal

Supervisor

Dr. Bharat Babu Shrestha

Professor

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Head of Department

Dr. Sangeeta Rajbhandary

Professor

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date: 7th July, 2023

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor Prof. Dr. Bharat Babu Shrestha, for his constant guidance, support and encouragement throughout my dissertation work. His keen supervision has enabled me to carry out the work smoothly and with high efficacy. I would like to acknowledge my co-supervisor Dr. Uttam Babu Shrestha director of GIIS for his invaluable assistance.

This work has been financially supported by a Collaborative Research Grant (Impacts of climate change on germination, growth, pollination, and distribution of Himalayan medicinal herbs *Aconitum spicatum* and *A. naviculare*; grant no.: CRG-77/78-S&T-1) from the University Grants Commission (UGC) of Nepal.

I am very thankful to Prof. Dr. Ram Kailash Prasad Yadav, former Head of Department and Prof. Dr. Sangeeta Rajbhandary, Head of Department, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal, for their administrative and moral support. In addition I'm extremely thankful to all the academic and administrative staff of Central Department of Botany, Tribhuvan University, for their help.

It gives me immense pleasure to acknowledge Assoct Prof. Dr. Chitra Bahadur Baniya for his indispensable support during data analysis. I am greatly appreciative and indebted to the Asst. Prof. Dr. Achyut Tiwari, Assoct. Prof. Dr. Anjana Devkota, and Asst. Prof. Dr. Babu Ram Paudel for their precious suggestions, support and guidance.

I am heartily thankful to my friends Prativa Masrangi, Sandesh Ghimire, Sharda Dhakal and Bishnu Sharma Gaire who supported me throughout the research. I am very grateful to my seniors Tulasa Chaudhary, Nisha Kharel, and Nita Kumari Somai for their profound help. I am also thankful to my batchmates Salina Nagarkoti, Mahendra Thapa, Rajaram Khang Khatbe, Dipa Paneru and my junior Sunraj Tamang Rumba.

My deep appreciation goes to my family members for all the love, inspiration, encouragement, care, and never-ending trust in me which gave me the strength for the completion of this work.

Sangita Bashyal

Date: 26 June 2023

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ABSTRACT

Plant functional traits strongly influence organisms' performance and fitness. Traits vary considerably within and among plant species. Elevation gradient provide unique opportunity to examine how plant functional traits vary with changing climate and resources availability. This research aims to assess the responses of functional traits along elevation gradients in two important medicinal plant of Nepal Himalaya *Aconitum spicatum* (Brühl) Stapf and *Aconitum naviculare* (Brühl) Stapf, growing in climatically contrasting habitats within Annapurna Conservation Area in central Nepal (moist, southern declivity, and dry, northern declivity of Annapurna mountain range, respectively). Several traits related to leaves (e.g. specific leaf area, leaf nitrogen content), plant growth (e.g. height, basal stem diameter), biomass allocations (e.g. root, stem and leaf mass fraction), and seed production (e.g. output) were measured along the elevation gradient. The relationship between functional traits and elevation were analyzed by generalized linear models. The results revealed that plant height and leaf area declined and root mass fraction increased in both species as elevation increased. However, specific leaf area declined with increasing elevation only in *Aconitum naviculare*. Leaf nitrogen content did not demonstrate any clear relationship with elevation. Moreover, the root-to-shoot ratio displayed opposite trends in two species, with *Aconitum naviculare* showing a positive relationship and *A. spicatum* showing a negative relationship with elevation. The observed patterns in functional traits provide valuable insights into the adaptive strategies of these species in response to changing climate along the elevation gradients. This knowledge can contribute to a better understanding of plant responses to environmental gradients in mountains and aid in their conservation and management efforts under climate change scenarios.

Key words: Annapurna Conservation Area, Biomass allocation, Himalaya, Medicinal plants, Specific leaf area

ABBREVIATION AND ACRONYMS

LMF	Leaf mass fraction
m asl	Meters above sea level
N_{mass}	Leaf nitrogen content per unit dry mass of leaf
N_{area}	Leaf nitrogen content per unit area of leaf
R/S	Root to shoot ratio
RMF	Root mass fraction
SLA	Specific leaf area
SMF	Stem mass fraction

CHAPTER 1. INTRODUCTION

1.1 Background

Plant functional traits are those morphological, physiological, and phenological traits that strongly influences organismal performance (Violle et al., 2007). Moreover, variations in morphology, chemistry, physiology, and phenology have an impact on the capacity of individuals to thrive, survive, reproduce, and disperse within a specific environment (Violle et al., 2007; Shipley et al., 2016). The examination of plant functional traits offers a profound understanding of the connections between community organization and the functioning of ecosystems (Díaz and Cabido, 2001). However, current knowledge of the interaction between traits that affect how plants respond to environmental factors like resources and disturbances (response traits), and traits that determine the consequences of plants on ecosystem functions (effect traits), such as biogeochemical cycling or vulnerability to disturbances, is limited and basic (Lavorel and Garnier, 2002). Plant intraspecific trait variation (ITV) plays a pivotal role in shaping various aspects of ecological dynamics. It exerts significant influence over individual and population performance, species interactions, community structure and assembly, as well as ecosystem properties (Westerband et al., 2021). It is generally assumed that functional traits, particularly of leaves, are closely linked to the regeneration niche (Poorter, 2007).

The behavior of plants is influenced by various factors that are associated with elevation rather than elevation itself (Körner, 2007). Exploring elevational gradients provides an opportunity to observe how different characteristics of plant species respond to changes in their biotic and abiotic surroundings (Midolo et al., 2019). Because the diverse range of abiotic and biotic factors such as irradiance, air temperature, aridity, competition and herbivory, the elevation significantly contribute to the variation in traits within plant populations (Westerband et al., 2021). These environmental factors serve as filters, selectively allowing species with specific combinations of traits to survive and thrive in a given ecosystem (Keddy, 1992). Understanding this process of filtering is crucial in determining the species' ability to adapt and thrive in their respective environments. Environmental condition at lower elevation is more favorable for plant growth and survival as compared to the higher elevation that is characterized by harsh environmental conditions. To combat the effect

of biotic and abiotic stresses plant shows variation in traits such as decrease in plant height, leaf size and specific leaf area (Pandey et al., 2021). Plants grown in nutrient-rich soil have larger inflorescences with more and heavier seeds. Additionally, plants in warmer environments also produce heavier seeds (Dainese, 2011).

1.2 Justification

A few previous studies conducted in the Himalayas (Chapagain et al., 2019; Pandey et al., 2021; Shah et al., 2019; Sigdel et al., 2023) consistently indicate that plant functional traits frequently respond to elevation dependent climatic variation. The way plants respond to changes in elevation shows their adaptive strategies for different habitats. The climatic variations across different elevation ranges cause shifts in resource availability and environmental conditions, which directly impact plant performance. In response to these changes, plants undergo environmentally-induced changes in their phenotype (Nicotra et al., 2010).

Both *Aconitum spicatum* and *Aconitum naviculare* are important medicinal plant species that exhibit endemism, particularly in the Himalayas (Ghimire et al., 2008). Their unique adaptations to the Himalayan environment, coupled with their medicinal properties, make them valuable resources for pharmaceutical and traditional medicine purposes. Medicinal plants play a vital role as a valuable natural resource, offering potential opportunities for the development of novel products and bioactive compounds in the field of drug discovery (Gangwar et al., 2010). The Himalayan Mountains are known for their abundant presence of medicinal plants (Lama et al., 2001).

However, there is ample evidence indicating that mountain regions have warmed at a faster pace than lower elevation areas, particularly during winter and spring (Díaz and Bradley, 1997; Rangwala & Miller., 2012; Liu et al., 2009). Focusing on the Himalayan region and the middle Mountains of Nepal, Shrestha et al., (1999) found that these areas have exhibited the highest warming trends, while the Terai and the Siwalik regions have shown the least warming or even cooling trends.

In order to predict how species will respond to environmental changes, it is essential to have a deep understanding of the mechanisms that influence the distribution of species along environmental gradient (Maharjan et al., 2021). This knowledge provides valuable insights into the potential responses of species to ongoing environmental

changes. Therefore understanding the variation in functional trait of medicinal plants under different environmental conditions has important implications for their conservation and sustainable utilizations. Similarly, it is very important to understand how climate change affect the growth, survival & distribution of plant species. Plant functional traits have been shown to vary with environmental condition. However we lack empirical data on how plant functional trait of different plant life forms respond to environmental factors. Therefore, studying the influence of environmental conditions on the distribution of plant functional traits in *Aconitum spicatum* (Brühl) Stapf and *Aconitum naviculare* (Brühl) Stapf, important medicinal plants of Nepal, would be useful to explore the patterns of functional trait variation along the elevation gradients.

1.3 Objectives

The objectives of the present study are as follows:

1. To measure the plant functional traits of two important medicinal plant of Nepal Himalaya *Aconitum spicatum* (Brühl) Stapf and *Aconitum naviculare* (Brühl) Stapf.
2. To analyze the pattern of functional trait variation in *A. spicatum* and *A. naviculare* along the elevation gradient.

1.4 Limitation

A potential limitation of the study is the variation in phenophasic stages (different developmental stages) of *Aconitum spicatum* across different elevations. This variability could introduce factors that might influence the measurements of plant traits.

CHAPTER 2. LITERATURE REVIEW

2.1 *Aconitum* species of Nepal Himalaya

Because of its potent therapeutic characteristics, the genus *Aconitum*, often known as monkshood or aconites, is one of the most valuable indigenous drugs (Agnihotri et al., 2015). In Nepal, a total of 28 species of *Aconitum* have been documented, of which eight species exhibit endemism to the region (Shrestha et al., 2022). The distribution of these species spans the western, central, and eastern regions of Nepal, primarily found at elevations ranging from 1800 to 4900 meters above sea level (m asl). Majority of the species are distributed in Eastern Himalaya and most of them are perennial herb (Shrestha et al., 2022).

The *Aconitum* tuber is used for antipyretic analgesic, tonsillitis, sore throat, gastritis and debility, antioxidant and anti-inflammatory, fever and headache, cuts and wounds, musculoskeletal problems, high blood pressure, fever, and jaundice, stomach ache, cough, cold, and intestinal problem as well as antidote in food poisoning and snake bite. (Lama et al., 2001; Bhattarai et al., 2010; Kunwar et al., 2010; Rokaya et al. 2010; Uprety et al., 2010; Shyaula, 2012).

2.1.1 *Aconitum spicatum* (Brühl) Stapf

Aconitum spicatum, a tall perennial herb endemic to the Himalayas and commonly referred to as *Bikh* by its local name, exhibits a distribution range encompassing Nepal, Sikkim, Bhutan, and Tibet (Ghimire et al., 2008; Lama et al., 2001). In Nepal, this species is found across the west, central, and eastern regions, with local occurrences reported in districts such as Dolpa, Kaski, Sindhupalchok, and Taplejung. The plant grows in open and moist conditions, typically found in forest scrublands, meadows, and along rivers and streams at elevation ranging from 1800 to 4800 m asl (Ghimire et al., 2008; Lama et al., 2001; Shrestha et al., 2022). In Nepal, root tubers are commercially in trade and it is among the highly traded species with 300 kg per year (Rana et al., 2020).

2.1.2 *Aconitum naviculare* (Brühl) Stapf

Aconitum naviculare is a small, perennial herb endemic to the Himalayas, and it is locally known as ponkar/bongkar (Shrestha & Jha, 2010). Its distribution spans Nepal,

Sikkim, Bhutan, and Tibet. In Nepal, this species has been reported to occur within specific regions, with Shey Phoksundo National Park located in the northwestern part of the country, and the Annapurna Conservation Area and Manaslu Conservation Area situated in the north-central region (Ghimire et al., 2008). The plant thrives at elevation ranging from 4000 to 4900 m asl (Shrestha et al., 2022). It grows in habitat characterized by sclerophyllous vegetation and thorny plant species. Additionally, it occurs in warm and dry conditions, specifically on slopes that face south (Shrestha & Jha, 2009). The population of the species was found fragmented and small due to collection by human and trampling damage by livestock (Shrestha & Jha, 2009). *Aconitum naviculare* is the most prioritized (Shrestha & Jha, 2009) and most effective (Shrestha et al., 2006) medicinal plant of Manang and used as Poisoning, fever due to poisoning, bile fever and infections (Lama et al., 2001), and fever and jaundice (Bhattarai et al., 2006).

2.2 Plant Functional traits

Plant functional traits are those traits that strongly influences organismal performance (Violle et al, 2007) and significantly affect ecosystem function and reflect the response of vegetation to environmental change (Lavorel and Garnier, 2002). Traits are considered to be major drivers shaping the assembly of communities and species diversity (Westoby et al., 2002). In addition, understanding and predicting ecological processes from species' traits has been considered a 'Holy Grail' in ecology (Funk et al., 2017). Traits in plants create trade-offs between competitive and non-competitive performance, which is important for the coexistence of different plant species. Within-species competition is stronger than between-species competition, and increasing trait dissimilarity between species has limited effect on reducing competition. (Kunstler et al., 2016). All traits are potentially linked to fitness depending on environmental variation (Sobral, 2021).

Plant height: Plant height, which is related to growth form, competitive vigor, reproductive size, whole-plant fecundity, potential lifespan, and other factors, is the shortest distance between the upper boundary of the primary photosynthetic tissues (excluding inflorescences) on a plant and the ground level (Pérez-Harguindeguy et al., 2013). As height is a major determinant of a plant's ability to compete for light it is crucial component of ecological strategy and carbon gain strategy (Moles et al., 2009).

Taller plants intercept more light but, trading off against that, construction and maintenance costs and risk of breakage increase with height (Poorter et al., 2008).

Specific leaf area (SLA): The one-sided area of a fresh leaf divided by its oven-dry mass is known as specific leaf area (SLA) which is inverse of leaf mass per area (LMA) (Pérez-Harguindeguy et al., 2013). Growth analysis typically uses SLA because it frequently has a positive relationship with relative growth rate across species. Specific leaf area tends to decrease when leaves live longer and contain more carbon-based compounds like tannins or lignin. However, SLA increases with higher photosynthetic rates and nitrogen concentration in leaves (Pérez-Harguindeguy et al., 2013). Long leaf life span necessitates toughness and low palatability which is associated with low specific leaf area (Shipley et al., 2006).

Leaf nitrogen content (LNC): The total amount of N per unit of dry leaf mass are known as leaf nitrogen content which is expressed in mg g^{-1} . LNC exhibit close correlations with SLA and with mass-based maximal photosynthetic rate (Pérez-Harguindeguy et al., 2013). In general, high LNC linked to excellent nutritional quality for the consumers in food webs. From the tropics to colder regions, there is a rise in leaf nitrogen (N) and phosphorus (P) levels, (Reich & Oleksyn, 2004). Since most leaf nitrogen is contained in metabolically active proteins, N_{mass} is directly connected to photosynthesis and respiration (Díaz et al., 2016).

Seed mass: The average seed mass of a species is its oven-dry mass (Pérez-Harguindeguy et al., 2013) which is a key characteristic of plants that affects dispersal and establishment. Larger seeds may be chosen over fewer seeds, especially in harsh settings, as they have a higher likelihood of producing established progeny (Pluess et al., 2005). Individuals grown on warmer sites have heavier seeds in *Dactylis glomerata* (Dainese, 2011). The most significant indicators for explaining seed mass variance were temperature, soil fertility, and soil pH; specifically, warmer, low-fertility, and alkaline grasslands displayed a bigger seed mass (Dainese and Sitzia, 2013).

Biomass allocation: The distribution of relative amount of biomass among the different organs, often known as "biomass allocation," may change over time, between environments, and between species; understanding these patterns is crucial to understanding plant ecology and evolution. (Poorter et al., 2012). The typical vascular plant consists of carbon-fixing leaves, supportive stems that serve as a hydraulic

system, and nutrient-absorbing roots that also provide anchorage for the plant (Poorter et al., 2012). Understanding how biomass allocation varies among different groups of organisms along an elevation gradient is indeed crucial for comprehending how individuals or populations respond to climate changes (Ma et al., 2010). Alpine plants' biomass allocation patterns were characterized by a decrease in above-ground reproductive organs and an increase in fine roots (Ma et al., 2010). Proportion of biomass in stem and leaves were generally higher in high-nutrient treatment, and root biomass percentages were generally higher under nutrient-poor conditions (Müller et al., 2000; Qi et al., 2019). Plant allocate more biomass to root when mean annual precipitation and temperature declines (Qi et al., 2019; Shipley & Meziane, 2002). The allocation of biomass in plants, specifically the proportion of total mass allocated to leaves (LMF), is influenced by both nutrient availability and light levels. The LMF shows the highest increase in response to increased nutrient availability and the strongest decrease in response to increased light levels (Poorter et al., 2012). Additionally, under high light intensity, plants exhibited a decrease in LMF and SLA while increasing their RMF (Freschet et al., 2015). Conversely, when plants received a higher nutrient supply, the RMF decreased while the LMF and SLA increased. These findings suggest that both light intensity and nutrient availability play significant roles in shaping the biomass allocation patterns of plants, with distinct responses observed in terms of LMF, SLA, and RMF. Similarly, root to shoot ratio varied among various biomes, vegetation types and plant functional types and it was inversely correlated with mean annual temperature and precipitation, plant height and shoot biomass, but was positively correlated with elevation and latitude (Qi et al., 2019).

2.3 Plant functional traits and Elevation

The plants are responding to a number of variables that covariate with elevation rather than directly to the elevation. As elevation increases atmospheric pressure and temperature decreases, solar radiation increases under cloudless sky, land area reduced per bioclimatic belt (Körner, 2007). While other meteorological variables precipitation, moisture, wind velocity, seasonality, hours of sunshine, geology doesn't show general trend with elevation. Elevational gradients offer a chance to see how different plant species' attributes react to biotic and abiotic environmental change (Midolo et al., 2019; Sigdel et al., 2023). The modifications to the features might provide advantage and

disadvantages that are balanced among individuals in varied circumstances, resulting in equivalent performance along environmental gradients (Umaña and Swenson, 2019).

The response of plant to elevation gradient reflects their adaptation strategy to different habitat. In challenging high-elevation environments with harsh climates, plants adapt by converging in terms of plant height and leaf area. In contrast, at lower elevations characterized by higher biotic pressure, there is a combination of unpredictable variations in plant height (Kergunteuil et al., 2018). *Quercus aquifolioides*, an evergreen alpine and subalpine shrub species, exhibits an increase in specific leaf area at elevations below 2800 meters, while it decreases at elevations above 2800 meters. While *Koenigia mollis* a perennial sub-shrub species of Himalaya shows decreasing pattern of plant height, specific leaf area, leaf area, length of floral axis and leaf nitrogen content with elevation (Sigdel et al., 2023). Additionally, there are contrasting patterns in leaf nitrogen content per unit area and carbon isotope composition (Li et al., 2006). Umaña and Swenson, (2019) describe that leaf thickness, leaf toughness, and specific leaf area generally varied with elevation, while wood density and leaf area exhibited constrained variation. A study carried out by Shah et al., (2019) in plant functional traits along elevation gradient and in Sagarmatha National Park and Buffer Zone, among 60 plant species by selecting eight traits found that, plants found at higher altitudes were predominantly characterized as short basal herbs, whereas lower altitudes exhibited the presence of spinescent plants and tall trees. Whereas Zhang et al., (2020 b) reported that at upper elevation there is a tendency for leaf dry mass per area (LMA) to increase. Similarly, the leaf carbon to phosphorus ratio (C/P) exhibits an upward trend as temperature decreases, and to some extent, the leaf carbon to nitrogen ratio (C/N) also follows a similar pattern in *Cyclobalanopsis gracilis*. In contrary that Cao et al., (2020) reported no significant trend of Leaf Nitrogen Content along the elevation in *Oxytropis ochrocephala*. In a meta-analysis conducted by Read et al., (2014) it was observed that along elevational gradients, there was a tendency for leaf dry mass per area (LMA) and leaf nitrogen content per unit area (N_{area}) to decrease as mean annual temperature (MAT) increased. However, there was no systematic variation observed in leaf nitrogen content per unit mass (N_{mass}) with changing MAT. According to Sharma et al., (2020), *Rhododendron campanulatum* exhibited higher specific leaf area (SLA) below the treeline compared to above it. And concluded that the species have adaptation strategy to cope with challenging environment at higher

elevation as indicated by the high juvenile density of plants, low seedling mortality, and low SLA observed above the treeline. Similarly *Rhododendron lepidotum* also shows smaller plant height with a low basal diameter, internodal length, leaf area & SLA at higher elevation (Pandey et al., 2021). A study by Shrestha & Jha, (2010) in *Aconitum naviculare* reported that individuals growing within juniper scrub exhibited greater plant height and petiole length. On the other hand, open areas had higher tuber mass, number of flowers per plant, and number of seeds per follicle. Stem mass and above-ground biomass decreased with increasing elevation. Chapagain, et al., (2019) studied the patterns of trait variation along the elevation gradient in *Aconitum spicatum* and found that the alpine population exhibited hindered growth, reduced biomass, a lower count of smaller leaves, and a diminished number and smaller reproductive structures, when compared to the subalpine and lower alpine populations.

2.4 Plant functional traits in climate change studies

Mountain regions have warmed at a greater rate than their low elevation counterparts often with greater increases in winter and spring temperature (e.g. Díaz and Bradley, 1997; Rangwala & Miller. 2012; Liu et al., 2009). Also in Nepal, the country experiencing a warming trend of 0.06°C per year (Shrestha and Aryal, 2011) with greater warming trends in the middle mountain than in the Terai and Siwalik region (Shrestha et al., 1999). Furthermore upper Mustang region has witnessed a yearly temperature increase of 0.13 °C on average (Aryal et al., 2014).

Medicinal plants are essential natural resource which constitutes one of the potential sources of new products and bioactive compounds for drug development (Gangwar et al., 2010). Himalayan mountains are rich in medicinal plants (Lama et al., 2001).

Traits have contrasting genotypic patterns and will be subjected to different climate selection pressures, which may lower the working optimum for functional traits and traits are independently associated with different climate factors, indicating that some trait correlations may be disrupted in the future (Ahrens et al., 2020). Functional traits are a non-dispensable tool to predict responses of plants and vegetation to environmental drivers like global warming, land use change or invasive species, and the effects of these drivers on community structure and ecosystem functions (Lavorel and Garnier, 2002). Trait-climate responses within and between biomes that help us understand which plant phenotypes may cope with or thrive under current and future

climate change (Kühn et al., 2021). The accurate characterization of the leaf economics spectrum and its relationship with the environment will be helpful in modeling nutrient fluxes and vegetation boundaries under changing land use and climate (Wright et al., 2004). Plant trait relationships are thus generalizable to the edge of global trait-space, informing prediction of plant community change in a warming world.

2.5 Research Gap

Plant intraspecific trait variation (ITV) shapes ecological dynamics and affects plant performance, species interactions, community structure, and ecosystem properties (Westerband et al., 2021). Most of plant functional trait variation studies in the past conducted on community level (Maharjan et al., 2021; Shah et al., 2019; Pescador et al., 2015) rather than a species level. Only a few studies in the Himalaya have analyzed intraspecific variations in functional traits along the elevation gradients. For example, Pandey et al., (2021) measured anatomical features, plant height, leaf area, specific leaf area, and stem diameter of *Rhododendron lepidotum* along elevation gradient in eastern and central Nepal. Plant traits of *Aconitum spicatum* (Chapagain et al., 2019) and *A. naviculare* (Shrestha & Jha, 2010) have been also analyzed along the elevation gradients. But, these previous studies have not measured some of the key traits such as specific leaf area and leaf nitrogen content. Meta-analyses on functional trait have also focuses on the leaf trait (Wright et al., 2004; Wright et al., 2005; Read et al., 2014; Midolo et al., 2019) while other traits remains less explored. Moreover, there is limited knowledge about the traits associated with intraspecific variation in biomass allocation along elevation gradients. Consequently, a significant gap exists in our understanding of how plant functional traits vary along elevation gradients across different species.

CHAPTER 3. MATERIALS AND METHOD

3.1 Study site

The study was conducted within Annapurna Conservation Area (ACA) which covers an area of 7629 km². The ACA stretches across Manang, Mustang, Kaski, Myagdi, and Lamjung districts. The present study focused on the trekking route to Annapurna Base Camp for measuring *Aconitum spicatum*, which is located along the banks of the Modi River. Additionally, *Aconitum naviculare* was sampled in upper Manang valley located on the trans-Himalayan region.

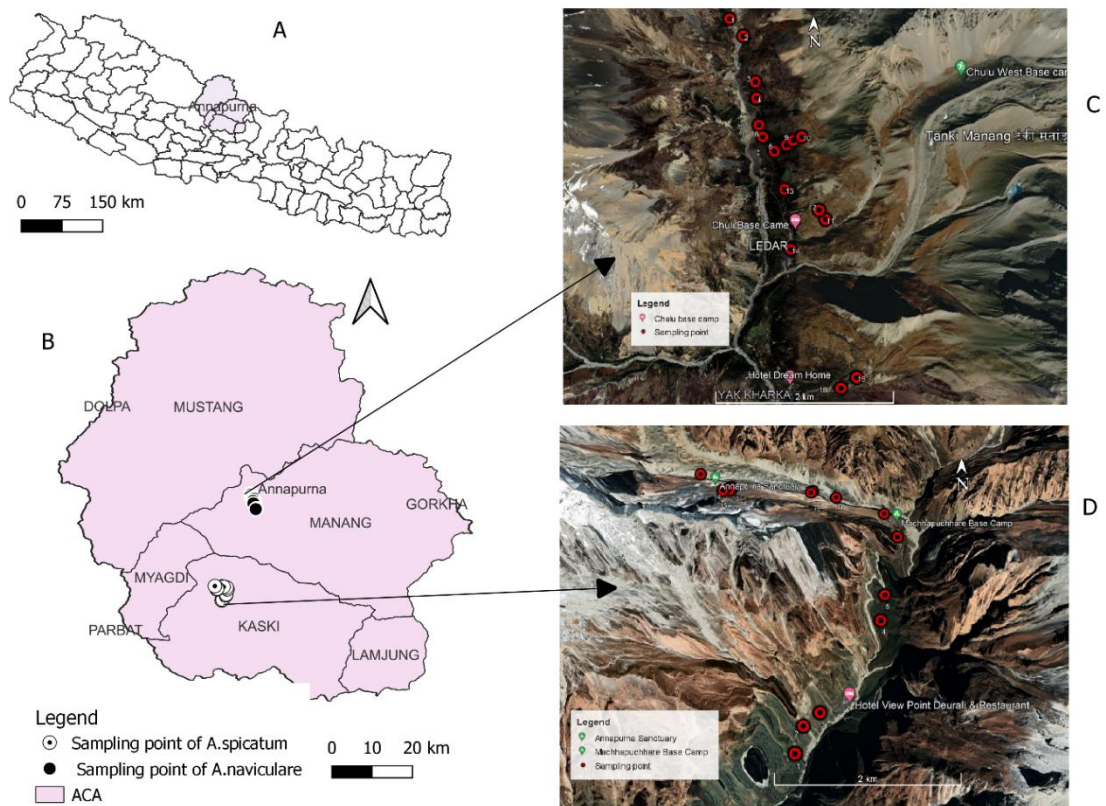


Figure 1: Map of the study area showing Annapurna conservation area in Nepal (A), Map of Annapurna conservation area showing sampling site in Kaski and Manang District (B), sampling site of *Aconitum naviculare* in google earth map (C), and sampling site of *Aconitum spicatum* in Google earth map (D).

3.1.1 Annapurna base camp area

Vegetation and physiography

The sampling of *Aconitum spicatum* was conducted along Modi River valley, within the altitude range of 3030 to 4465 m, along the trekking route to the Annapurna base camp. The sampling sites lie between 28.49°N to 28.53°N latitude and 83.87°E to 83.90°E longitude. The vegetation in the area exhibits distinct characteristics based on elevation. At lower elevations (>3,000-3,500 m), there are upper temperate coniferous and mixed broad-leaved forests. Moving up, at and below the tree line (>3,500-4,000 m), sub-alpine mixed forests take dominance, primarily with *Abies spectabilis* and *Betula utilis*. Beyond the tree line, around 4,000 m, the landscape transitions to lower alpine thickets consisting mainly of dwarf bushes from the *Rhododendron* species. Above 4,000 m, the terrain transforms into alpine meadows and grasslands (Chapagain et al., 2019). The associated vegetation of *Aconitum spicatum* consisted of *Cotoneaster acuminatus*, *Arundodanx*, *Meconopsis*, *Pedicularis* sp, *Rumex nepalensis*, *Thalictrum*, *Saxifraga*, *Fragaria*, *Anaphalis*, *Rhododendron anthopogan*, *Bistorta* along with various grasses and pteridophyte species.

Climate

The Modi River originates from the glaciers of the Annapurna range, along with other tributaries, streams, and waterfalls in the catchment areas. The study area encompasses the temperate to the alpine bioclimatic zone. The Modi River watershed experiences a typical monsoon climate, with Nepal's highest precipitation levels. Analysis of meteorological data from the nearest station (Lumle) over the past 11 years (2012-2022) revealed a mean annual precipitation of 5255mm (Figure 2). The highest monthly precipitation occurs in July with 1511 mm, while November has the lowest with 4 mm. The mean annual minimum temperature is recorded as 12.04°C, and the maximum temperature is 27.09°C. January exhibits the lowest monthly mean temperature at 4.54°C, while August reaches the highest at 24.23°C.

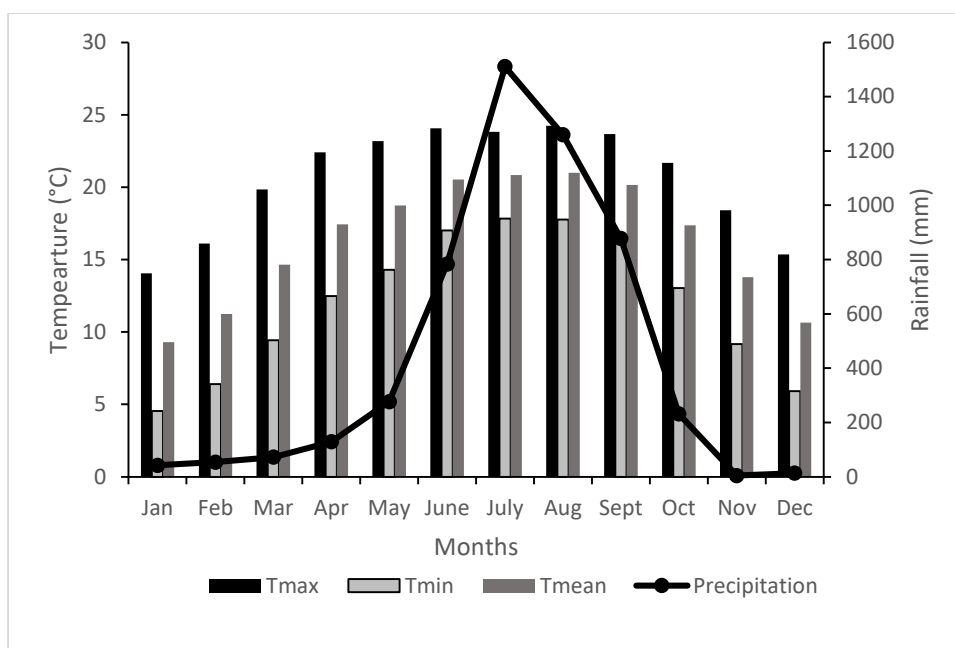


Figure 2: Average monthly temperature and precipitation of past 11 year (2012-2022) of Lumle meteorological station, Kaski (Data from Department of Hydrology and Meteorology).

3.1.2 Upper Manang valley

Vegetation and physiography

The study area is situated in the upper part of Manang district. The sampling site spans from 28.72°N to 28.76°N latitude and 83.96°E to 83.98°E longitude, with an altitude ranging from 4250 m to 4500 m. The sampling site extends from Yakh Kharka and Ledar to near Thorong Phedi. The Valley divides the Tibetan Plateau to the north and the major Himalayan axis of the Annapurna range to the south, and it is part of the country's trans-Himalayan dry zone (Chhetri et al., 2017). At the upper part the moist alpine scrub beyond the tree line on the north-facing slope is primarily characterized by dominant species such as *Rhododendron anthopogon*, *Juniperus indica*, and *Caragana* species. In contrast, the alpine scrub on the dry southern slope is composed of dwarf and prostrate junipers (*Juniperus indica*, *J. recurva*, *J. squamata*), *Rhododendron lepidotum*, *Rosa* spp., *Berberis* spp., *Ephedra gerardiana*, and other similar plant varieties (Shrestha & Jha, 2009). The associated vegetation in the study area, along with *Aconitum naviculare*, includes *Juniperus* sp, *Rhododendron lepidotum*, *Berberis*, *Caragana*, *Ephedra gerardiana*, *Cotoneaster microphyllus*,

Potentilla sp, *Cremanthodium* sp, *Stellera* spp, *Anaphalis*, *Spirea*, various grasses and other vegetation.

Climate

The Manang district experiences a climate that ranges from temperate to cold alpine. The study site specifically exhibits a cold alpine climate. Due to its location north of the massive Annapurna range, the area receives limited rainfall from the southeast monsoon. Based on the climatic data collected from the Humde meteorological station over the past six years (2016 to 2022), the average annual precipitation was recorded at 448 mm. The highest monthly precipitation occurs in July with 79.36 mm, while November receives the lowest with 1.54 mm. The mean annual minimum temperature is 0.35°C, while the maximum temperature reaches 14°C. January records the lowest monthly mean temperature at -10°C, while July exhibits the highest at 27.27°C. The area remains covered in snow from November to March.

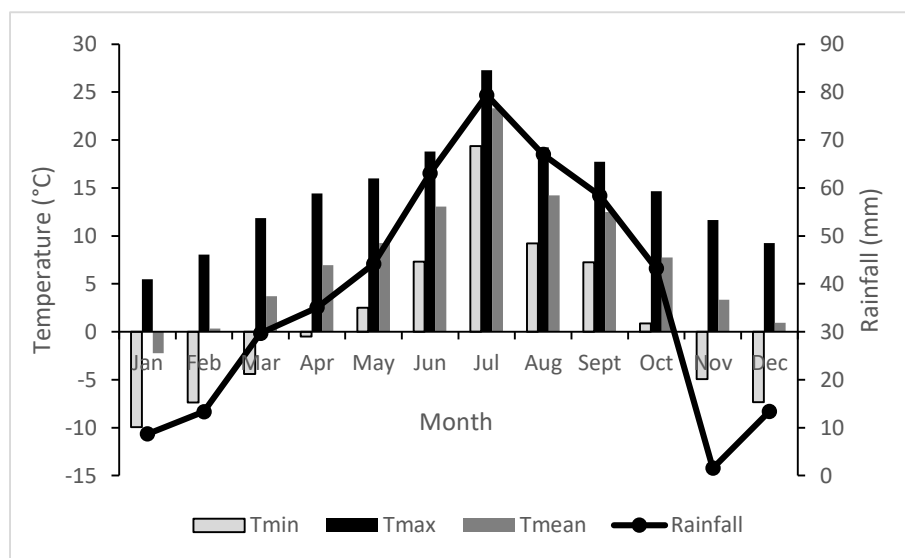


Figure 3: Average monthly temperature and precipitation of past 6 year (2016-2022) of Humde meteorological station, Manang (Data from Department of Hydrology and Meteorology).

3.2 Study species

3.2.1 *Aconitum spicatum* (Brühl) Stapf

Aconitum spicatum (Brühl) Stapf is a perennial herb of family Ranunculaceae, with paired tuberous roots and erect stems 1- 2m (Ghimire et al., 2008; Lama et al 2001).

The aerial part of the plant is annual but the tubers are perennial (Chapagain et al., 2019). The leaves of *Aconitum spicatum* are deeply lobed, exhibiting an orbicular-cordate or reniform shape. The ovate lobes are further divided into pointy or serrated segments. Deeply lobed, orbicular-cordate or reniform leaves; ovate lobes further divided into pointy or serrated segments. Flowers on a dense terminal spike are dark blue, violet to greenish white, tinted with purple, and veined; the spur is recurved. Lower surface of the sepal is densely pubescent. The largest and broadest upper sepal has a helmet-like form, a small beak, and an s-shaped petal head. Hairy and swollen follicles. Flowering period of plant is Jul-Sep and fruiting in Aug.-Nov. Commonly occurs on open and wet areas in forests scrubland, meadows, river, and stream side along 3000-4300 m altitude (Ghimire et al., 2008; Lama et al 2001). It is used as medicine for cough, bile fever, lung and intestine infection, headache, cuts and wounds, tubers are highly poisonous (Lama et al., 2001). It is detoxified by boiling with the extract of *Terminalia chebula* Retz.

3.2.2 *Aconitum naviculare* (Brühl) Stapf

Aconitum naviculare (Brühl) Stapf (Ranunculaceae) is a small perennial herb endemic to Himalaya and distributed in Nepal, Bhutan, and Tibet (Lama et al., 2001; Ghimire et al., 2008). It has biennial tuberous roots and annual aerial part. The stem and tuber were remain connected only with meristematic tissue (Shrestha & Jha, 2010). The plant is characterized by spherical kidney-shaped leaves that are primarily basal, deeply (palmately) split into 3-5 segments, with 2-3 entire or serrated lobes (Ghimire et al., 2008). A single individual consist of 1-17 flower and 160 seeds with average 4-16 follicle (Shrestha & Jha, 2010). Flowers borne on the thin pedicels, reddish blue with darker veins, bulbous spur, boat-shaped top sepal. Follicles are covered in persistent sepals and are hairy. Flowering season runs from July to October (Lama et al., 2001; Ghimire et al., 2008)). The smaller seeds were dispersed during low temperature and moisture condition exists (Shrestha & Jha, 2010). It is found in south facing warm, open and dry areas, as well as within sclerophyllous vegetation and thorny plants (Shrestha & Jha, 2009). It is used against poison effect, fever due to poisoning, bile fever and infections (Lama et al., 2001) and fever and jaundice (Bhattarai et al., 2006).

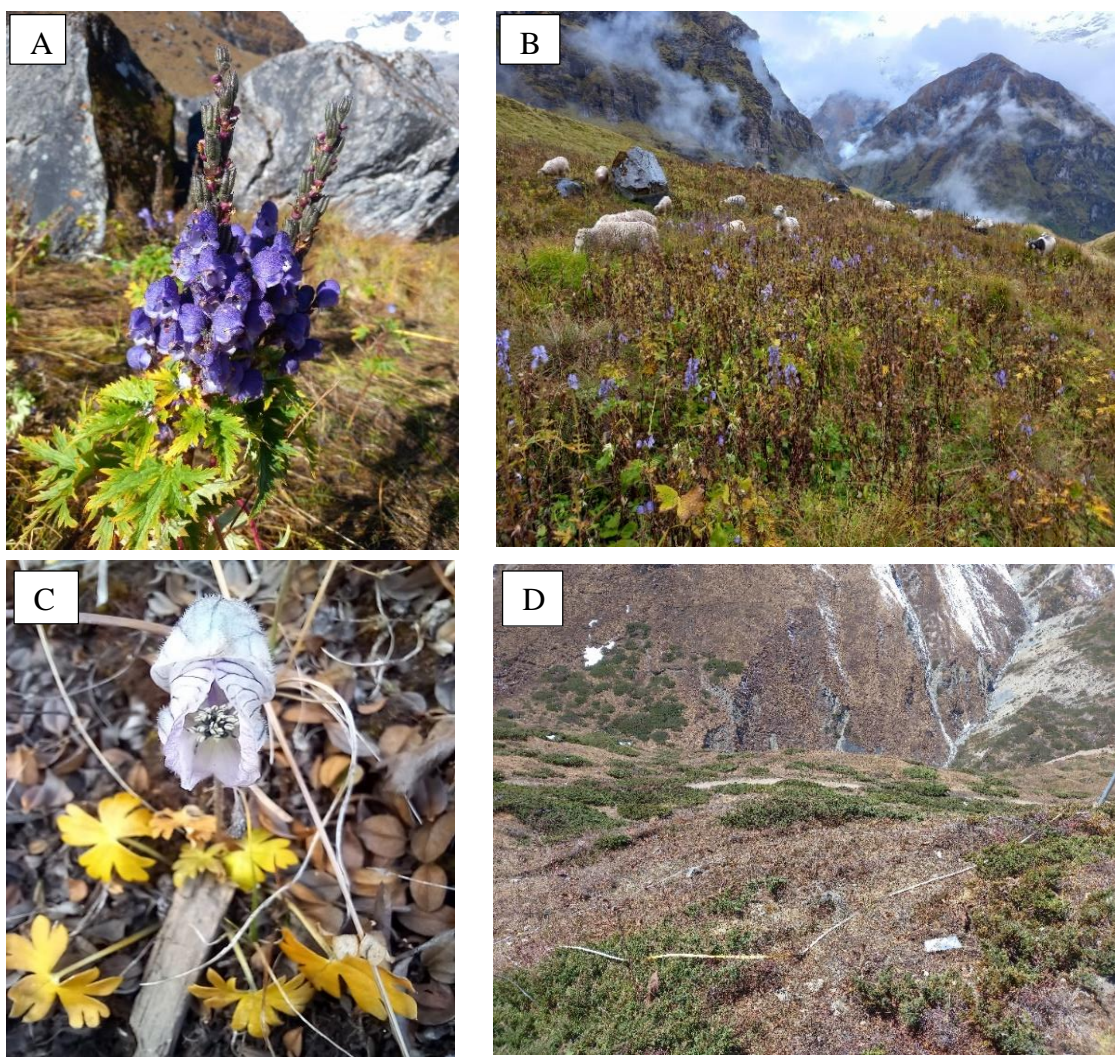


Figure 4: Photograph of study species and their habitat. A) *Aconitum spicatum*, B) Habitat of *Aconitum spicatum* in Annapurna base camp, Kaski, C) *Aconitum naviculare* and D) habitat of *Aconitum naviculare* in Ledtar, Manang.

3.3 Methods

3.3.1 Selection of plant functional traits

Altogether 14 traits were selected to understand their responses to elevation gradient. Ecological significance was taken into consideration while selecting the traits (Table 1), and those that were simple to assess in the field were given much more emphasis. The selected traits were plant height, basal stem diameter, leaf area, specific leaf area, leaf number, leaf nitrogen content (LNC) (N_{mass} : leaf nitrogen content per unit dry mass of leaf and N_{area} : Leaf nitrogen content per unit leaf area), seed mass, floral axis length, flower number, root to shoot ratio, root mass fraction (RMF), stem mass fraction (SMF), leaf mass fraction (LMF) and reproductive mass fraction (ReMF).

Table 1. Ecological significance of plant trait

Plant traits	Significance
Plant height	Related to growth form, competitive vigor, reproductive size, whole-plant fecundity, potential lifespan (Pérez-Harguindeguy et al., 2013)
Leaf area	Linked with various environmental stressors, ecological strategy with regard to disturbance, nutrient stress and also associated with allometric factor (plant size, twig size, anatomy, architecture, leaf number and number of lateral bud produced (Pérez-Harguindeguy et al., 2013).
Specific leaf area	Associated with relative growth rate that's why commonly used in growth analysis and also related with photosynthetic rate, leaf life span, leaf nitrogen content and other (Pérez-Harguindeguy et al., 2013).
Leaf nitrogen content	Associated with nutritional quality in the food web, mass based photosynthetic rate and SLA (Pérez-Harguindeguy et al., 2013).
Seed mass	Related with survival and establishment of seedling
Biomass allocation	Response to environmental stress, nutrient stress, competition and so on (Poorter et al., 2012)

3.3.2 Sampling design

Sixteen plots were sampled for each of two species (*Aconitum spicatum* and *Aconitum naviculare*). Due to patchy distribution of plant a place where the plant population was found high were selected for the sampling. At each sites a square plots of 10m×10m were defined for *A. spicatum* and 5m×5m for the *A. naviculare*. The difference in plot sizes for sampling *Aconitum spicatum* and *Aconitum naviculare* was due to the inherent differences in their individual sizes. *Aconitum spicatum* exhibits a notably greater stature compared to *A. naviculare*. Sampling was done at an elevation difference of ca. 100 m where suitable populations were available.

3.3.3 Field measurements and sample collections

The fieldwork for data collection of *Aconitum spicatum* in the trekking route of Annapurna Base Camp was conducted from 21st September to 30th September. Additionally, the data collection for *Aconitum naviculare* in Manang was carried out from 15th October to 3rd November. Height of 138 individual of *Aconitum spicatum* and 149 individual of *Aconitum naviculare* were measured from ground level to upper boundary of the main photosynthetic tissue (excluding inflorescence) (Pérez-Harguindeguy et al., 2013), with the help of measuring tape and basal stem diameter was measured at base of stem with the help of digital vernier caliper (Berrylion, GB/T1212. 2-1996). Floral axis length was measured from base of floral axis. From each sampling plot healthy looking ten individual were selected subjectively and then 2 or 3 fully expanded healthy leaves with no noticeable indications of pathogen attacks were plucked to determine specific leaf area and leaf nitrogen content. Altogether 302 leaf of *Aconitum spicatum* and 209 leaf of *Aconitum naviculare* were collected. Leaf petioles were removed and photographs of all fresh leaf samples were taken in the field. To improve data accuracy, photographs of the same leaf sample were taken twice. Then, the leaves were put between blotting papers for drying transportation from field to the laboratory. One or two individual from each plot were collected for the measurement of biomass allocation. Each individuals were uprooted carefully so that the all the daughter and mother tubers remained intact. Soil and debris attached in the plant part including tubers were cleaned with water. Normal roots were removed Plant part such as rhizome, leaf, stem and inflorescence were separated and kept in the paper pouch or cotton bags during transportation.

Soil moisture and soil pH were measured using Kelway Soil Acidity and Moisture Tester (Kel Instruments, HB-2). Soil samples from 5-10 cm depth were collected from four corner and center of each plot, and mixed to make a single sample. Then the soil was allowed to air dry and sieved through the 0.425 mm sieve. The fine soil samples were stored in plastic zipper bags until further chemical analyses.

With the help of GPS receiver (Garmin, GPSMAP 64s) elevation, latitude and longitude of each sampling plot were recorded and slope and aspect were measured by using clinometer. While vegetation cover and disturbance were recorded by visual observation.

3.3.4 Laboratory measurements

Specific leaf area

For the determination of specific leaf area, the leaf area was measured from photographs taken in the field using software ImageJ (Ferreira and Rasband, 2011; Weraduwege et al., 2015). Subsequently, the leaf samples were oven dried at 60°C for 72 hours. After drying, the samples were weighed using a digital balance (0.0001g) to determine biomass.

Seed mass

Hundred healthy looking seeds free from insect and fungal infestation and damage were carefully selected from each sampling plot. The seeds were subjected to an oven-drying process at a temperature of 60°C for a duration of 72 hours (Pérez-Harguindeguy et al., 2013). To ensure accuracy, triplicate samples were prepared and weighed through digital balance (0.0001g). Due to early snow fall in the study area in this year seed output of *Aconitum naviculare* was disturbed and we can't collect the seed.

Plant biomass allocation

Stem, leaf and reproductive parts were oven dried at 60°C for 72 hours. In the present study, the tuber represents root of the plant. We did not include the old tuber of the previous year in the calculation. Due to larger size and flesh nature of the tubers, they were oven dried at 60°C for 120 hours. Dry biomass were measured using digital balance (0.001 g).

3.3.5 Laboratory chemical analyses

Soil organic carbon

The Walkley and Black method was applied for the determination of soil organic carbon (Gupta, 2000). First of all, all the reagents required for the performing experiment were prepared. Then, 0.25 g soil was taken in a dry 500 ml conical flask. After that, 10 ml of 1 N $K_2Cr_2O_7$ was pipetted and swirled a little. Then, 20 ml of H_2SO_4 was added and swirled again two or three times. The flask was allowed to stand for 30 minutes and then 200 ml of distilled water was added to it. Then, 10 ml of phosphoric acid and 1 ml of diphenylamine indicator was added respectively. Finally, the contents

was titrated with 0.5 N ferrous ammonium sulphate solution till the colour changes from blue-violet to green. Simultaneously, a blank is run without soil.

$$\text{Soil organic carbon (\%)} = 0.003 \times \frac{10 (\text{Blank reading} - \text{Titration reading})}{\text{Blank reading} \times \text{wt. of soil (g)}} \times 100$$

Soil and leaf nitrogen content

The same oven dried leaves used in SLA measurement were used for the determination of LNC. Out of 10 leaf samples from each plot, only three samples were selected systematically (e.g. sample 1, 4 and 7 from first plot; sample 2, 5 and 8 from second plot) to reduce the expenses of chemical analyses. All leaves from one plant were missed to make a single leaf sample for LNC determination. Hence a total of 96 leaf samples with 48 leaf sample for each species were proceeded for LNC determination. We followed the micro-Kjeldahl method for determination of soil nitrogen (Kalra, 1998) and leaf nitrogen content (Horneck and Miller, 1998) which involved following three steps.

Digestion: Oven dried leaves were crushed into powder by hand crushing. 0.25g of crushed leaf sample was taken in digestion tube with 2g of the catalyst (a mixture of 100g Potassium Sulphate: 10g Copper Sulphate: 1g selenium powder) and 6ml concentrated Sulphuric acid. Then the mixture was kept in the digester with gentle shaking at 350°C for about 30-40 minutes until green coloration appeared. one blank sample without plant material was also run for each batch of six samples. Then the tube with digest was allowed to cool.

Distillation: After cooling 60 ml distilled water added with constant shaking and the digest was transferred to distillation flask (300ml). Regulator of heating mantle was set to 50. Then, 10 ml of boric acid indicator was taken in a clean and dry beaker (100 ml) and placed below the nozzle of the condenser in such a way that the end of nozzle dip in to the indicator. Once the digest became warm 30 ml sodium hydroxide (40%) and the temperature was raised by setting the regulator to 70. The black color of the mixture in the distillation flask was noticed. Once the distillation began to condense the color of boric acid indicator changed from purplish red to green. Distillation was continued until the volume of distillate in beaker reached to about 50 ml.

Titration: The beaker containing distillate was removed and titrated it with hydrochloric acid (0.1N). The volume of HCl consumed was recorded. The same procedure was followed for other samples.

The given formula was used to calculate Leaf N.

$$\text{Lea N (\%)} = \frac{14 \times N \times (S-B)}{M} \times 100$$

Where, N = normality of HCl

S = volume of HCl consumed with sample (ml)

B = volume of HCl consumed with blank (ml)

M = mass of leaf sample (mg)

From the above method we determined mass-based leaf nitrogen content (N_{mass}). According to Wright et al., (2004) N_{mass} and N_{area} are inter-convertible using LMA (or SLA). Therefore, N_{area} was estimated indirectly by using following formula:

$$N_{\text{area}} = N_{\text{mass}}/\text{SLA}$$

All the procedure for analyzing soil nitrogen content is also same as that of leaf nitrogen content determination except the catalyst used in the digestion procedure. For soil nitrogen content determination, 1 g of soil was taken, 0.4g of Copper Sulphate and 3.5g Potassium Sulphate as catalyst, and 6ml of concentrated Sulphuric acid for digestion.

3.4 Data analysis

SLA was calculated as the fraction of one sided area of fresh leaf (LA) to dry mas of the same leaf (LM) and determined by using the following formula (Pérez-Harguindedguy et al., 2013)

$$\text{SLA} = \frac{\text{Area of leaf (LA)}}{\text{dry mass of leaf (LM)}}$$

For the determination of biomass allocation pattern, the Root to shoot ratio(R/S), Root mass fraction (RMF), Stem mass fraction (SMF), Leaf mass fraction (LMF) and Reproductive mass fraction (ReMF) were calculated using the following formulae (Poorter et al., (2012):

$$R/S = \frac{\text{Tuber dry mass}}{\text{Stem dry mass} + \text{Leaf dry mass} + \text{Reproductive dry mass}}$$

$$\text{RMF} = \frac{\textit{Tuber dry mass}}{\textit{Total plant dry mass}}$$

$$\text{SMF} = \frac{\textit{Stem dry mass}}{\textit{Total plant dry mass}}$$

$$\text{LMF} = \frac{\textit{Leaf dry mass}}{\textit{Total plant dry mass}}$$

$$\text{ReMF} = \frac{\textit{Reproductive dry mass}}{\textit{Total plant dry mass}}$$

Independent Sample t-test and Mann-whitney U-test were used to compare the mean values of the traits of two species. Log10 transformations were performed for the variables that did not meet the assumption of normality (R/S, Organic carbon, soil nitrogen). For those variable that didn't meet the assumption of normality even after transformation, non-parametric Mann-whitney U-test was performed. Generalized linear model (GLM) was used to determine the pattern of trait variation along the elevation gradient. Correlation was done to know the relationship between specific leaf area (SLA), leaf nitrogen content per unit dry mass of leaf (N_{mass}) and leaf nitrogen content per unit leaf area (N_{area}). All statistical analyses were done using SPSS version 25 (IBM Corp, 2017) and R studio 4.2.2. (R Core Team, 2021).

CHAPTER 4: RESULTS

4.1 Functional traits of *Aconitum spicatum* and *A. naviculare*

The study found significant differences in the mean of all traits except root mass fraction, root-shoot ratio, reproductive mass fraction, and soil nitrogen content between two species. The mean values of plant height, floral axis length, basal stem diameter, number of leaves, specific leaf area, stem mass fraction, rhizome mass, leaf nitrogen content, and soil organic carbon were greater in *Aconitum spicatum*. Whereas leaf mass fraction have revealed higher values in *Aconitum naviculare* (Table 2).

Table 2: Variation in traits between *Aconitum spicatum* and *Aconitum naviculare*

Functional traits	<i>Aconitum spicatum</i> (Mean±S.E)	Sample size (N)	<i>Aconitum naviculare</i> (Mean±S.E)	Sample size (N)	P value
Plant height (cm)	102 ±4.42	139	11.46±0.57	149	<0.001
Floral axis length (cm)	26.59±1.55	136	1.92±0.22	149	<0.001
Basal stem diameter (cm)	0.86±0.03	139	0.12±0.004	149	<0.001
No of leaves	20.4±0.79	139	9.15±0.3	149	<0.001
No of flower	45 ±4.0007	139	2.71±0.19	149	<0.001
Specific leaf area (cm ² /g)	251±4.17	138	194±3.79	148	<0.001
Root to shoot ratio	10.66±1.56	31	5.42±0.52	20	0.656
Root mass Fraction	0.46±0.02	31	0.51±0.02	20	0.137
Stem mass fraction	0.30±0.01	31	0.12±0.01	20	<0.001
Leaf mass fraction	0.14±0.003	31	0.25±0.01	20	<0.001
Reproductive mass fraction	0.09±0.08	31	0.11±0.01	20	0.2
Seed mass (g)	0.104±0.029	39			
No of seed	78±1.318	159			
Rhizome mass (g)	16.360 ±3.72	31	0.43±0.05	20	<0.001
Soil organic carbon (%)	0.8202±0.031	32	0.7361±0.19	32	0.018
Soil Nitrogen (%)	0.529±0.038	32	0.438±0.024	33	0.138
Leaf nitrogen content (%)	2.42±0.11	47	1.83±0.19	47	0.009

4.2 Variation of functional trait along the elevation

The generalized linear model revealed that the trait variation along elevation reflects various pattern.

4.2.1 Vegetative and reproductive traits

Plant height of both species, *Aconitum spicatum* (Figure 5, Appendix 1) and *Aconitum naviculare* (Figure 6, Appendix 2), was negatively associated with elevation. The mean height of *A. spicatum* ranged from 31.7 cm to 219 cm, and the mean maximum height was recorded at 3043 m elevation and the minimum height at 4351 m. whereas the maximum height of *Aconitum naviculare* (25 cm) was recorded from 4347 m altitude and the minimum (6.31 cm) from 4422 m.

While basal stem diameter (BSD), leaf number (Lfno), floral axis length (FAL), and flower number (Flno) showed significant relationships with elevation only in *Aconitum spicatum*, where BSD and leaf number decreased with an increase in elevation, (Figure 5, Appendix 1), and floral axis length (FAL) and flower number (Flno) also show a significant negative relationship with elevation; however, a slightly increasing trend was observed up to 3700 m.

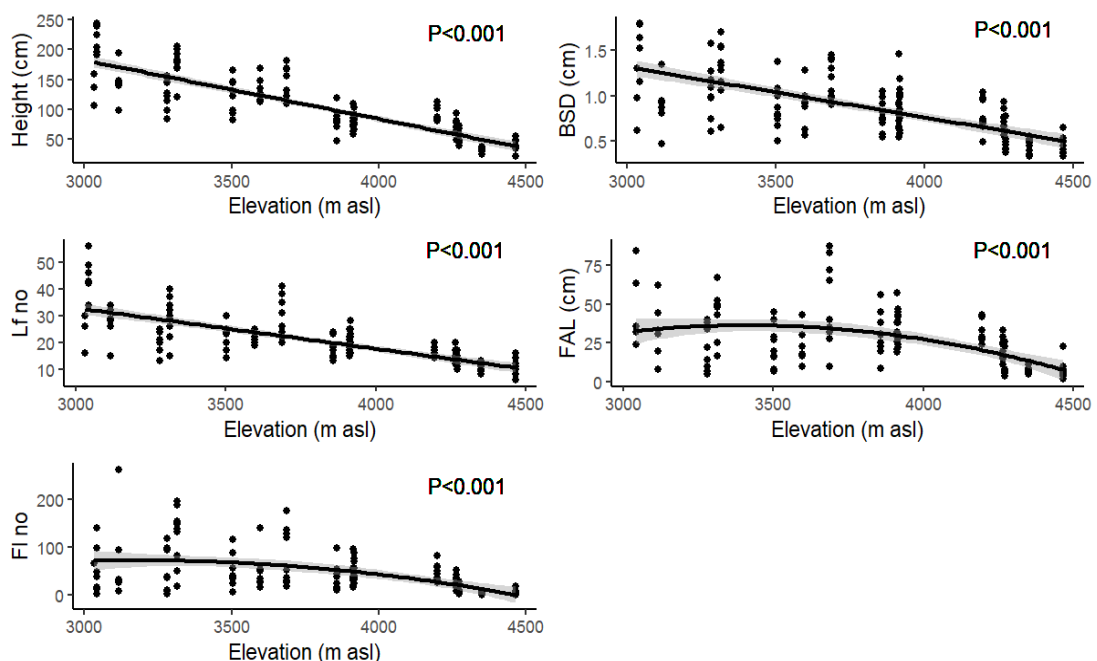


Figure 5: Variation of plant functional traits of *Aconitum spicatum* with elevation. The fitted lines were based on generalized linear model. Where P indicates significance

level. Note: BSD - Basal stem diameter, Lfno - Leaf number, FAL - Floral axis length, Flno - Flower number.

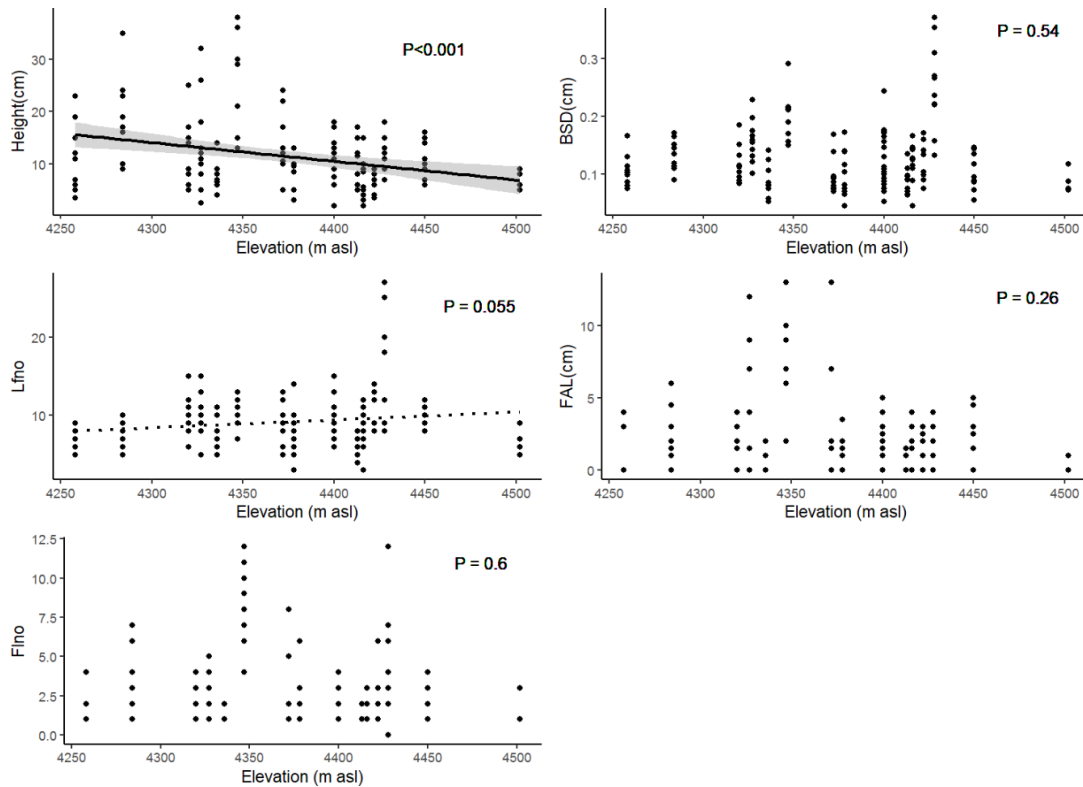


Figure 6: Variation of plant functional traits of *Aconitum naviculare* with elevation. P indicates significance. The fitted lines were based on generalized linear model. Note: BSD - Basal stem diameter, Lfno - Leaf number, FAL - Floral axis length, Flno - Flower number.

Leaf area was negatively associated with elevation in both species. The specific leaf area of *Aconitum spicatum* didn't reveal any significant trend with elevation (Figure 7, Appendix 1); however, a slightly decreasing trend was observed up to 4200 m altitude. While in *A.naviculare*, it decreased with an increase in elevation (Figure 7, Appendix 2). In *Aconitum spicatum* seed mass decreased with increased in elevation while seed number increased with increased in elevation (Figure 8, Appendix 1).

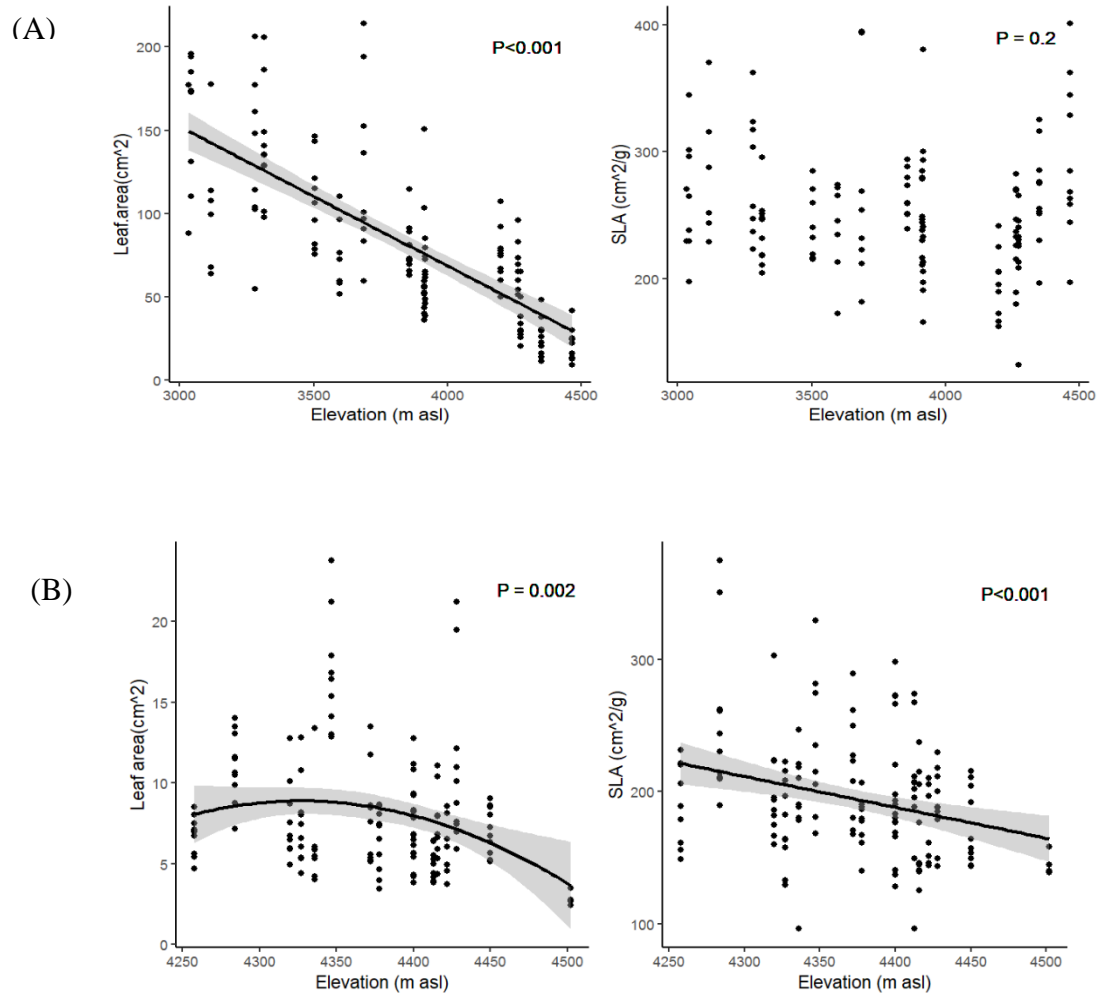


Figure 7: Variation of leaf area and the specific leaf area (SLA) along elevation gradient in *Aconitum spicatum* (A) and *A. naviculare* (B). The fitted lines were based on generalized linear model. P indicates significance.

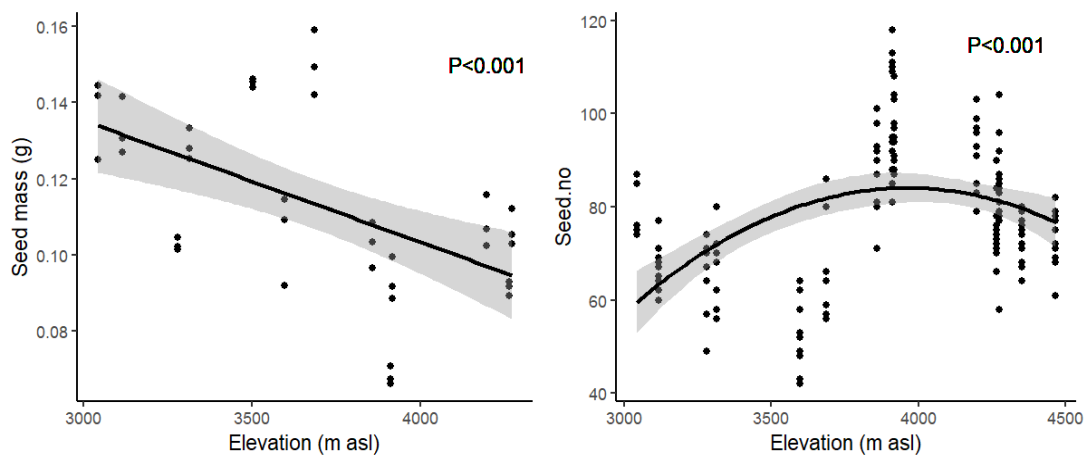


Figure 8: Scatter plot shows relationship between Elevation and Seed mass, and Elevation and Seed number per follicle of *Aconitum spicatum*. The fitted lines were based on generalized linear model. P indicates significance level.

4.2.2 Biomass allocation pattern

Rhizome mass, root-shoot ratio (R/S), root mass fraction (RMF), leaf mass fraction (LMF), and stem mass fraction (SMF) showed significant patterns of variation with elevation in *Aconitum spicatum*. The rhizome mass, root-shoot ratio, and stem mass fraction decreased and root mass fraction and leaf mass fraction increased with increase in elevation (Figure 9, Appendix 1). While only the RMF and R/S varied with elevation in *Aconitum naviculare*, both traits showed a positive relationship with elevation (Figure 10, Appendix 2).

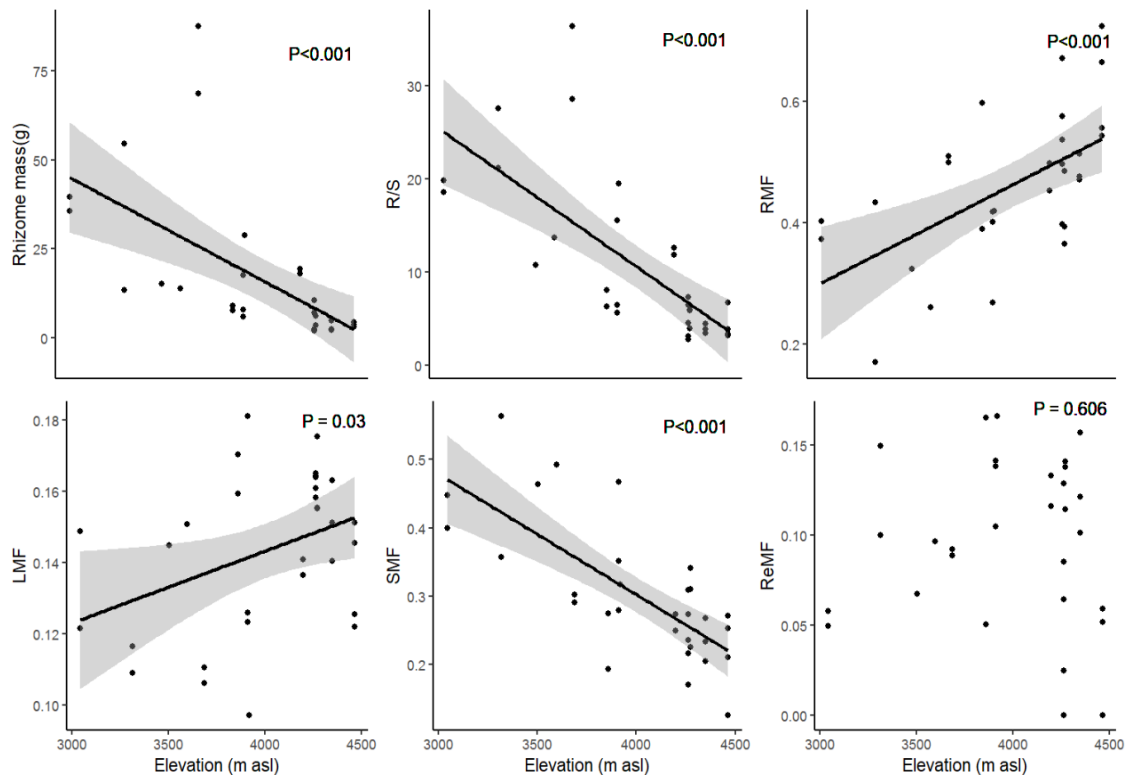


Figure 9: Variation of biomass allocation pattern along the elevation in *Aconitum spicatum*. The fitted lines were based on generalized linear model. Where P indicate the significance level. Note: R/S- Root to shoot ratio, RMF- Root mass fraction, SMF- Stem mass fraction, LMF- Leaf mass fraction and ReMF- Reproductive mass fraction

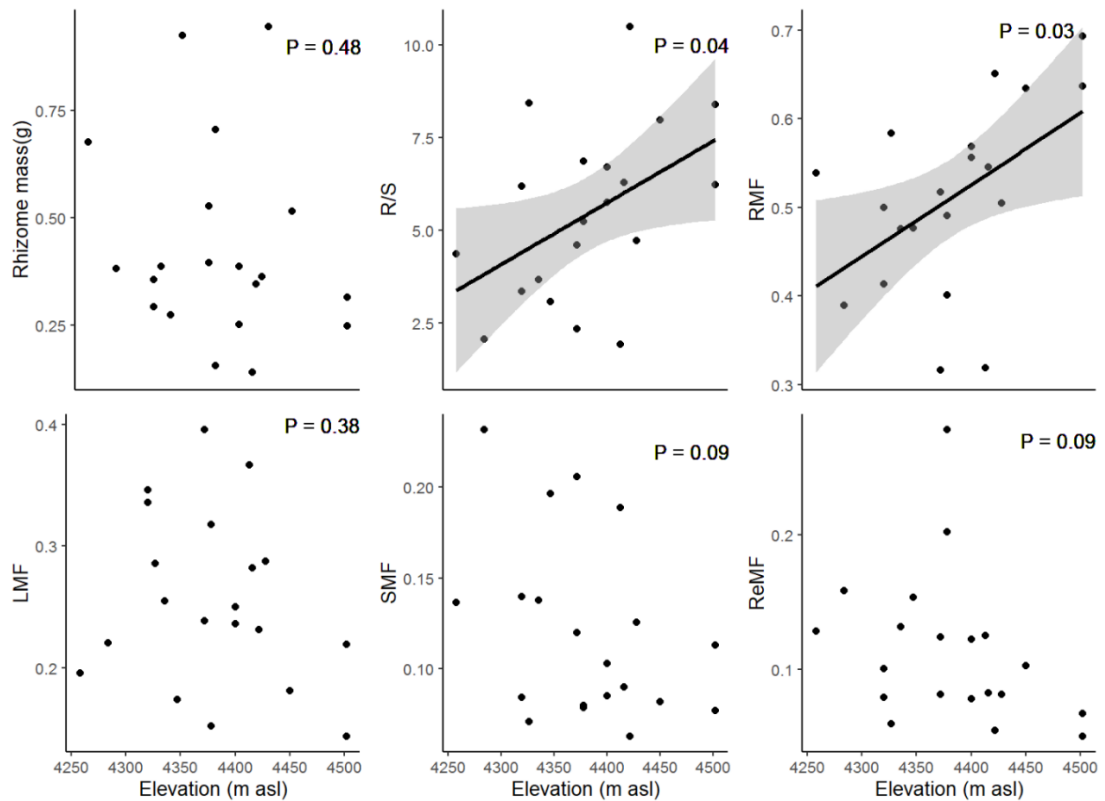


Figure 10: Variation of biomass allocation pattern along the elevation in *Aconitum naviculare*. The fitted lines were based on generalized linear model. Where P indicates significance level. Note: R/S- Root to shoot ratio, RMF- Root mass fraction, SMF- Stem mass Fraction, LMF- Leaf mass fraction and ReMF- Reproductive mass fraction.

4.3 Relationship between leaf traits

Overall Specific leaf area was negatively correlated with Leaf nitrogen content along the elevation gradient. This relationship was significant for SLA with N_{area} ($P = 0.0001^{***}$, $P = 0.02^*$) not with N_{mass} ($P = 0.78$, $P = 0.84$) in both species *A.spicatum* and *A.naviculare* respectively (Figure 11).

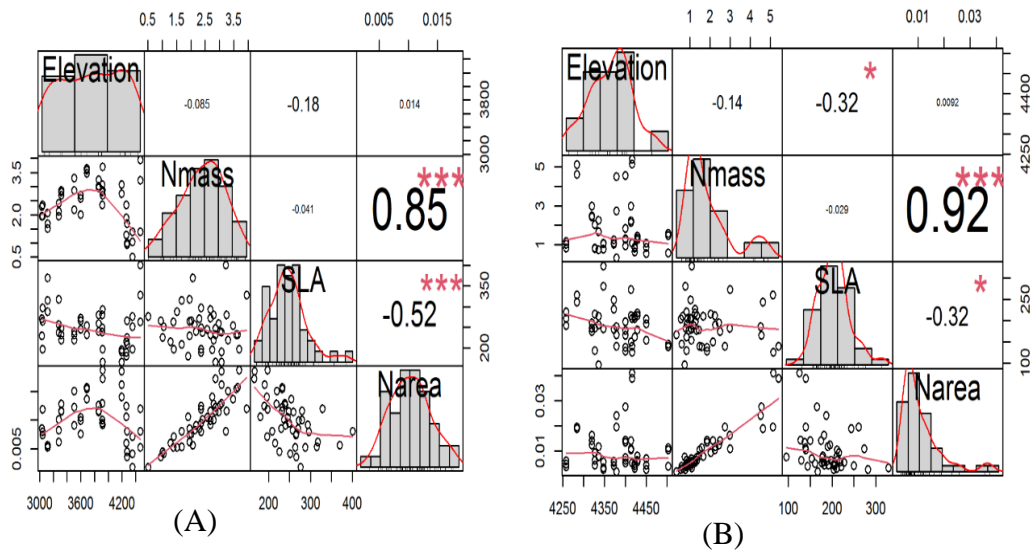


Figure 11: Correlation chart among Elevation, N_{mass}, N_{area} and SLA of (A): *Aconitum spicatum* and (B): *Aconitum naviculare* where Asterisks (*) indicates Significance at P = <0.001 ‘***’, P = 0.01 ‘*’ and SLA- Specific leaf area, N_{mass}- Leaf nitrogen content per unit mass and N_{area}- Leaf nitrogen content per unit leaf area.

4.4 Soil attribute

Mean soil pH and moisture were found to be 6.8 and 65, respectively, in *Aconitum naviculare* and 6.58 and 71.56, respectively, in *Aconitum spicatum*. Soil organic carbon (SOC), soil nitrogen content (SNC), carbon to nitrogen ratio (C/N) and soil pH didn't vary significantly with elevation in *A. spicatum* (Figure 12, Appendix 1), whereas soil organic carbon increased with increase in elevation and soil nitrogen content, carbon to nitrogen ratio and pH didn't vary with elevation in *A. naviculare* (Figure 12, Appendix 2).

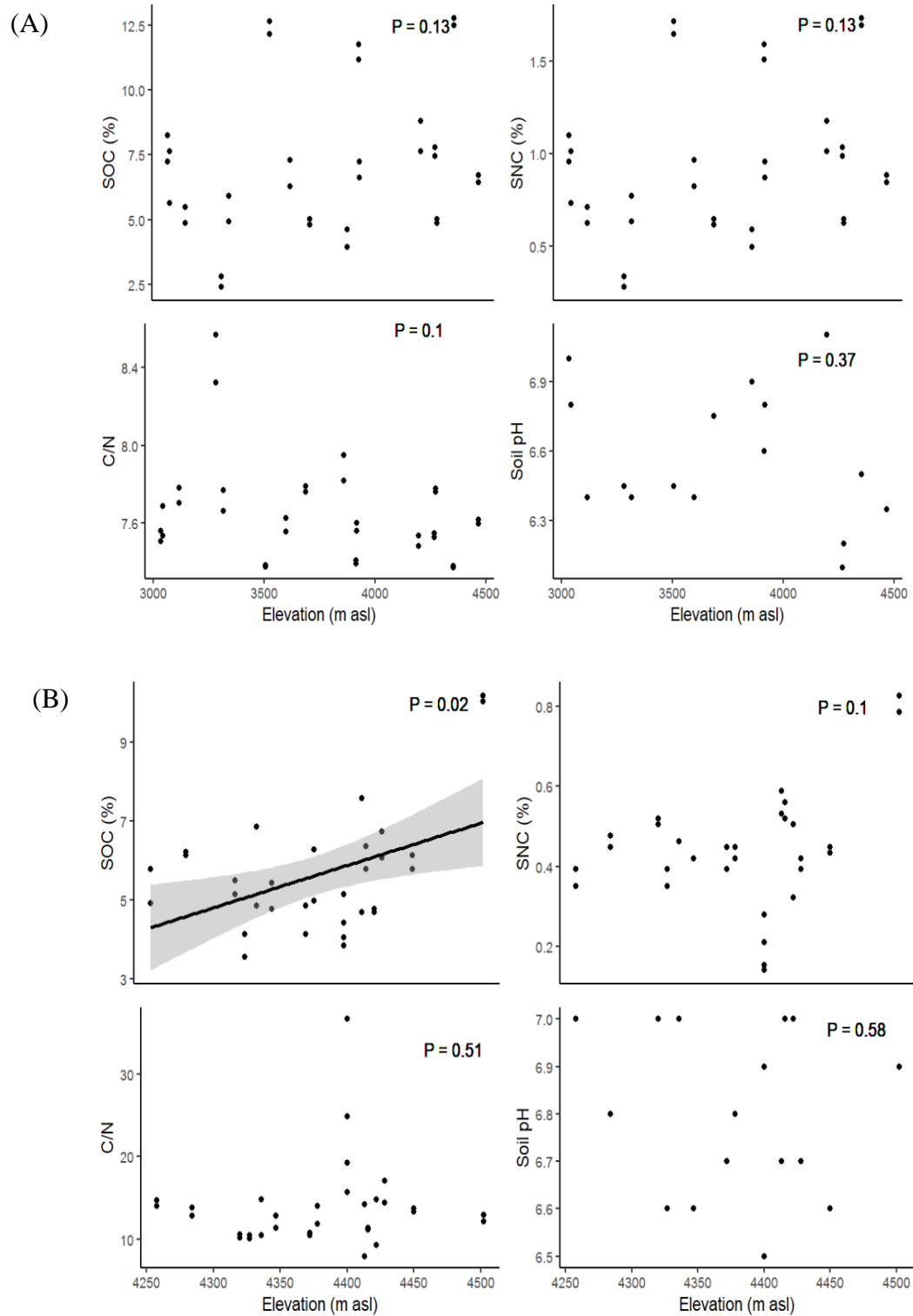


Figure 12: Variation of soil nutrients and pH along the elevation *Aconitum spicatum* (A) and *Aconitum naviculare* (B). Where P indicates significance level. The fitted lines were based on generalized linear model. Note: SOC- Soil organic carbon, SNC- Soil nitrogen content and C/N- Carbon to Nitrogen ratio.

CHAPTER 5. DISCUSSION

5.1 Functional traits of *Aconitum spicatum* and *Aconitum naviculare*

The mean values of most of the measured traits were found to be significantly higher in *Aconitum spicatum* compared to *Aconitum naviculare*. This difference in trait values could be attributed to the contrasting climates that prevail in habitat of these two species. Although both species are found in the Annapurna conservation area, they occupy different habitats. The sampling sites of *Aconitum spicatum* lies on the southern declivity of Annapurna mountain range, which receive the highest amount (>5000 mm per annum) of precipitation in Nepal. On the other hand, the study site of *Aconitum naviculare* is situated on the northern declivity of Annapurna mountain range, which represents a rain-shadow area (trans-Himalayan region) with mean annual precipitation of only 448 mm. Plants that thrive in extremely high elevation often constrained by a combination of factors such as inadequate nutrient absorption caused by underdeveloped soils and scarcity of water resources (MacEk et al., 2012). And it has been observed that increased precipitation has a significant positive effect on plant photosynthetic rates, and subsequently on growth vigor (Song et al., 2016). Moreover, precipitation is recognized as a key driver of various ecosystem processes (Wu et al., 2011). Further, elevation range (3100 to 4500 m asl) of the sampling sites of *A. spicatum* spanned from subalpine to alpine climatic belts while those of *A. naviculare* were all in the alpine region (4250 to 4500 m asl). Height and other growth parameters often decline while going from subalpine to alpine climatic belts due to stressful environment prevalent at higher elevation. Therefore, the substantial difference in precipitation levels between the habitats of *Aconitum spicatum* and *Aconitum naviculare* as well as the difference in elevation range of their distribution might have contributed to the large difference in their measured traits. Though the two study species are congeneric, the genetic difference between two species could have also contributed for the measured differences in traits.

5.2 Variation in Functional traits along the elevation gradient

5.2.1 Vegetative and reproductive trait

The results revealed that elevation had strong effects on some of the measured functional traits such as plant height, leaf area and root mass fraction in both species.

Along with elevation, several environmental factors such as air pressure, temperature, and solar radiation also covariate (Körner, 2007), which can influence plant growth and development. Previous studies have shown that as elevation increases, there is a decrease in plant height and stem diameter in species such as *Aconitum spicatum* (Chapagain et al., 2019), *Rhododendron lepidotum* (Pandey et al., 2021), *Koenigia mollis* (Sigdel et al., 2023), as well as in a group of species studied by Kergunteuil et al., (2018). The greater plant size observed at lower elevation compared to higher elevation could be a result of increased competition for resources and competition with other greater heightened associated plants. Our field observations support this pattern, as we have noticed that associated species at the same elevation also tend to have greater average height.

Low stature vegetation has the ability to resist the flow of air, snow fall effectively and warm up significantly under sunlight. This character allows them to perform photosynthesis optimally even at lower temperature (Körner, 2007). This suggests that decrease in plant height along elevation gradient represents an adaptive response to the unique environmental condition encountered at higher elevation. This adaptation enables plants to thrive and persist in a mountainous regions with challenging climates and limited resources. The soil in the alpine treeline ecotone does have higher levels of nutrients, but factors like cold temperatures, inhibited microbial activity, and a slower rate of nitrogen mineralization limit the availability and utilization of these nutrients by plants and other organisms (Thébault et al., 2014). Consequently, the availability of resources, such as nutrients and water, tend to decrease with increasing elevation. By maintaining a smaller size, plant can optimize resource utilization and survive in nutrient –poor soils and limited water availability.

Leaf area and SLA decreases with increasing elevation in *Aconitum naviculare*. Our finding align with prior research conducted on *Rhododendron lepidotum* (Pandey et al., 2021), *Rhododendron campanulatum* (Sharma et al., 2020), *Micropholis garciniifolia* (Umaña & Swenson, 2019) as well as a community level analyses conducted by (Midolo et al., 2019) and Cruz-Maldonado et al., (2021). The reduction in SLA was connected to an increase in leaf thickness and higher leaf dry matter content (Cruz-Maldonado et al., 2021). This change in leaf structure and composition is considered an adaptation to colder temperatures and a drier environment.

There was no significant trend of variation in SLA of *Aconitum spicatum* with respect to elevation; however there was a slight decreasing trend up to 4200 m followed by slight increase beyond this point. The reason behind this trend might be due to the fact that plants above 4200m were in the stage of early senescence. During senescence various physiological parameters including photosynthetic activity, chlorophyll content, nitrate reductase activity, vegetative growth and nitrogen content undergo a reduction (Matos et al., 2012), which might have attributed to loss of leaf mass without proportional decrease in leaf area which ultimately results in the increase in SLA.

Seed mass

Generally heavier seed may be selected over the seed number in the stressful environment (Pluess et al., 2005) because the presence of abundant resources within large seeds typically enhances the ability of young seedlings to withstand and establish themselves in the presence of various environmental challenges (Pérez- Harguindeguy et al., 2013). The most significant indicators for explaining seed mass variance were temperature, soil fertility, and soil pH (Dainese and Sitzia, 2013). Contrary to the previous findings reported by Chapagain et al., (2019), our data revealed a decreasing trend in seed mass as elevation increases. However, our results align with the studies conducted by Pluess et al., (2005) and Ge et al., (2020). This suggests that while there is a preference for species with heavier seeds at higher elevation, indicating a selection pressure, it is worth noting that this pattern is not consistently observed in all situations.

5.2.2 Biomass allocation

Since biomass allocation may vary across different environments, species, and over time, the plant must precisely allocate its resources among leaves, stems, and roots to effectively support the physiological activities and functions carried out by each of these organs (Poorter et al., 2012). Results of the present study revealed a significant increase in root mass fraction (RMF) and leaf mass fraction (LMF) with increasing elevation, while there was a consistent decrease in stem mass fraction (SMF) and root-shoot ratio in *Aconitum spicatum*. These findings align with previous studies conducted on other plant species, including *Carex brevicuspis* (Chen et al., 2015) and *Gentiana rhodantha* (Zhang et al., 2020 a) in which an increasing trends of underground storage organ along the elevation gradient have been reported. In nutrient-depleted areas, it is expected that there will be a relatively greater allocation of biomass in the root system

compared to the above-ground shoots (Pérez- Harguindeguy et al., 2013). Moreover, a decline of SMF with increasing elevation align with the findings of Ma et al., (2010) and Xu et al. (2020). Additionally, the increase in LMF and decrease in SMF observed in *Aconitum spicatum* are consistent with the findings of Xu et al., (2020) in *Fritillaria*. Furthermore, Zhang et al., (2020 a) examined *Gentiana rigescens* and reported an increase in both LMF and SMF with elevation. In general, the RMF tends to increase, whereas the SMF tends to decrease when plants are subjected to wind exposure and mechanical effects on shoots, such as stem flexure (Niklas, 1998).

In environments where resources are limited, plants may prioritize survival and growth over reproduction. By investing more in root and leaf development, plants increase their chances of survival and optimize resource acquisition (Shipley & Meziane, 2002), which can indirectly benefit future reproductive efforts. Therefore, the observed patterns of biomass allocation in *Aconitum spicatum* along the elevation gradient support the notion that plants adapt their allocation strategies based on nutrient availability.

The R/S ratio and RMF of *Aconitum naviculare* were found to increase with elevation, while the LMF, SMF and ReMF remained stable. This insignificant variation might be due to the narrow elevation range of distribution, and therefore the sampling. The observed increase in R/S ratio with elevation aligns with previous studies conducted by Qi et al., (2019) and Ma et al., (2010). Conversely, Zhang et al., (2020 a) found an isometric scaling relationship between aboveground and belowground biomass in two *Gentiana* species.

5.3 Relationship between traits

The key leaf traits showed inter-correlation, and specifically, SLA demonstrated positive correlation with leaf nitrogen content per unit mass (N_{mass}) and negative correlation with nitrogen content per unit unit area (N_{area}) (Wright et al., 2004). The result of this study observed a negative correlation between specific leaf area (SLA) and nitrogen content per unit leaf area (N_{area}) in both species and there is no such correlation with N_{mass} . This suggests that plants with thinner and larger leaves tend to have lower nitrogen content per unit area, while those with thicker leaves have higher nitrogen content. Our findings align with previous studies conducted in tropical rainforests of Brazil (Carswell et al., 2000) and Terra firme forest in Venezuela (Reich

et al., 1994), which also reported a negative relationship between SLA and leaf nitrogen per unit area. The lower CO₂ concentration inside leaves in the species from semi-arid sites was linked with higher N_{area}, but the high N_{area} corresponds to less robust tissue (Wright et al., 2004). Under low light conditions, high-SLA species exhibited higher photosynthetic nitrogen use efficiency due to their lower nitrogen content per unit of leaf area. In contrast, low-SLA species had an excessive investment in photosynthetic nitrogen under these conditions (Poorter and Evans, 1998). This represent adaptation strategy that allows them to efficiently utilize and balance their nitrogen resources based on the prevailing light conditions, optimizing their photosynthetic performance and survival in diverse environmental setting. Leaf nitrogen content didn't reveal any significant relation with elevation and this result was supported by Cao et al., (2020) and Zhang et al., (2020 b) while MacEk et al., (2012) reported decrease in nitrogen content with elevation.

CHAPTER 6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Most of the measured functional traits in both *Aconitum spicatum* and *Aconitum naviculare* exhibited a clear pattern of variation along the elevation gradient. In both *Aconitum spicatum* and *Aconitum naviculare*, plant height and leaf area declined with increasing elevation. However, specific leaf area declined with increasing elevation only in *Aconitum naviculare*, while no trend was found in *Aconitum spicatum*. The analysis of both area-based and mass-based leaf nitrogen content did not indicate any relationship with elevation. However, area-based leaf nitrogen content exhibited a negative relationship with specific leaf area. Interestingly, the pattern of variation in root-to-shoot ratio differed between the two species. *Aconitum naviculare* demonstrated a positive relationship with elevation, while *A. spicatum* displayed a negative relationship. In terms of biomass allocation, we found an increase in the root mass fraction with elevation, suggesting that *Aconitum spicatum* and *Aconitum naviculare* allocate more biomass belowground than in the aboveground biomass to cope with harsh environmental conditions at higher elevation. This finding leads us to conclude that the reproductive success of these plants depends also on vegetative reproduction, such as regeneration through rhizomes, rather than sexual reproduction. This variation in intraspecific traits suggests that elevation-dependent climate variations play a crucial role in shaping functional traits, indicating the significance of elevation and associated environmental factors in plant growth. These adaptive changes in functional traits enable these species to withstand the diverse climatic conditions experienced across different elevations. Furthermore, this variation in functional trait helps to understand response how a species occupying a wide range of elevations can effectively adapt to climate change by modifying their traits and strategies for utilizing resources. As elevation gradient is also about temperature gradient the determination of trait responses along elevation gradients is help us to anticipate the potential shifts in species' distribution ranges under global warming scenarios.

6.2 Recommendation

- Many easy to measure plant traits were frequently observed. However, there was a lack of information regarding more complex traits, such as the rate of photosynthesis, leaf Carbon / Phosphorus content, and leaf dry matter content. Conducting detailed studies on these traits can also provide a deeper understanding of how plants perform.
- Trait variation along the elevation gradient suggest that the both species have specific adaptation strategy to expose environmental condition. To confirm whether it is because of phenotypic plasticity or genetic variation, this study recommend to conduct common garden experiment in future research.

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APPENDICES

Appendix 1: Summary of GLM showing interaction between Elevation and traits of *Aconitum spicatum*, where elevation and plant traits are predictor and response variable respectively.

Traits	Model	Polynomial order	Residual degree freedom	Residual of deviance	Estimate		AIC value	P value
					Intercept	Elevation		
Height	Linear	1	137	11.6	8.4	-0.001	1304	<0.001
BSD	Linear	1	137	11.74	2.36	-0.0006	-5.05	<0.001
Leaf no	Linear	1	137	10.21	5.87	-0.0007	851	<0.001
FAL	Polynomial	2	132	47.25	3.20	-3.15	1073	<0.001
Fl no	Polynomial	2	134	98	3.6	-4.71	1254.8	<0.001
Leaf area	Linear	1	136	25.4	8.5	-0.001	1333	<0.001
SLA			136	5	5.7	-<0.001	1460	0.196
Rhizome mass	Linear	1	29	17.002	11.03	-0.02	214	<0.001
RSratio	Linear	1	29	7.15	8.08	-0.001	178	<0.001
RMF	Linear	1	29	0.28	-0.20	<0.001	-51	<0.001
LMF	Linear	1	29	0.012	0.006	<0.001	-148	0.03

SMF	Linear	1	29	0.14	1.006	-0.0001	-73.2	<0.001
ReMF	-		29	0.07	0.13	-0.00001	-94.5	0.606
SOC	-		30	4.6581	1.14	0.0002	156	0.134
SNC	-		30	5.32	-0.96	0.0002	30.9	0.134
C/N	-		30	0.03	0.02	-0.00002	4.81	0.1
Soil pH			14	1.16	7.1	-0.0001	9.53	0.3
Seedno	Poly	2	156	31171	78.5	-59.3	1298	<0.001
Seedmass	Linear	1	37	0.015	0.23	-<0.001	-189	<0.001

Appendix 2: Summary of GLM showing interaction between Elevation and traits of *Aconitum naviculare*, where elevation and plant traits are predictor and response variable respectively.

Traits	model	Polynomial order	Residual freedom	degree of	Residual deviance	Estimate		AIC value	P value
						Intercept	Elevation		
Height	Linear	1	147		44.66	16.41	-0.003	934	<0.001
BSD			147		25.32	-3.71	0.0003	-467	0.54
Leaf no	Linear	1	147		18.54	-2.78	0.001	762	0.055
Floralaxis length	-	-	147		55.86	6.54	-0.0008	1095	0.26
Fl no	-	-	147		114.66	9.3	-0.004	1277	0.6
Rhizome mass	-	-	18		4.73	5.23	-0.001	-4	0.48
R/S	Linear	1	18		82.26	-67.62	0.01	91	0.04
RMF	Linear	1	18		0.158	-3.03	-3.03	-34	0.03
LMF	-	-	18		0.09	1.25	-0.0002	-44.8	0.38

SMF	-	-	18	2.49	8.66	-0.002	-66.11	0.09
ReMF	-	-	18	3.24	11	-0.003	-64	0.09
Leaf area	Poly	2	146	24.83	2.06	-1.44	753	0.001
SLA	Linear	1	146	7.09	10.6	-0.001	1529	<0.001
SOC	Linear	1	30	1.54	-5.494	0.01	108	0.02
SNC	-	-	30	3.48	-7.17	0.001	-30.16	0.09
C/N	-	-	30	2.98	-0.5	0.0007	186	0.514
Soil pH	-	-	14	0.009	1.14	0.0001	-6.41	0.34

PHOTOPLATES

Photo plate 1: Field work



Plant height and diameter measurement



Counting of flower and fruit



Uprooted whole plant for biomass allocation measurement

Photo plate 2: Laboratory measurement



Leaf sample analyzed through image J for determination of leaf area (upper two) and weighing of dry leaf mass (lower one)

Photo plate 3: Laboratory work



Digester



Distillation apparatus