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# What Shapes the Comeback? Environmental conditions and associated vascular plants as ecological indicators for the orchid *Herminium monorchis*

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Master in Ecology

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Ås, 15th of May 2025

Thea Aurland Storholt

# Abstract

In a rapidly changing world, biodiversity loss is accelerating and now represents one of the most significant global threats. Among the primary drivers are land-use change and climate change. Despite various international initiatives aimed at reversing this trend, evident results remain limited. One increasingly applied strategy to help reverse the declining trend is species reintroduction. This study contributes to such efforts by investigating the habitat requirements of the critically endangered orchid *Herminium monorchis,* and addressing the knowledge gap associated to ecological indicators. This study aims to investigate how the number of individuals of *H. monorchis* relate to soil moisture, soil depth, vegetation cover, vegetation height and species richness. As well as analyzing species composition and identifying indicator vascular plants species associated with the presence of *H. monorchis*.

In Norway, *H. monorchis* exists at four known locations on Asmaløy in Hvaler municipality. This study focuses on two of these locations: Skjellvik and Skipstadsand. Vegetation surveys and environmental measurements were conducted during the summer of 2024.

Higher soil moisture showed a significant negative relationship with the number of individuals of *H. monorchis*, suggesting a preference for intermediate soils moisture. Conversely, deeper soil and higher vascular plant cover were positively associated with the number of individuals of *H. monorchis,* indicating that *H. monorchis* may favor locations with neighboring vegetation and stable soil conditions beneficial for root development. No significant relationship was found between vegetation height or species richness and the number of individuals of *H. monorchis*, though these results may be influenced by ongoing habitat management at both locations. *Carex distans* was positively associated with *H. monorchis*, implying that areas where *C. distans* occurs may offer suitable conditions for future reintroduction. Additionally, eight species were negatively associated with *H. monorchis*, indicating that such habitats may be less favorable and should be avoided in a reintroduction. Additionally, a NMDS ordination showed differences in species composition between the two locations, which indicate that *H. monorchis* may tolerate some fluctuations in species composition,

A potential reintroduction of *H. monorchis* would therefore require intermediate soil moisture, high soil depth and high vascular plant cover, preferably at locations where *C. distans* occur.

# Sammendrag

I en verden i rask endring øker tapet av biologisk mangfold, noe som nå utgjør en av de største globale truslene. Blant de viktigste drivkreftene er arealbruksendringer og klimaendringer. Selv om flere internasjonale initiativer er iverksatt for å motvirke denne trenden, har de foreløpig gitt begrensede resultater. En strategi som oftere blir brukt er reintroduksjon/bevaringsutsettelse av arter. Denne studien bidrar til slike tiltak ved å undersøke habitatkravene til den kritisk truede orkidéen *Herminium monorchis*, og utforsker kunnskapshullet om nærliggende økologiske faktorer. Studien har som mål å undersøke hvordan antall individer av *H. monorchis* har en sammenheng med jordfuktighet, jorddybde, vegetasjonsdekke, vegetasjonshøyde, artsrikdom og artssammensetning, samt å identifiserekarplante indikatorer knyttet til forekomsten av *H. monorchis*.

I Norge finnes *H. monorchis* på fire kjente lokasjoner, alle lokalisert på Asmaløy i Hvaler kommune. Denne studien fokuserer på to av disse lokalitetene, Skjellvik og Skipstadsand. Vegetasjonsanalyser og registrering av miljøvariabler ble gjennomført sommeren 2024.

Høyere jordfuktighet viste en signifikant negativ relasjon med antall individer av *H. monorchis*, noe som antyder en preferanse for moderat fuktige jordforhold. Derimot var dypere jordsmonn og større dekning av karplanter positivt assosiert med antall individer, noe som kan indikere at *H. monorchis* foretrekker lokaliteter med omkringliggende vegetasjon og stabile jordforhold som er gunstige for rotdannelse. Det ble ikke funnet noen signifikant sammenheng mellom vegetasjonshøyde eller artsrikdom og antall individer av *H. monorchis*, men disse resultatene kan være påvirket av pågående skjøtselstiltak ved begge lokalitetene. Resultatene fra en indikator art analyse viste at *Carex distans* var positivt assosiert med *H. monorchis*, noe som antyder at områder der *C. distans* forekommer kan ha egnede forhold for fremtidig reintroduksjon. Åtte arter negativt assosiert med *H. monorchis*, noe som peker på mindre gunstige habitater og bør unngås ved planlegging av reintroduksjon. I tillegg viste en NMDS analyse variasjoner i artssammensetningen mellom lokasjonene, noe som viser *at H. monorchis* kan tåle noen endringer i artssammensetningen.

Ved en mulig reintroduksjon av *H. monorchis* vil middels jordfuktighet, dypt jordsmonn og høy dekning av karplanter være grunnleggende. Et godt utgangspunkt kan være lokasjoner der *C. distans* forekommer.

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# Introduction

In a rapidly changing world, the loss of biodiversity is increasing (Butchart et al., 2010; Keil et al., 2015). High biodiversity provides stable ecosystems, and higher resilience in face of climate change (IPBES, 2019). A decline in biodiversity threatens ecosystem services such as pollination, food supply, freshwater and clean air (Butchart et al., 2010; IPBES, 2019). According to FN's Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) (2019), global biodiversity has never been as threatened as it is today, and major drivers for this are climate change, land-use change, and other human activities that enhance species' extinction rates. The conservation of threatened species is an international obligation (Swarts & Dixon, 2016), and in 1993, the Convention on Biological Diversity (CBD) was signed by 150 countries. The main objectives for the CBD are to conserve biological diversity and sustainable use of the components of biodiversity (CBD, 1993). However, the goals from the CBD are not yet met, and several countries are moving away from their goals regarding biodiversity (Secretariat of the Convention on Biological Diversity, 2020; Leadley et al., 2022). As a result, the decline in biodiversity is happening faster than ever (Butchart et al., 2010; Leadley et al., 2022). A step in the right direction regarding CBD was the creation of the red list.

To slow down the rapid decline in biodiversity, the red list was made to investigate trends regarding biodiversity and help with conservation politics (Mace et al., 2018). The red list is a list with information regarding threatened species globally, with over 169 000 species represented (IUCN, 2025). In Norway 4957 of 23 405 assessed species are red-listed, of these 2752 are critically endangered, endangered, or vulnerable (Artsdatabanken, 2021c). From 2015 to 2021, there has been an 0.9% increase in the number of threatened species added to the Norwegian red list, indicating a gradual decline in biodiversity (Artsdatabanken, 2021a). The majority of species are threatened due to land-use change, followed by pollution and threats from other species (Artsdatabanken, 2021b). This suggests that negative pressures continue to impact Norway's biodiversity. Furthermore, a general trend is that more species are moving upwards on the threatened scale, meaning their conservation status is worsening. (Artsdatabanken, 2021a), emphasizing the need for strengthened conservation efforts. Increased protection and restoration of nature, alongside tackling the drivers for the land-use changes, can help reduce the declining trend (Leclère et al., 2020; Kyrkjeeide et al., 2023).

To reverse the downward spiral regarding biodiversity, several measures have been suggested, and increased knowledge has been collected and developed (Mace et al., 2018; Kyrkjeeide et al., 2023). Reintroduction of species has become an increasingly important conservation strategy as it can help ensure viable populations of threatened species (IUCN, 1998; Godefroid et al., 2011). Reintroduction is a term often used when trying to establish or reestablish a species in an area that was once a part of its historical range, or to introduce them to new appropriate locations (IUCN, 1998; Godefroid et al., 2011). A selection of appropriate locations with preferred environmental conditions and careful monitoring are mentioned as some of the most important strategies for a successful reintroduction (Yam et al., 2010; Segovia-Rivas et al., 2018). However, even with increased importance and use of this method, there is a lack of reports of the results from reintroductions, limiting our understanding on how to successfully reintroduce species (Bottin et al., 2007; Godefroid et al., 2011). Nonetheless, there is enough knowledge to continue using this method, while further developing reintroduction as a conservation tool and deepening our understanding of variables needed for successful usage (Haskins, 2015). Examples of successful reintroductions are the reintroduction of *Arenaria grandiflora* in France (Bottin et al., 2007) and *Spiranthes brevilabris* in the United States (Stewart, 2008). The successes were connected to the consistency between environmental variables of the reintroduction location and the reintroduction species, an enhanced population size and sufficient genetic variability to maintain a strong population when facing shifts in environmental variables and use of soil from suitable locations in the germination (Bottin et al., 2007; Steward, 2008).

*Orchidaceae,* orchid, is often used in a reintroduction effort due to their high rate of species on the IUCN red list (Swarts & Dixon, 2009). Orchids are one of the largest plant families and are found in a broad diversity of habitats worldwide (Fay & Chase, 2009; Hsiao et al., 2011). They have unique flower morphology and a remarkable diversity in life strategies (Zhang et al., 2017). A common trait for orchids is their symbiosis with mycorrhizal fungi, from which they obtain nutrients like nitrogen and phosphorus (Favre-Godal et al., 2020; Genre et al., 2020). Still, the family has several threatened species, more than any other plant family, often due to some orchids' special plant-pollinator interactions and land-use change (Fay & Chase, 2009; Swarts & Dixon, 2009). Lack of awareness remains regarding orchid reintroduction, particularly concerning species-specific habitat requirements, mycorrhizal associations, and the environmental variables influencing establishment success (Swarts & Dixon, 2009; Andriamihaja et al., 2021). Vegetation analysis and key environmental variables, such as soil moisture and vegetation height, play a crucial role in identifying suitable habitats for reintroduction by revealing plant community composition, competition levels, and environmental conditions that influence orchid survival and growth (Bottin et al., 2007; Yam et al., 2010). Such studies are important for enhancing conservation strategies and increasing the likelihood of successful reintroduction of threatened orchid species (Swarts & Dixon, 2009).

In Norway 24 of 35 orchid species are threatened (Artsdatabanken, 2021c). One of these is *Herminium monorchis,* which is a small, perennial, less competitive and light demanding species, often found in temperate grasslands, shrublands and wetlands (Rankou, 2011). The species has a vast spatial distribution across Eurasia, from Italy in the south, France to the west, Japan to the east, and Norway in the north (Rankou, 2011; Raskoti et al., 2017; Solstad et al., 2021). *Herminium monorchis* experiences a decreasing trend globally (Rankou, 2011; Ekelund, 2021b) and the species has become extinct in some European countries and is classified as threatened on several national red lists, including, as mentioned, in Norway (Solstad et al., 2021b).

In Norway, historically, *H. monorchis* were located at around 70 different locations (DN, 2010; Solstad et al., 2021b). However, the population is now limited to four coastal, relatively moist locations, all situated short distances from each other in Hvaler municipality (Solstad et al., 2021b; Evju et al., 2022a; Roos et al., 2024b). Nationally, the greatest threats against *H. monorchis* are land-use changes, increased fertilizer use causing overgrowth and higher competition, drainage, and fragmentation of natural habitats (Rankou, 2011; Ekelund, 2021b; Solstad et al., 2021b).  Since the species is not particularly competitive, such changes and severely increased nutrient levels negatively affect its population (Kravdal, 2015). *Herminium monorchis* is categorized as critically endangered on the Norwegian red list due to its limited geographical range (Solstad et al., 2021b). Thus, *H. monorchis* is a prioritized species on a national level and was granted special protection in the Nature Diversity Act (Naturmangfoldloven, 2009).

Due to the drastically decline in species abundance, monitoring of *H. monorchis* started in the late 1990s and early 2000s in Norway (Ekelund, 2021a). The monitoring has documented trends regarding *H. monorchis* populations, and conservation management has been done accordingly. The short-term goal is to ensure the species survival with viable populations, with the long-term goals being a reintroduction of populations of *H. monorchis* and increasing its abundance (DN, 2010; Kyrkjeeide et al., 2023). To achieve these goals, maintaining the current population and increasing the abundance is vital, and collecting information needed to help ensure a successful reintroduction (DN, 2010; Kyrkjeeide et al., 2023).

Detailed monitoring of the populations were not established until 2014 (Kravdal, 2015) and has been carried out annually since then (Roos et al., 2024b). The monitoring shows that ensuring a growing population remains a challenge, and there are some fluctuations in the number of individuals of *H. monorchis* between years (Roos et al., 2024b). Reintroduction could serve as a viable method for boosting populations, alongside other measures (Tingstad & Endrestøl, 2021; Evju, Roos, et al., 2022; Kyrkjeeide et al., 2023). To successfully reintroduce *H. monorchis,* a thorough understanding of several factors, including environmental threats and preferred environmental conditions, geographic distribution, habitat specializations and reproductive biology is important, as well as detailed knowledge about the species ecological needs and interactions (Swarts & Dixon, 2009; Roos et al., 2023).

A critical knowledge gap in the conservation of *H. monorchis* is the limited understanding of which species it may be ecologically associated with. This information is vital for guiding reintroduction efforts, where the identification of indicator species, those that reflect specific environmental conditions and often share species-specific habitat requirements, is particularly valuable (Bal et al., 2018). Such species can serve as ecological signals of habitat suitability, helping to identify locations that meet the environmental needs of *H. monorchis*. Addressing this gap is essential for selecting reintroduction locations that are ecologically compatible, thereby improving the likelihood of long-term establishment and success (Bottin et al., 2007; Yam et al., 2010).

To improve understanding of *H. monorchis* requirements for reintroduction, conducting an indicator species analysis can provide valuable insight into the species it is commonly associated with, or rarely found near, thus highlighting both potential threats and habitat preferences (Bal et al., 2018). Although direct habitat assessments are essential, the indicator species can help clarify what conditions *H. monorchis* depend on. A study from 2008 has investigated how neighboring species significantly correlated with the abundance of orchids and can help in the conservation of orchids by assessing habitat quality, ecological relationship and environmental impacts (Landi et al., 2008). As such, indicator species can aid in identifying potential positive and negative relationships and help find suitable habitats for reintroduction (Landi et al., 2008; Lamothe et al., 2019).

This study investigates how environmental variables shape *H. monorchis'* abundance and how the species interact with surrounding vascular plant species. By analyzing species composition and richness, and variables such as vegetation height, vegetation cover, soil depth, and soil moisture, the study aims to understand the conditions that influence habitat suitability for *H. monorchis*. A key focus of this study is identifying specific indicator species that are critical for the presence of *H. monorchis*. It aims to determine which species can reliably serve as indicators, granting valuable information that can guide targeted management strategies, and provide useful information when looking for potential locations for reintroduction of the species. These insights provide the foundation for addressing the following research questions:

1. **How do cover of vascular plants, species richness, species composition, vegetation height, soil moisture, and soil depth influence the number of individuals of *H. monorchis*?**

2. **Are there any vascular plants serving as indicator species for *H. monorchis*, and if so, which ones?**

I hypothesize that in areas with higher soil moisture and soil depth there is an increase in the number of individuals of *H. monorchis*, especially since *H. monorchis* commonly is found in moist habitats such as beach meadows. Moreover, I hypothesize that the cover of vascular plants, species richness and vegetation height will negatively affect the number of individuals of *H. monorchis* due to its low competitive ability. Lastly, I expect that some species share similar ecological traits and may indicate suitable habitats for *H. monorchis*, while others might indicate habitats where *H. monorchis* is absent.

# Methodology

## Study species

This study focuses on the species *Herminium monorchis* (Figure 1), which belongs to the Orchidaceae family, one of the most species-rich plant families (Harrap & Harrap, 2009; Swarts & Dixon, 2009). Most members of this family are perennials and are typically monoecious, meaning they possess both male and female reproductive organs on the same plant (Lid & Lid, 2005, p. 905). They are primarily insect-pollinated, and their seeds are minute and dispersed by wind. Due to their minimal nutrient reserves, orchid seeds often rely on symbiosis with fungi during germination (Bidartondo et al., 2004; Favre-Godal et al., 2020; Genre et al., 2020). For many species, this mycorrhizal relationship continues beyond the seedling stage, with mature plants depending on carbon obtained from fungal partners (Rasmussen, 2002).

*Figure 1: Herminium monorchis in bloom at Skjellvik, Hvaler. Photo by Thea Aurland Storholt*

Like several other orchids, *H. monorchis* is pollinated by insects, and a study from Sweden shows that various species of flies, beetles, and solitary wasps visited the *H. monorchis* population (Rudall et al., 2013). To attract pollinators, *H. monorchis* produces nectar, (Stroh, 2015), giving it a honey-like scent. Typically, it grows between 5 and 20 cm tall, featuring a straight stem, a centrally located round tuber, and strong bilateral symmetry with yellow-green, bell-shaped flowers (Lid & Lid, 2005, p. 914). The individuals are not in full bloom until mid-July. *Herminium monorchis* can also reproduce vegetatively, by producing new tubers at the tip of a long stolon (Wells et al., 1998; Artsdatabanken, 2010; Stroh, 2015). Some suggest that vegetative reproduction is more favorable for *H. monorchis,* but it is often dependent on the environmental conditions and stress (Lei, 2010). *Herminium monorchis*, like many other orchids, occasionally go dormant during the growing season, for one to two years. In other words, no above-ground biomass is produced (Shefferson, 2009), which makes it harder to estimate the population size (Wells et al., 1998).

*Herminium monorchis* is highly tolerant to salinity, moist soil and high pH and is often found in damp, calcareous, alkaline meadows, wetlands and beach meadows (Rankou, 2011; Ekelund 2021b; Solstad et al., 2021b). In addition to habitat conditions, belowground interactions, through their mycorrhizal relationships, are important for *H. monorchis*. The most common fungi for *H. monorchis* to be associated with are species within the *Ceratobasidiaceae* family (Roos et al., 2024a).

## Study site

In Norway, only four locations with *H. monorchis* remains, all located within a small area in Hvaler municipality (Figure 2). This study only includes Skjellvik and Skipstadsand as study locations due to time constraints, to allow for a more in-depth investigation of the species composition within the available timeframe. Skjellvik were chosen because it is the largest of all the locations and in 2021 had the largest population of *H. monorchis* while Skipstadsand is the largest of the two populations on the eastern part of Asmaløy (Evju et al., 2022).

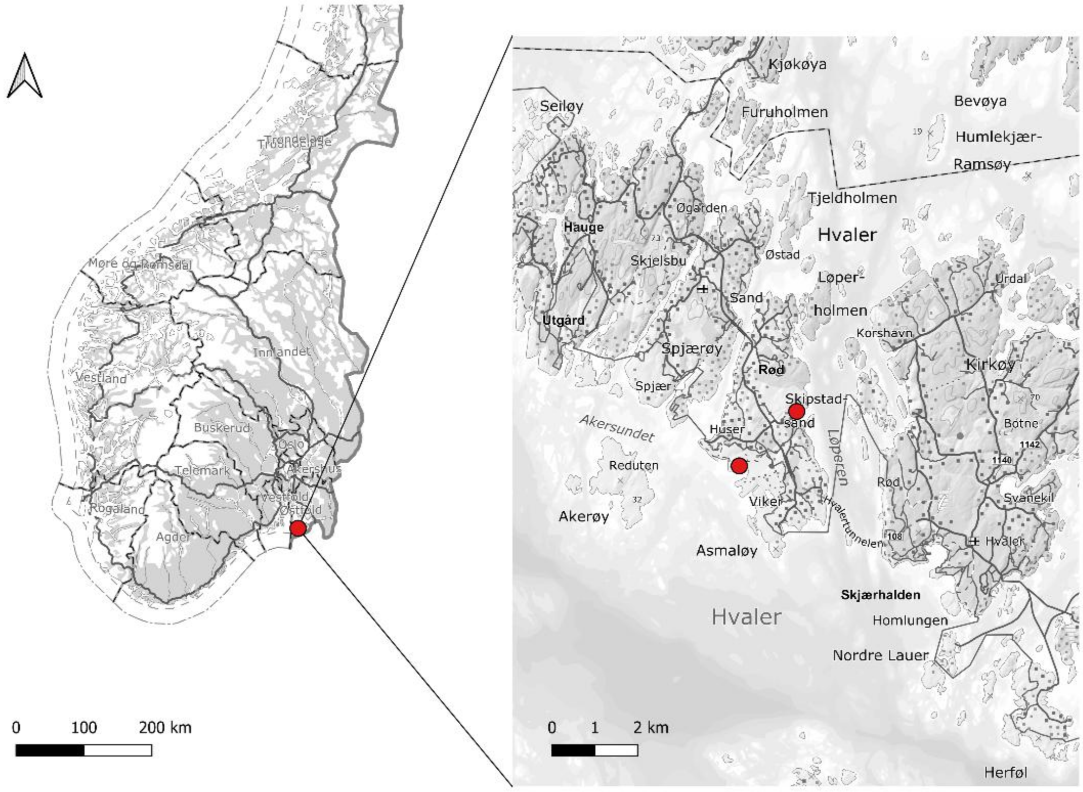


*Figure 2: Map of the two study sites, Skjellvik and Skipstadsand. Skjellvik is located on the western side of Asmaløy, close to Teneskjær (not included in the study). Skipstadsand is located on the eastern side, close to Filletassen (not included). Map made in QGIS version 3.32 by Thea Aurland Storholt (QGIS.org, 2025), background map: Topographical map of Norway (Norwegian Mapping Authority, 2025).*

***Skjellvik***

Skjellvik is located on the western side of Asmaløy and is a part of "Ytre Hvaler" National Park (Figure 2). This national park is the eldest maritime national park in Norway (Ytre Hvaler Nasjonalpark, 2022; Miljødirektoratet, N.A.-b). The main goal of the establishment of this National Park was to preserve nature on land as well as in the ocean (Ytre Hvaler Nasjonalpark, 2022), and a management plan was developed. This management plan mentioned *H. monorchis* as a species with extra consideration (Ekelund, 2021b).

The nature types at Skjellvik includes semi-natural meadows, semi-natural wetlands, and semi-natural beach meadows (Ekelund, 2021b; Miljødirektoratet, N.A.-a). This diverse landscape creates a mosaic of microhabitats, which in turn influences biodiversity. The bedrock consists of granite, a thick layer of shell sand and overlaying peat, creating calcareous conditions (Ekelund, 2021b; NGU, N.A.-a). Shell sand, a carbonate-rich material formed from crushed shells, clams, and other chalk-covered organisms (NGU, N.A.-b) enhances soil fertility and promotes biodiversity. Skjellvik is influenced by wind and salt exposure, as well as high soil moisture. In 2019 a total of 78 different vascular plant species were registered at Skjellvik (Ekelund, 2021a, 2021b), and several of these species were red-listed in Norway (Artsdatabanken, 2021b). Traditional management has been important in shaping these habitats, and many species, including *H. monorchis*, depend on continued management practices like grazing and mowing.

Some documents contain information on grazing in this area already from 1657 (Ekelund, 2021b; Evju et al., 2022a). This clearly indicates a long history of human land use. From 1950 heavier cow species were introduced (Ekelund, 2021b). It is likely that the cattle did not graze over longer periods, but pinpointing the exact endpoint remains challenging (Ekelund, 2021a). Active management by the management authorities has been carried out since 1995, but the management method has changed during the last 30 years. Today, the area is mowed with a two-wheel mower twice a year, at the beginning of the season, and again at the end of the season. At the end of the season, the hay is left to dry for a few days before being collected, allowing the seeds to disperse naturally (Evju et al., 2022a). The area where *H. monorchis* is located at Skjellvik is fenced with an electrical fence to keep cows from grazing in that specific area, and to be protected against trampling (Evju et al., 2022a). The trampling is believed to be non-beneficial for *H. monorchis*. Today's management is more related to conservation rather than exploitation of resources as done earlier.

***Skipstadsand***

Skipstadsand is located within Skipstadsand Nature Reserve and is located at the eastern side of Asmaløy (Figure 2). Skipstadsand Nature Reserve became a preserved area to maintain the species diversity, especially the red-listed species, including *H. monorchis* (Bjar, 2013). The Nature Reserve was established in 2010. The area is defined as a semi-natural beach meadow with species typical for this nature type, such as *Centaurium littorale* and *Trifolium fragiferum* (Bjar, 2013). The geological composition consists of granite, and organic soil and peat (Roos et al., 2024; NGU, N.A.-a). Environmental variables crucial for the species composition at Skipstadsand are salt-, wave- and wind exposure (Artsdatabanken, N.A.-c), and the area has been regularly managed to ensure suitable conditions for *H. monorchis.*

The grazing of cows stopped already in 1965. However, it was not until 2003 that the area was managed by humans with scythes once a year, mostly to prevent overgrowth and higher competition towards *H. monorchis* and other red-listed species. The management method used today is the same as that of Skjellvik, but the area at Skipstadsand is not fenced in (Ekelund, 2021a; Evju et al., 2022a). Additionally, other management efforts such as the removal of *Rosa rugosa* and other small bushes and trees are done when necessary (Bjar, 2013; Ekelund, 2021a). Furthermore, some of the vegetation around *H. monorchis* individuals are removed early in the season, before *H. monorchis* comes in flower, to prevent competition and simulate the effects of grazing (Ekelund, 2021a; Evju et al., 2021).

## Study design

Three weeks of fieldwork were done, from the 22nd of July to the 8th of August. This project is part of an ongoing project.

In 2014, 40 field plots were established (Figure 3), 20 at each location, in a cooperation between Norwegian Institute of Nature Science (NINA) and Norwegian University of Life Science (NMBU). At each location, ten plots were selected based on the presence of *H. monorchis,* each paired with a nearby control plot to ensure similar vegetation conditions (Kravdal, 2015). The control plots have not been monitored after 2014. In 2021 some adjustments were made and transects at each location were established, including more plots to ensure the environmental gradient is incorporated. At Skjellvik, four transects were established, two measuring 40 meters and two measuring 50 meters. Two of the transects were established in a northeast - southwest direction and had a soil moisture gradient, while the other two had a northwest - southeast direction. Some new plots were established, as well, and there are now 27 plots at Skjellvik (Figure A1). At Skipstadsand, three transects were established, and each transect measuring 30 meters in an east-west direction, with 15 plots in total (Figure A2) (Evju et al., 2022a). All plots were marked with

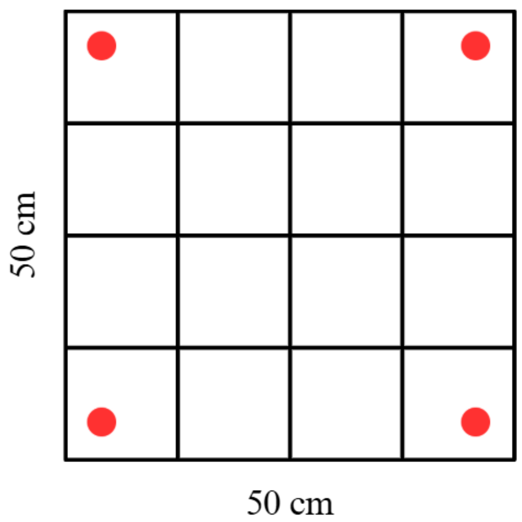


*Figure 3: An illustration of a plot divided into 16 quadrats. The red dots indicate where the height measures were taken. The illustration is made by Thea Aurland Storholt with [Home - Canva]*

aluminum pipes in the ground in all four corners, and in 2019 high-precision GPS locations were established to locate each plot with a marker in two corners (Evju et al., 2022a). By doing this, the plots are easier to find between years, even if the aluminum pipes slide deeper into the ground due to the wet conditions. Further explanations of the plot and transect establishments can be found in Kravdal (2015) and Evju et al., (2022a).

Every year from 2014 until 2024, except 2018, the population numbers of *H. monorchis* have been counted and different variables related to *H. monorchis* have been recorded, such as vegetation heigh, soil moisture and cover of over-growth species. In 2014, 2016 and 2017 soil moisture data were collected, and in 2023 soil samples, including the soil depth, were taken (Kravdal, 2015; Roos et al., 2024a; Roos et al., 2024b). This data has been used in this study (Kravdal, 2015; Evju et al., 2022a; Roos et al., 2024a; Roos et al., 2024b).

## Sampling plan

The high-precision GPS and aluminum pipes helped locate each plot, by using a handheld metal detector. At least two aluminum pipes at each plot had to be found. Thereafter, yellow sticks were put in the aluminum pipes or directly in the ground to mark each plot. Each plot was divided into 16 quadrats (Figure 3).

Vegetation analyses of each plot were done. I recorded all species present in each quadrat and noted their frequency across quadrats (Figure B1). Species were identified following Lids Flora (Lid & Lid, 2005). In cases where I was unsure, “Artskart” and “Artsorakelet” were used to help identify species, but the results were always double checked in Lids flora (Lid & Lid, 2005; Artsdatabanken, N.A.-a, N.A.-b). Some species were collected and brought home for further inspection, and pictures of some individuals were sent to Marianne Evju and Ruben Erik Roos at NINA. However, some individuals remained unknown.

In addition, the cover of vascular plants, bare soil, litter and bryophytes were recorded in all plots. The cover of vascular plants (%), bare soil (%) and litter (%) were combined to sum up to 100%, while the cover of bryophytes (%) were in addition since, they can be beneath the vascular plants cover in the bottom layer. The cover was estimated by using the quadrats, each quadrat consisted of 6.25% of the vegetation, which helped to remain quite consistent. Lastly, the vegetation height was measured in each corner by selecting an individual plant. The individuals were selected based on being approximately average in height for that specific corner (Figure 3). The measurements were collected with a ruler.

## Statistical analysis

All statistical analyses were conducted in R Statistical Software version 4.1.1(R Core team, 2021) and R-studio version 2024.12.0+467 (Posit, 2024). The packages that were used to load and organize data were *readxl* (Wickham & Bryan, 2023) and *tidyverse* (Wickham et al., 2019).

ChatGPT (OpenAI, 2025) and Microsoft Copilot (Microsoft, 2025) were used to aid in code development and error correction for the statistical analyses performed in RStudio and as aid for proofreading paragraphs and translations.

To examine if the data were normally distributed, I used a Shapiro-Wilks test and visual evaluations of histograms. The results showed that none of the explanatory variables, except species richness, were normally distributed (Table C1). Furthermore, I used a standard correlation test from the *corrr* -package (Kuhn et al., 2022) to check if any of the explanatory variables correlated with each other. Since cover of litter and vascular plants had a correlation of 0.97, I conducted two Akaike Information Criterion (AIC) tests with all variables and either of the correlated variables in each test. The model with cover of vascular plants had the lowest AIC, meaning that this model was a better fit for the data compared to the other test, and cover of vascular plants was therefore used in the analyses (Table C2). Additionally, I removed all unknown species from the dataset.

Since the soil moisture data were collected from three different years (2014, 2016 and 2017), I chose to do a Kendall's rank correlation tau to investigate the relationship between years and how much they differ from each other (Table C3) (Puka, 2011). This test showed a strong correlation, and all three years were included.

To test the hypothesis on how the number of individuals of *H. monorchis* is related to species richness, vegetation height, cover of vascular plants, soil moisture and soil depth, a Generalized Linear Mixed Model (GLMM) with a negative binomial type 2 dispersion was performed using the *glmmTMB* - package (Brooks et al., 2017).

$$log(\lambda_{ij}) = \beta_0 + \sum_{k+1}^{n} \beta_k X_{ik} + b_j$$

Using the abundance of *H. monorchis* as response variable ($\lambda_{ij}$), while soil moisture, soil depth, vegetation heigh, vegetation cover and species richness as explanatory variables ($X_{ik}$). In addition, it accounts for variation between locations through a random effect ($b_j$) (Salinas Ruiz et al., 2023). I selected this model since the abundance of *H. monorchis* is count data, and due to overdispersion, a negative binomial distribution was the most appropriate choice (Hoef & Boveng, 2007), and after testing the model using the *simulateResiduals* - function from the *DHARMa* – package (Hartig, 2024), it proved to be the best fit (Figure C1). The overdispersion was detected by comparing the mean and the variance of the distributions, and the variance exceeded the mean (Hoef & Boveng, 2007; Linden et al., 2011). Locality was included as a random effect to account for unexplained variability beyond what is captured by fixed effects (Salinas Ruiz et al., 2023). The results were visualized by creating a scatterplot with a polynomial trend-line, using the *ggplot2* – package (Wickham, 2016). The variables cover of bryophytes and cover of bare soil were excluded from this thesis as the GLMM showed they had no significant relation with the number of individuals of *H. monorchis* (P - values = 0.95 and 0.99, respectively). The variables species richness and vegetation height were not significant, but were included due to the interesting relevance this information may have regarding conservation management and a potential reintroduction.

To investigate how the numbers of individuals of *H. monorchis* are associated with the species composition and the environmental variables, I used Nonmetric Multidimensional Scaling (NMDS). The ordination analyses were conducted using the *vegan* – package (Oksanen et al., 2024). The stress for this analysis was 0.14, and the NMDS is therefore two-dimensional. For the optimum presentation of the species in the ordination, I used the 25 most frequently observed species. To include the environmental variables the *envfit* – function, from the *vegan* – package (Oksanen et al., 2024), were used. *Envfit* fit environmental variables onto the ordination, illustrating their correlation with the ordination axes (Oksanen, 2025). The following variables were included: the number of individuals of *H. monorchis*, vegetation height, species richness, average soil moisture, average soil depth, and the cover (%) of bryophytes, vascular plants, litter, and bare soil. Further, I used the results from the *envfit* - function to investigate how these variables may explain the variation in the ordination. Lastly, I conducted Canonical Correspondence Analysis (CCA) to examine the relationship between the environmental variables and the species composition. I used CCA based on the length of the axis from a Detrended Correspondence Analysis (DCA), which indicated that the distribution of species is unimodal (Table C4). The CCA and DCA were conducted by using *cca* - and *decorana*- functions from the *vegan* – package (Oksanen et al., 2024). Additionally, I conducted a permutation test, and an analysis of variance (ANOVA) to test if the results from the CCA were statistically significant.

To investigate the hypothesis if, and which, indicator species related to *H. monorchis* occurred, I used an Indicator Species Analysis (ISA) from the *Indicspecies* - package (De Cáceres & Legendre, 2009). Indicator Species Analysis investigates the association of *H. monorchis*, and the rest of the species present at the locations (Siddig et al., 2016; Severns & Sykes 2020). I used the species matrix as an x-variable and created a matrix for *H. monorchis* which served as a y-variable. The matrix with *H. monorchis* is categorized into two groups depending on whether *H. monorchis* is present or not. The function *multipatt*, from the *Indicspecies*– package (De Cáceres & Legendre, 2009), was used to find the associated species from the species matrix and connect these to the two groups to test whether they were good indicators for *H. monorchis*. The indicator value is represented by the following formula:

$$IV_{kj} = 100(RA_{kj}\ X\ RF_{kj})$$

$IV_{kj}$ refers to the indicator value of species (j) within each group (i). $RA_{kj}$ is the average of the relative abundance of each species within each group, and $RF_{kj}$ is the relative frequency of each species within each group (Severns & Sykes 2020). The calculation is multiplied with 100 to give an indicator value in percent. The association is represented as an *IndVal* – score (stat) ranging from 0 to 1 and is used to identify the indicators based on this score (Dufrene & Legendre, 1997).

# Results

In total, for both locations there were 42 plots, and in these 96 species were registered (Table B1). At Skjellvik the average amount of species within each plot was 19.4, with a standard deviation of 5.7 species, while at Skipstadsand the average was 12.3 with a standard deviation of 3.6 species. *Herminium monorchis* occurred in 13 of 27 plots at Skjellvik, and in 8 of 15 plots at Skipstadsand.

## Species richness, vegetation height, cover of vascular plants, soil moisture and soil depth and their relationship with *H. monorchis*

There was a significant relationship between the number of individuals of *H. monorchis* and soil moisture, soil depth and the cover of vascular plants (Table 1). The GLMM showed little variation between the two locations Skjellvik and Skipstadsand (Table 1).

*Table 1: Results from a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution. Fixed effects include species richness, soil depth, vegetation height, soil moisture, and vascular plant cover. Estimates, standard errors, F-values, and P-values are shown, with significance levels indicated. Location is included as a random effect.*

| Term | Estimate | Std. Error | F-value | P-value |
|---|---|---|---|---|
| (Intercept) | - 3.94 | 5.15 | - 0.74 | 0.444 |
| Soil moisture | - 0.18 | 0.04 | - 4.35 | **0.003** |
| Soil depth | 0.16 | 0.05 | 2.90 | **< 0.001** |
| Cover of vascular plants | 0.10 | 0.05 | 1.90 | 0.052 |
| Vegetation height | - 0.06 | 0.06 | - 0.92 | 0.345 |
| Species richness | 0.17 | 0.11 | 1.49 | 0.129 |
| Random: Location | 0.00004 | - | - | - |

The relationship between soil moisture (%) and the number of *H. monorchis* individuals showed a significant negative trend (Figure 4, Table 1). The abundance remained relatively stable at moderate soil moisture levels (approximately 30–40%) but began to decline noticeably when moisture exceeding 50% (Figure 4). This suggests that *H. monorchis* prefers intermediate moisture conditions, and that higher soil moisture negatively affects its occurrence (Figure 4, Table 1)

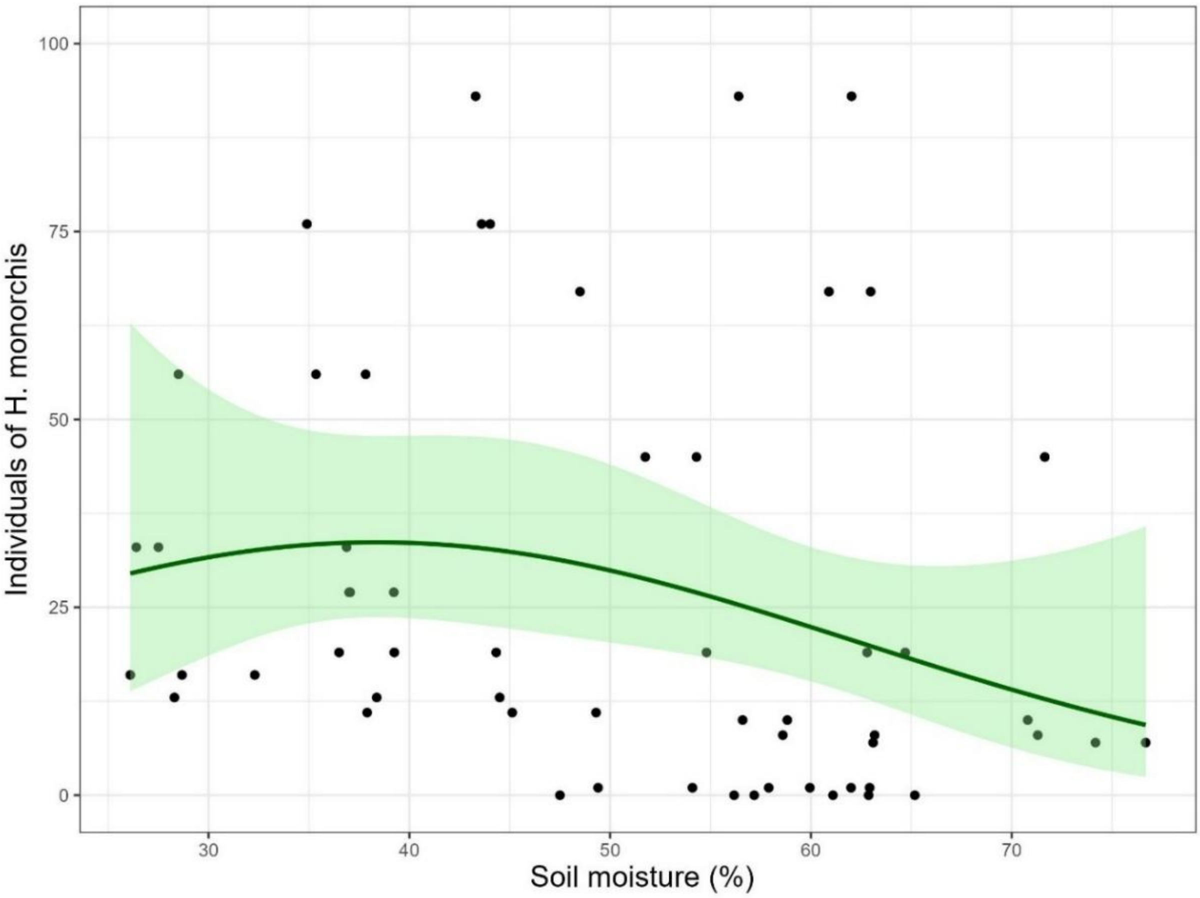


*Figure 4: Relationship between soil moisture and individuals of H. monorchis. The dark green area represents a 95% confidence interval. The trend line is a second-degree polynomial line, suited to follow the trends in the figure (green line).*

The relationship between soil depth and the number of individuals of *H. monorchis* showed a significant positive relationship with increased soil depth (Figure 5, Table 1). Observations of *H. monorchis* were present with a soil depth between 10 cm to 50 cm (Figure 5).

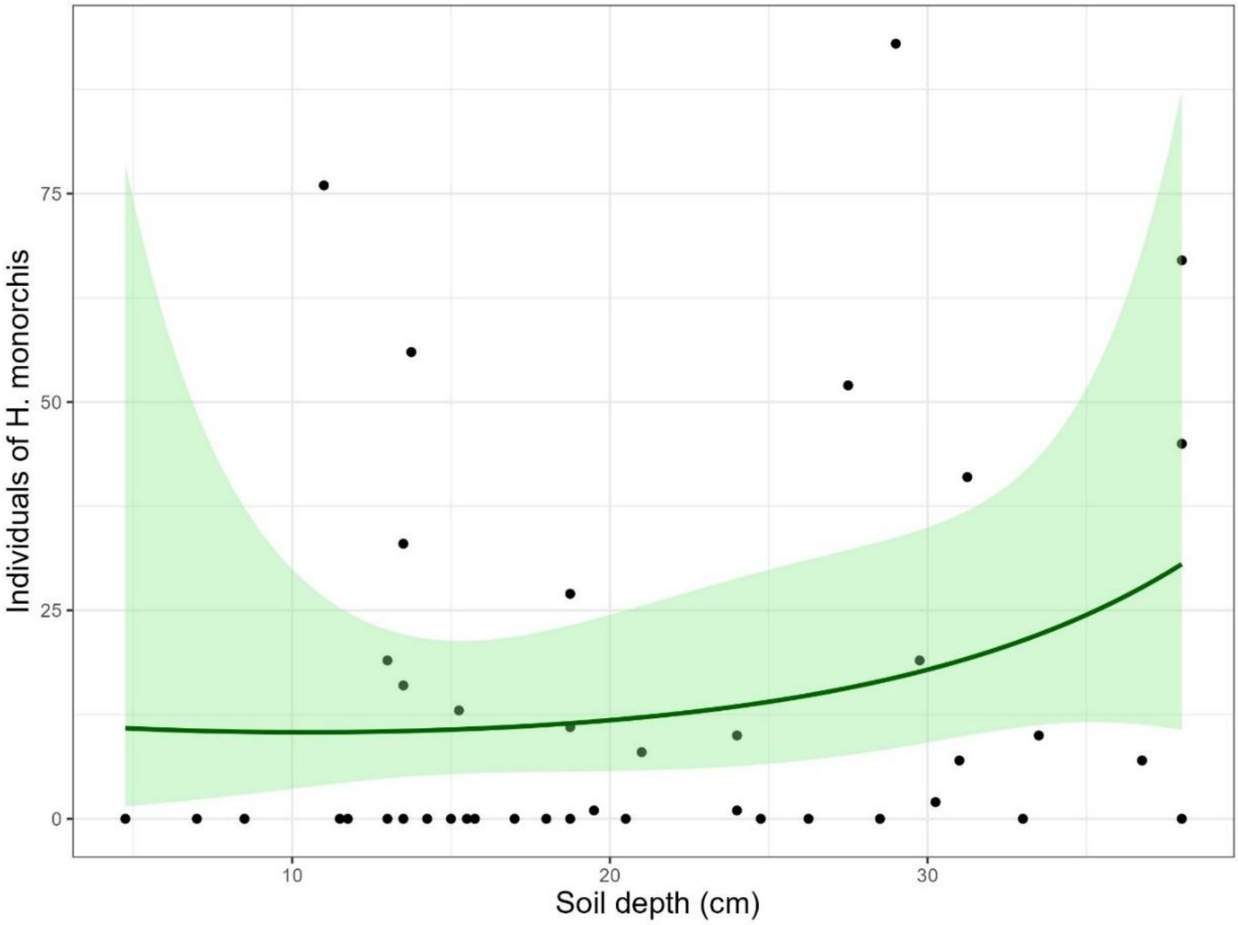


*Figure 5: The relationship between soil depth and individuals of H. monorchis. The dark green area shows the 95% confidence interval. The green trend line is a second-degree polynomial line to find the trends between the data points.*

The cover of vascular plants had a significant positive relationship with the number of individuals of *H. monorchis* (Figure 6, Table 1). Most of the investigated plots with the presence of *H. monorchis* have at least 95% cover of vascular plants and there is a slight increase in the abundance *of H. monorchis* at a higher vegetation cover (Figure 6).

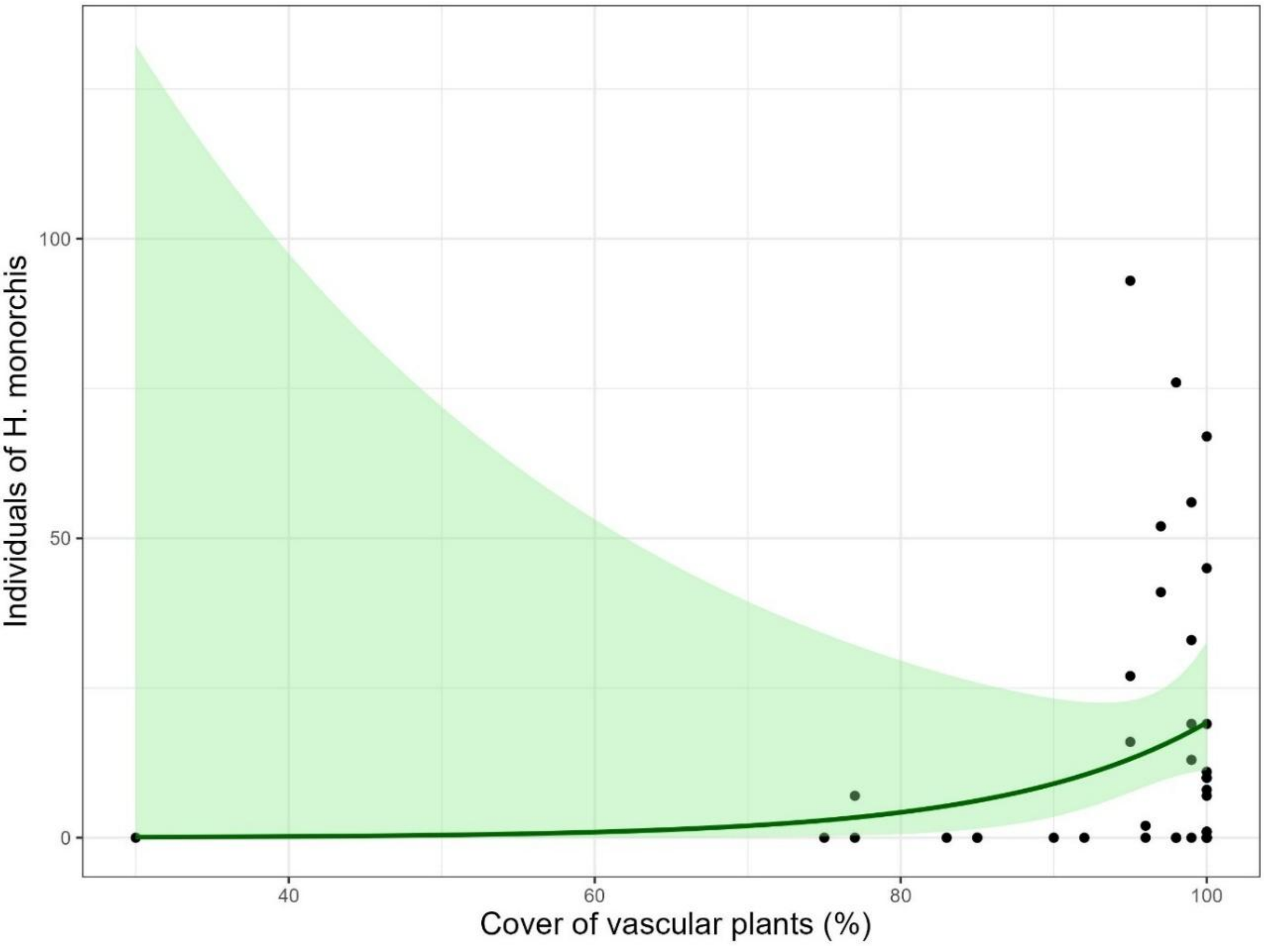


*Figure 6: Relationship between cover of vascular plants and individuals of H. monorchis. The dark green area represents the 95% confidence interval, with the highest uncertainties and largest error margins occurring at lower cover of vascular plants. The green trend line is a second-degree polynomial, highlighting the overall trends in the data*

Vegetation height and the number of *H. monorchis* individuals showed a non-significant relationship (Figure 7, Table 1). Across the 42 plots at Skjellvik and Skipstadsand, vegetation height ranged from 4 to 45 cm, and *H. monorchis* was found in the range between 12 to 26 cm (Figure 7). The bell-shaped trend suggested most individuals occurred where vegetation was approximately 10–20 cm tall (Figure 7).

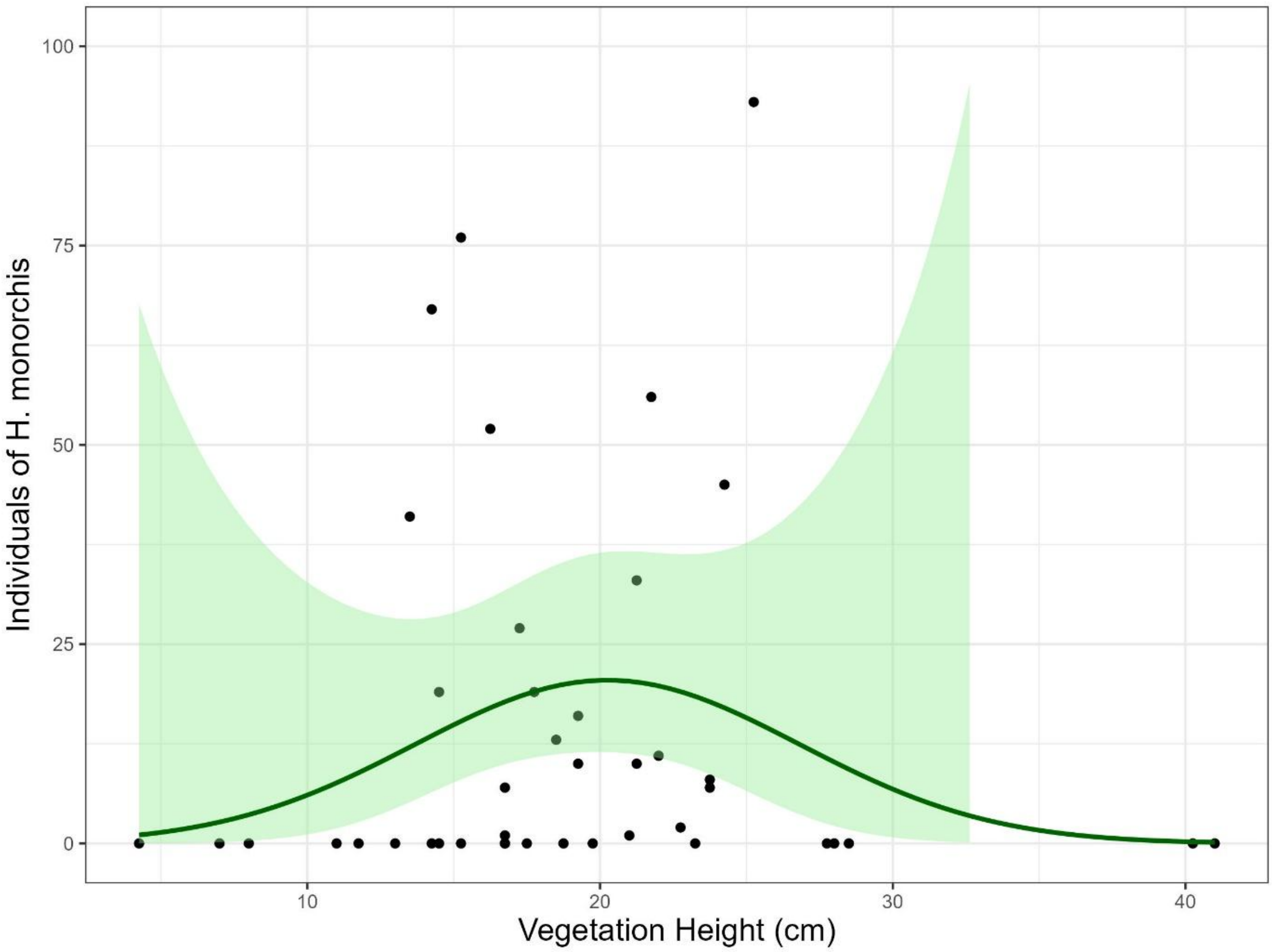


*Figure 7: Relationship between vegetation height (cm) and individuals of H. monorchis, with a second-degree polynomial line fitted to highlight the non-linear trend (green line). The dark green areas show a 95% confidence interval, with the wide edges showing uncertainties.*

The relationship between species richness and the number of individuals of *H. monorchis* were non-significant (Figure 8, Table 1). The relationship between species richness and individuals of *H. monorchis* were bell-curved, with an optimum of approximately 18 species (Figure 8). There was an increase in the total abundance of *H. monorchis* from approximately ten species, after which it began to decline at approximately 20 species (figure 8).

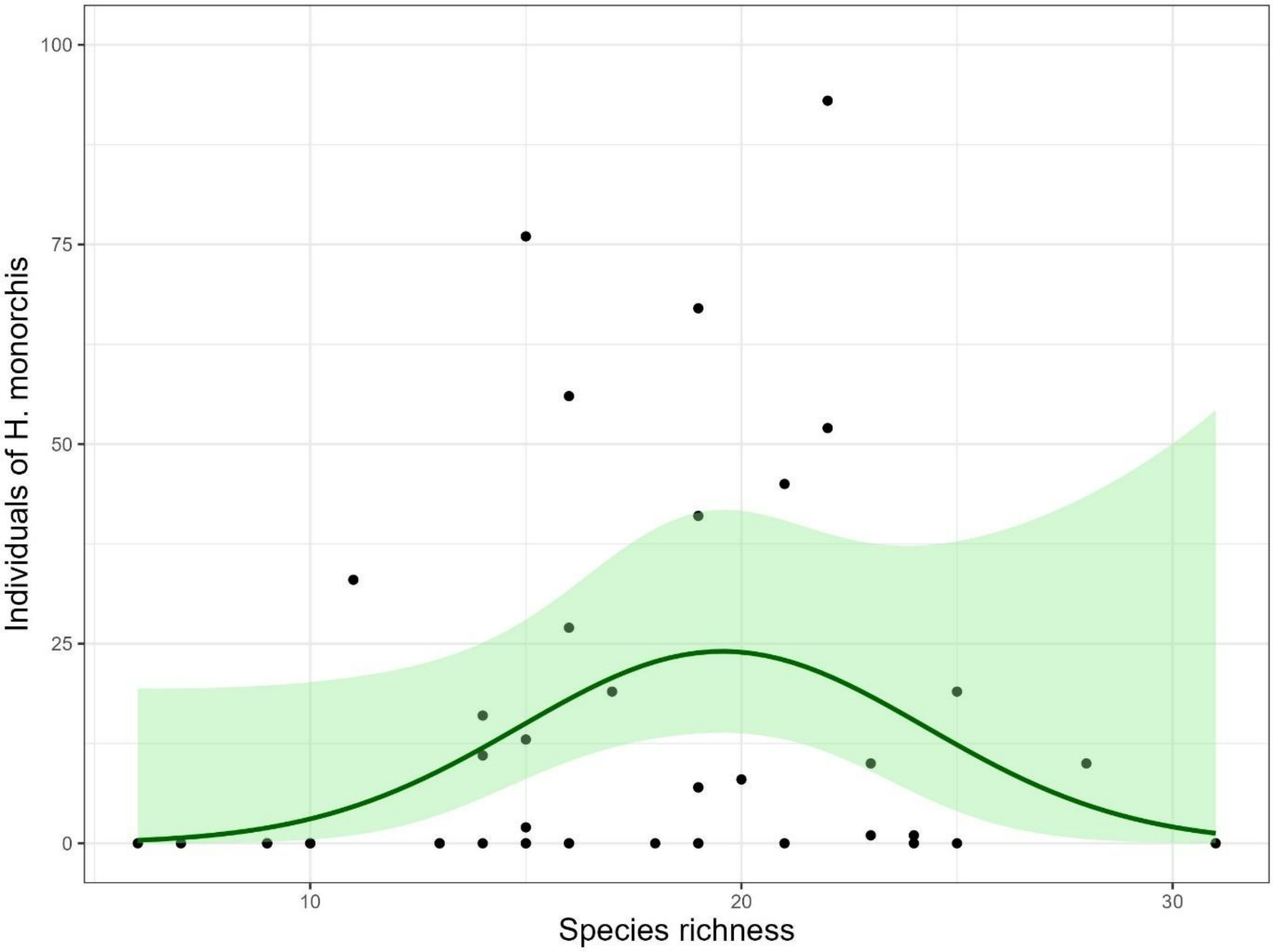


*Figure 8: Relationship between species richness and individuals of H. monorchis with a second-degree polynomial line (green). The light green area is a 95% confidence interval.*

## Species composition in relation to *H. monorchis* and associated environmental variables

The NMDS ordination with the fitting of environmental variables showed that vegetation height, cover of vascular plants, bryophytes, litter and bare soil were particularly related to variation in species composition along axis 1, while soil depth, soil moisture and species richness was related to variation in species composition along axis 2 (Figure 9, Table 2). Bare soil, litter and bryophytes were negatively related to the abundance of *H. monorchis.*

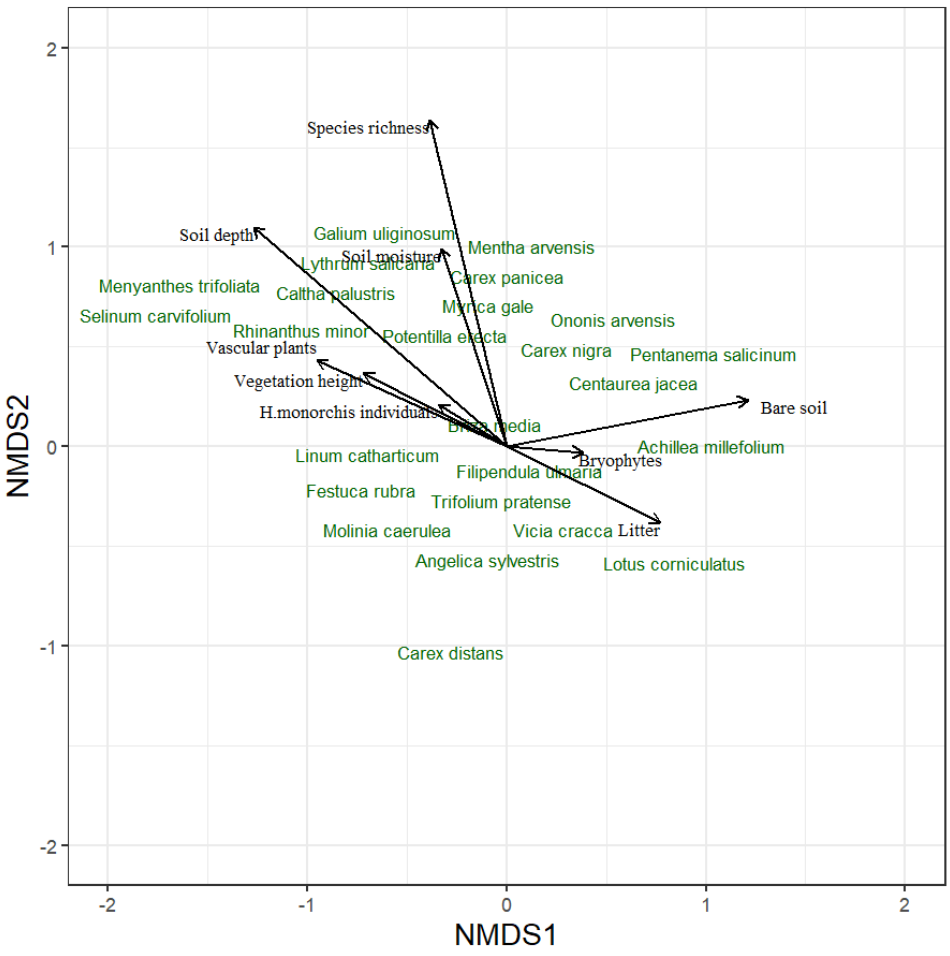


*Figure 9: Nonmetric multidimensional scaling (NMDS) of top 25 species (green) and the species closer together are more related to each other. With the non-linear method similar species are closer together, and the distance between them represents the likeness to each other. The arrows are different factors correlating with the NMDS-axes. The direction of the arrows indicates whether the correlation is positive, negative, or neutral, while the length of the arrows reflects the strength of the correlation with the environmental variable.*

The variables explaining most of the variation in species composition were species richness and soil depth (Table 2). Vegetation height, cover of vascular plants, cover of litter, cover of bare soil and soil moisture showed a significant impact related to the top 25 species present (Table 2). *Herminium monorchis* individuals and bryophytes had the least impact on the species composition and were the only two variables with a non-significant P-value (Table 2)

*Table 2: The effects each environmental variable has on the species composition with belonging $R^2$ and P - value. $R^2$ represents how much of the variation which can be explained by each variable, and the P – value states the significance.*

| **Environmental variables** | **NMDS 1** | **NMDS 2** | **$R^2$** | **P-value** |
|---|---|---|---|---|
| Soil moisture | -0.31 | 0.95 | 0.27 | **0.005** |
| Soil depth | - 0.76 | 0.66 | 0.71 | **0.001** |
| Cover of vascular plants | - 0.91 | 0.41 | 0.27 | **0.005** |
| Vegetation height | - 0.89 | 0.46 | 0.16 | **0.038** |
| Species richness | - 0.23 | 0.97 | 0.71 | **0.001** |
| Cover of litter | 0.90 | - 0.44 | 0.19 | **0.029** |
| Cover of bryophytes | 1.0 | -0.10 | 0.04 | 0.498 |
| Cover of bare soil | 0.98 | 0.19 | 0.38 | **0.001** |
| Individuals of *H. monorchis* | - 0.85 | 0.53 | 0.04 | 0.413 |

There was a clear grouping within each location, except for four plots at Skjellvik which were noticeably different. This implies clear differences between the species' composition and the relationship with environmental variables at the different locations. The clear difference between the four plots and the rest at Skjellvik is due to the placement of the four plots being in the driest areas. The arrows for higher soil depth, soil moisture and species richness moved towards the blue dots, which shows that these variables had higher values at Skjellvik than Skipstadsand (Figure 10). The grouping of the dots for Skipstadsand (red) showed less species richness and higher values of cover of litter compared to Skjellvik (Figure 10).

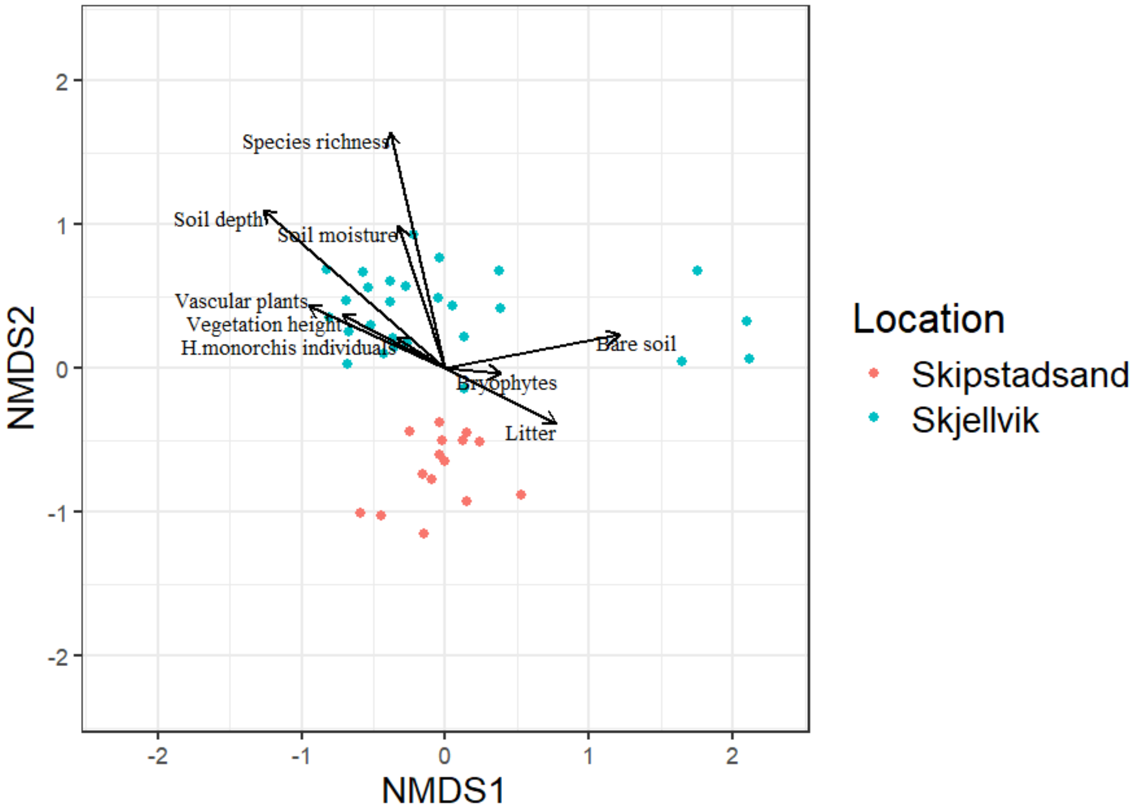


*Figure 10: A Nonmetric multidimensional scaling (NMDS) ordination that visualizes how the environmental factors (black arrows) are represented at each plot (red and blue dots). Skjellvik is represented by blue dots, and Skipstadsand by red dots. The direction of the arrows indicates whether the correlation is positive, negative, or neutral, while the length of the arrows reflects the strength of the correlation with the environmental variable. Longer arrows have a greater impact on species composition, whereas shorter arrows indicate a weaker influence.*

The CCA, a method used to explore relationships between species composition and environmental variables, revealed that the combined impact of all measured environmental factors had a significant influence on species composition. Together, these variables explain a substantial portion of the variation observed in the dataset (Table 3). The results of the CCA were assessed using permutation-based ANOVA.

*Table 3: Results from a CCA, presented by an ANOVA, testing the significance between the variation can be explained by environmental factors. Random Permutations = 999.*

| **Term** | **Df** | **Chi Square** | **F-value** | **P-value** |
|---|---|---|---|---|
| Model | 9 | 1.73 | 2.59 | **0.001** |
| Residual | 32 | 2.37 | - | - |

## Indicator species analysis

The ISA identified nine species with a significant association with *H. monorchis* (Figure 11, Table 4). *Carex distans* were the only species that had a positive association, while the remaining eight species had a negative association with *H. monorchis* (Table 4). The significant species were divided into two groups, based on a negative or positive relationship (0 and 1, respectively) (Table 4). *Pentanema salicium* and *Festuca ovina* were the species with the highest negative associations to *H. monorchis* (Table 4). *Molinia caerulea* and *Festuca rubra* showed strong association, as they were present in most plots where *H. monorchis* occurred. Since they were also found in plots where *H. monorchis* were absent, they do not serve as reliable indicators of the presence of *H. monorchis,* which was also shown in the non-significant P-value. The species with purple bars were less associated with *H. monorchis* and were observed less frequently (figure 11).

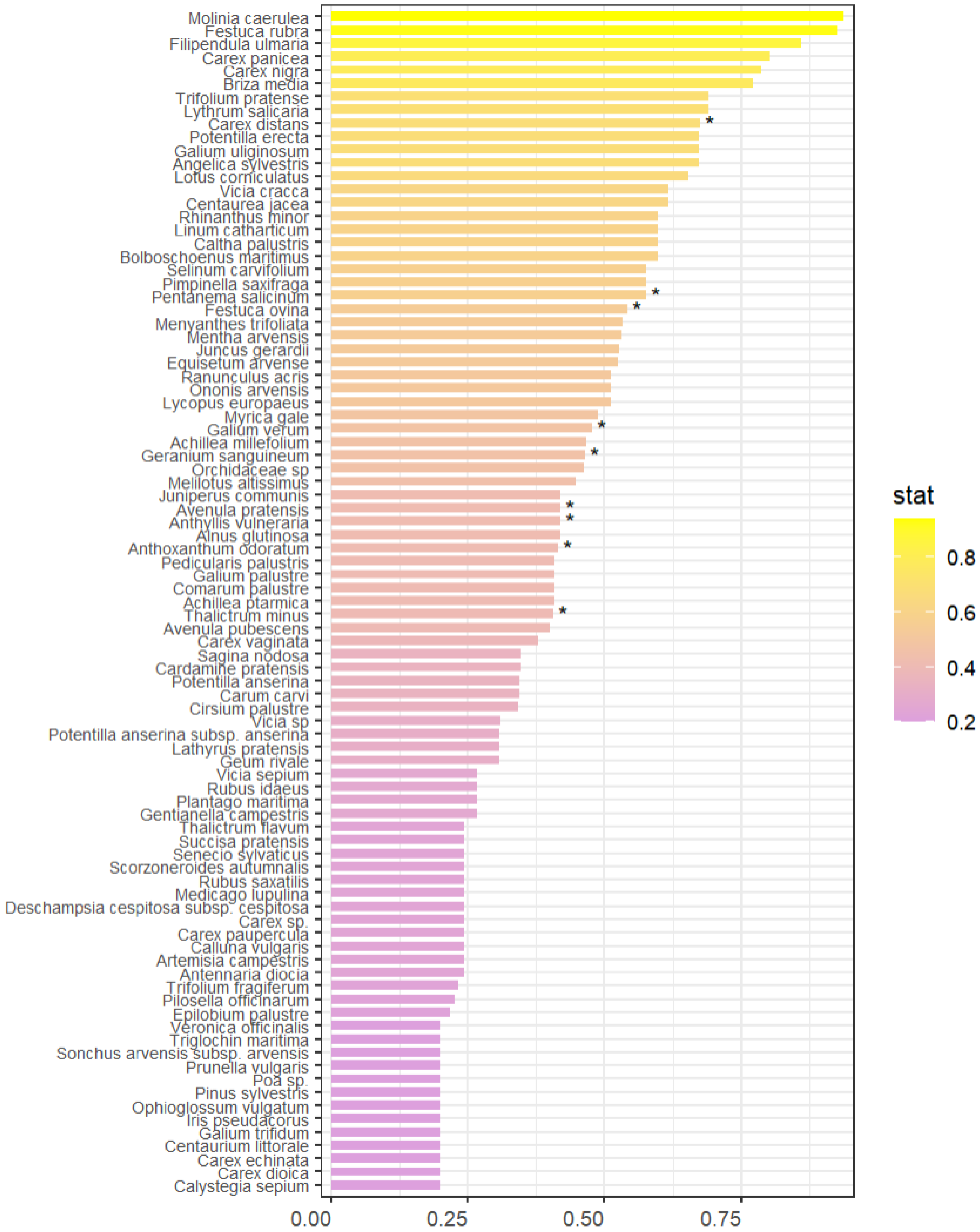


*Figure 11: The results from the Indicator Species Analysis (ISA) display the indicator values of species in relation to H. monorchis. The color gradient represents the strength of their association with H. monorchis, ranging from low (purple) to high (yellow). A limitation of this visualization is that it does not differentiate between positive and negative associations,*

*meaning species negatively associated with H. monorchis may still appear with a high stat value. Stat (Indval) = the association with H. monorchis ranging from 0 to 1. * on the right corner of the bars shows a significant relationship between the given species and H. monorchis. Carex distans is the only one of the nine with a positive, significant association with H. monorchis.*

*Table 4: The statistically significant results from the indicator species analysis. Group states the negative (0) or positive (1) association. Stat (Indval) shows the strength of the association, ranging from 0 to 1.*

| Group | Species | Stat | P-value |
|---|---|---|---|
| 0 | *Pentanema salicium* | 0.58 | **0.025** |
| 0 | *Festuca ovina* | 0.54 | **0.005** |
| 0 | *Galium verum* | 0.478 | **0.040** |
| 0 | *Geranium sanguineum* | 0.464 | **0.030** |
| 0 | *Anthyllis vulneraria* | 0.420 | **0.040** |
| 0 | *Anthoxanthum odaratum* | 0.425 | **0.040** |
| 0 | *Thalictrum minus* | 0.406 | **0.040** |
| 1 | *Carex distans* | 0.675 | **0.015** |

## Discussion

The aims of this study were to investigate how certain environmental variables might influence the number of individuals of *H. monorchis,* and if there were any indicator vascular plant species related to *H. monorchis*. The main findings showed that the number of individuals of *H. monorchis* had a negative relationship with higher soil moisture, while deeper soil and higher cover of vascular plants was positively associated. The indicator species analysis identified nine species closely related to *H. monorchis*. Among these, *Carex distans* showed a positive relationship with *H. monorchis* and appeared to have similar habitat preferences. The remaining eight species had a negative association with *H. monorchis*

### Intermediate soil moisture, deep soil and dense plant cover favor *H. monorchis*

The results showed that *H. monorchis* preferred medium soil moisture. These results were the contrary to what hypothesized. Earlier observations of *H. monorchis* in their natural habitat indicate that this species prefers areas affected by saline water or other water impacted areas such as wetlands and moist grasslands (Økland & Økland, 1996; Sharma et al., 2015). The locations included in this study, Skjellvik and Skipstadsand, align with these expectations, due to the previously mentioned coastal influences on the field sites. Additionally, the distribution of *H. monorchis* across Europe reveals a similar pattern of establishment in moist, occasionally waterlogged/flooded areas (Økland & Økland, 1996; SLU Artsdatabanken, 2025). Even though my findings suggested the contrary, it is important to note that in the driest areas where *H. monorchis* are found, and during dry years, it struggles (Wells et al., 1998; Roos et al., 2024b). Therefore, it can be argued that the findings in this study showed that there is an optimal soil moisture under which the species has the highest abundance, but it can tolerate other conditions as well. Several ecological and methodological factors may explain this.

Firstly, it is important to distinguish between habitat preference and habitat tolerance. While *H. monorchis* may prefer medium soil moisture at Skjellvik and Skipstadsand, it does not mean that it is not present in wetter conditions at the same locations. The species may tolerate wetter conditions, and a year with high precipitation and flooding may not severely harm the species. Plant species must adapt to local environmental variation, as their immobility prevents them from relocating to more favorable habitats. It is also important to acknowledge that my findings showed that the number of individuals of *H. monorchis* is close to zero at low soil moisture and therefore indicate little preference for such conditions. Wells et al. (1998) stated that drought will severely affect the flowering abundance of *H. monorchis*, and in years with less precipitation the species will struggle. These findings align with the results of my study.

Secondly, ecological interactions are also important to consider. Increased competition might occur in wetter conditions, and *H. monorchis* may therefore favor intermediate moist areas at Skjellvik and Skipstadsand. Certain adaptations to a specific environment between populations might occur, and it may influence the number of individuals of *H. monorchis* living in certain areas (Ahmad & Prasad, 2011; Lascoux et al., 2016), which in turn can cause dissimilarities between studies. For instance, the presence of *H. monorchis* in other studies might not be due to a preference for wet conditions but rather reduced competitive species in the given area (Kennedy & Sousa, 2006). Additionally, the symbiosis with fungi might differ between the populations of *H. monorchis,* which can cause different adaptations and may create opportunities for *H. monorchis* that allows it to survive even in suboptimal conditions.

An important consideration is that the soil moisture data were collected in 2014, 2016, and 2017, while the counting of *H. monorchis* was conducted in 2024. The results may be affected by the soil moisture measurements not being taken in the same years as the *H. monorchis* count. There is, however, a strong correlation between the years, leading to the assumption that the soil moisture data is still valid, in the absence of extreme variations. Temperature fluctuations and rainfall patterns may affect the abundance of *H. monorchis* (Wells et al., 1998; Sharma et al., 2015), and these might fluctuate between the measurements of soil moisture and the counting of *H. monorchis*. Precipitation data indicates some fluctuations between the years, however, the average precipitation in July and August across the three measured years was 105 mm, compared to 112 mm in the same months in 2024 (Norsk Meteorologisk Institutt, N.A.), overall, the precipitation levels are relatively similar. But due to climate change, and more intense periods of precipitation and followed by drought causing more extreme weather conditions, it cannot be stated with certainty that fluctuations in precipitation and temperature did not affect the results.

Increased soil depth had a positive relationship with the numbers of individuals of *H. monorchis,* which aligns with my hypothesis. This may be due to reduced risk of drought during years with less precipitation, and lower seedling mortality, as the temperatures are more stable in deeper soil and less affected by the above ground temperature (Gülser & Ekberli, 2004). The preferred increased soil depth might, also, be because of root establishment and mycorrhizal relationships. Some studies suggest that the root system of orchids is shallow and are often 10 – 15 cm below ground (Rasmussen, 2002). Therefore, the significant positive relationship between soil depth and the numbers of individuals of *H. monorchis* might be influenced by other factors such as the symbiosis with fungi which can increase the network drastically. However, to my knowledge, there have not been any studies investigating the relationship between soil depth, mycorrhiza, and *H. monorchis.* Furthermore, the larger soil volume might lead to less belowground competition between species by allowing root systems to occupy different soil layers (Homulle et al., 2022) and optimize the likelihood of *H. monorchis* survival.

Cover of vascular plants showed a slightly significant, positive relationship with the number of *H. monorchis* individuals, which contrasts with the initial hypothesis. Increased vascular plant cover can lead to greater interspecific competition (Jeong et al., 2024), potentially reducing habitat suitability for *H. monorchis*, a species known to be less competitive. However, it is also important to note that higher vascular plant cover does not necessarily imply greater vegetation height or species richness. The species contributing to the increased cover are likely less competitive and may coexist with *H. monorchis* under the current management regimes at Skjellvik and Skipstadsand, which is important information in a potential reintroduction of *H. monorchis*. The cover of vascular plants might also create microhabitats suitable for *H. monorchis* by regulating soil moisture and cause higher ecosystem stability and resilience, especially when impacted by environmental stress (Brunbjerg et al., 2018).

## Vegetation height and species richness did not affect the abundance of *H. monorchis*

Vegetation height and species richness did not have a significant effect on the number of *H. monorchis* individuals, in contrast to what hypothesized. This differs from several studies showing that *H. monorchis* is not highly competitive (Harrap & Harrap, 2009; Ekelund, 2021b), and requires sunlight (Wells, 1998; Raskoti, 2017). These unexpected results can be explained by the conservation management done at both Skjellvik and Skipstadsand. With measures to keep the vegetation low, removal of dead organic material, and removal of invasive species, the conditions are designed for *H. monorchis* to survive according to established literature. However, there is no certainty that today's management practices are best suited for *H. monorchis*, but in general low-intensity human influences can be positive for biodiversity, including orchids (Wallis de Vries, 2016; Leten et al., 2022). A study from Belgium found that human interference had a positive impact on the abundance of *H. monorchis* (Leten et al., 2022). The role of low-intensity human management in shaping vegetation dynamics is particularly relevant, as my results indicate a negative trend in the number of individuals of *H. monorchis* with increasing vegetation height. Although the observed trends suggest a potential negative impact of increased vegetation height, these results were not statistically significant and should therefore be interpreted with caution. However, similar patterns reported in other studies support this assumption (Wells et al., 1998; Leten et al., 2022). For instance, a study conducted by Wells et al. (1998) in England, found *H. monorchis* in areas with an average vegetation height not exceeding 10 cm, and therefore *H. monorchis* experienced less competition for light. Additionally, *H. monorchis* locations in Sweden have relatively low vegetation, often due to grazing, which limits the competition for light (SLU Artdatabanken, 2019). In contrast, my findings showed that *H. monorchis* individuals were present at locations with vegetation heights exceeding 20 cm. This suggests that the management practices at Skjellvik and Skipstadsand are effectively timed to maintain conditions that allow the species sufficient access to sunlight. These insights imply that the importance of maintaining an open vegetation is important for the conservation of *H. monorchis.*

The number of *H. monorchis* individuals showed an optimum preference at intermediate species richness. However, the results were not statistically significant and cannot be stated with high certainties, but the trends predicted are interesting regarding management methods. The intermediate preference for species richness may be due to competition or less suitable conditions for *H. monorchis* at lower or higher species richness. The unimodal curve can be explained by the increased competition at lower species richness because of some highly competitive species outcompeting the rest. Especially taller and fast-growing species may outcompete others by creating shade and reducing light availability and therefore cause a lower species richness. The decline at high species richness might be because of less optimal conditions regarding nutrients and water availability. Nutrient-adapted species outcompete species less tolerated for such conditions (Muehleisen et al., 2023). For instance, a study found that increased nutrient availability in grasslands changed the species composition with over 30% of the original species lost within one year, and nutrient-loving species colonizing the area within the same year (Muehleisen et al., 2023). Such conditions are often unfavorable for less competitive species found in cultural landscapes, including *H. monorchis*, due to the increase in nutrients and the decline in light. As a small, light demanding and non-competitive species, *H. monorchis* may be excluded from areas where nitrophilous, tall species dominate and therefore prefer intermediate species richness.

## Indicator species as an ecological tool

The findings from the ISA suggested that areas where *C. distans* were found can be suitable habitats for *H. monorchis*, and its absence can predict potential risks for *H. monorchis* (Bal et al., 2018). *Carex distans* is mostly found in coastal areas such as beach meadows (Lid & Lid, 2005, p. 1009) and in damp and wet areas (Ali, 2010). These habitat preferences align well with those of *H. monorchis*. Additionally, a good indicator species can help with conservation management (Bal et al., 2018; Virkkala et al., 2022). For instance, if *C. distans* responds positively to a specific management practice, the same management practice may have a positive effect on *H. monorchis*. On the other hand, the negatively associated plants have a higher tolerance for drought and drier habitats, with preference for low intensity grazing and alkaline soils (Lid & Lid, 2005, p. 285, 517, 1040; Solstad et al., 2021a). This contrasts with the habitat preference of *H. monorchis,* which showed that dry environments are not suitable habitats for *H. monorchis* and supports my earlier findings regarding *H. monorchis´* preference for soil moisture. Thus, the presence of *Pentanema salicium*, *Festuca ovina, Galium verum*, *Geranium sanguineum*, *Anthyllis vulneraria*, *Anthoxanthum odaratum* and *Thalictrum minus* can act as an indicator tool for areas less suitable for establishment of H. *monorchis* populations.

Indicator species can be valuable tools for assessing ecosystem quality, as their presence, absence, or condition often reflects underlying environmental conditions (Bal et al., 2018; Legendre, 2024). For example, the diversity and composition of species in an area can provide insight into factors such as soil moisture, disturbance regimes, or nutrient availability. In this way, indicator species help identify key ecological characteristics and changes within a habitat

### ***Differences in species composition across H. monorchis locations***

Based on the NMDS results there were a clear difference between Skjellvik and Skipstadsand in species composition, indicating that *H. monorchis* tolerates differences in the species composition and related environmental variables. The alignment of environmental variables with the distribution of plots in the ordination suggests that variation in species composition is primarily structured by underlying environmental gradients, rather than the presence of specific species. This pattern aligns with the broader adaptability observed in orchids, which are generally a highly successful plant family with numerous species and ecological strategies (Fay & Chase, 2009; Hsiao et al., 2011). Some of these adaptations may be seen in *H. monorchis*. Historical data reports that *H. monorchis*, has been found at 70 different locations in Norway, all the way up to Gudbrandsdalen (Innlandet county), but due to land-use change there has been a drastic decline in the abundance (DN, 2010; Solstad et al., 2021b). Several of the 70 locations lie near water streams (Artsdatabanken, N. A.-a), in nature types such as wetlands, hayfields, pastures and coastal meadows (DN, 2010). This might suggest that *H. monorchis* may be found in different habitats if the ecological conditions and environmental conditions are beneficial for less competitive, sun-loving plants. This indicates that *H. monorchis* can tolerate some fluctuations within species communities and environmental conditions, which is important to consider in a reintroduction, but it still has narrow limits in what the species can endure and still be able to survive and reproduce.

## Reintroduction

Based on the findings in this study, relatively moist areas with high soil depth and a well-developed cover of vascular plants appear to be important for the abundance of *H. monorchis*. These factors likely support the habitat stability, suitability and resource availability that *H. monorchis* requires. In addition, the presence of certain indicator species, such as *C. distans* can indicate favorable environmental conditions and therefore serve as a useful proxy when identifying potential reintroduction sites. Given that *H. monorchis* is a small, light-demanding orchid vulnerable to drought and shade caused by taller vegetation, the factors investigated in this study, soil moisture, soil depth, and vegetation cover and height, are particularly relevant to understanding its habitat requirements (Økland & Økland, 1996; Wells et al., 1998; Adamowski & Kęczyński, 2009). These characteristics together suggest that a potential successful reintroduction should prioritize relatively moist and deep soil with an intermediate level of competition. Moist soils with sufficient depth may provide stable conditions for root development and nutrient uptake, while a well-vegetated but not overly competitive ground layer can help maintain the microclimate and prevent drought (Padilla & Pugnaire, 2007; Wright & Francia, 2024). Prioritizing such conditions in location selection can enhance the likelihood of successful reintroduction and establishment of viable populations, while also improving the efficiency and ecological impact of conservation efforts (Bottin et al., 2007; Bal et al., 2018).

Globally, reintroduction of threatened species is not commonly successful, mainly because of lack of information about environmental variables and therefore choosing unsuitable locations with too few individuals being used in the reintroduction (Bottin et al., 2007; Godefroid et al., 2011). The lack of success in some reintroduction efforts may reflect an incomplete understanding of the whole ecological context in which species are reintroduced. Historically, there have been few reports on the results and protocols of reintroduction efforts (Bottin et al., 2007; Godefroid et al., 2011), perhaps because of failed experiments. However, with more successful attempts there has been an increase in the international documentation regarding reintroduction in the last decade (Stewart, 2008; Godefroid et al., 2011).

In Norway a suggestion for a national guideline (Tingstad & Endrestøl, 2021) involving reintroduction has been made (Tingstad & Endrestøl, 2021). In these guidelines it is stated that a deep knowledge is crucial for even considering a reintroduction. Therefore, when considering reintroduction of *H. monorchis* information about ecological preferences, such as neighboring plants and associated fungi, how the species respond to management and which negative factors affect the population of *H. monorchis* are vital (Bottin et al., 2007; Tingstad & Endrestøl, 2021). Suitable reintroduction locations for *H. monorchis* may be found where *C. distans* is located. According to species registrations in "Artskart", *C. distans* is located along southern and south-west parts of the Norwegian coast (Artsdatabanken, N.A.-a), and some of these habitats, such as, coastal meadows can be a potential location for a reintroduction of *H. monorchis*. "Artskart" can be used to find potential locations for a reintroduction of *H. monorchis* (Artsdatabanken, N.A.-a). By searching for *C. distans*, and where it occurs, potential suitable locations can be found for reintroduction of *H. monorchis*. However, when using "Artskart" it needs to be used with some caution since some of the registrations are done by amateurs and are not necessarily double checked (Artsdatabanken, N.A.-a).

As *H. monorchis* historically occurred in over 70 locations in Norway, some might still be suitable for a reintroduction, especially if key environmental conditions are intact or can be restored, such as soil moisture, light availability and low vegetation height. Locations with ongoing or potential conservation management, such as low intensity grazing with lightweight animals or mowing, could be promising if it is adapted to the ecological needs of *H. monorchis*. However, further data on historical locations should be collected before proceeding with a decision.

After the reintroduction, close monitoring is crucial to gain success and implement or change the conservation management. Considering ongoing climate and land-use changes, reintroductions should also incorporate future habitat suitability projections. A successful reintroduction for *H. monorchis* will rely on combining ecological insight and suitable habitats, and long-term commitment, as emphasized in other reintroduction frameworks (Bottin et al., 2007; Stewart, 2008; Godefroid et al., 2011).

# Conclusion

Protecting endangered species and their ecosystems is crucial for maintaining biodiversity and ecosystem resilience in the face of global environmental change. *Herminium monorchis* serve as a reminder of how habitat degradation and small population sizes can push species to the brink of extinction. This study identifies key environmental conditions and their relation to *H. monorchis*, as well as indicator vascular plant species associated with *H. monorchis,* offering valuable insight into its habitat preferences. These findings suggest that *H. monorchis* thrive in relatively moist areas with deep soil and high vascular plant cover and recommended that *C. distans* should be used as an indicator species when assessing potential habitats for *H. monorchis*. These insights provide an important foundation for future reintroduction efforts in Norway. With much of the necessary ecological knowledge now available, the next step is to identify suitable reintroduction sites and assess whether current management practices support long-term population growth of *H. monorchis*. As the populations of *H. monorchis* remain relatively stable but show no signs of growth, reintroduction can help species recover, if it is based on a good understanding of its ecological needs and careful management of its habitat over time. Ensuring the survival of *H. monorchis* will not only help preserve a unique species but also strengthen our commitment to protecting the ecosystems it depends on.

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# Appendix A: Maps of transects

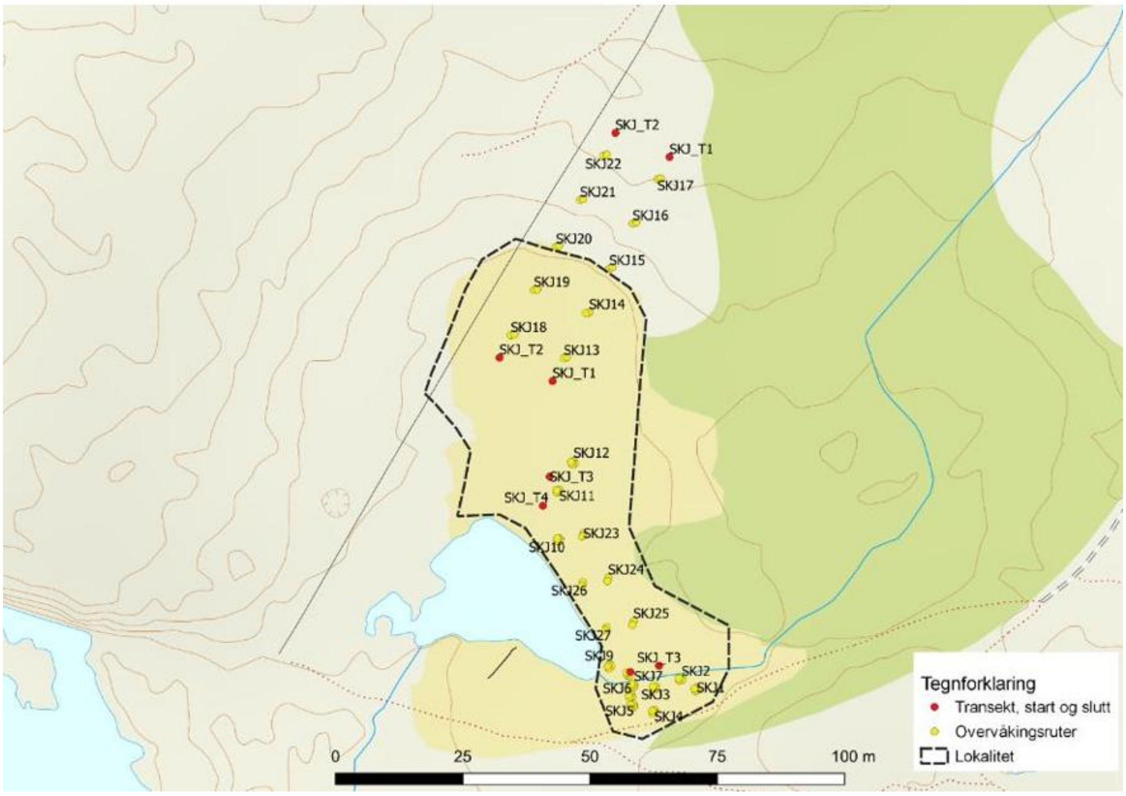


*Figure A1: A map over the transects at Skjellvik. The red dots symbolize the start and end of each transect, while the yellow dots illustrate the GPS-points. The black dotted lines show which parts of the location that are fenced in. Map taken from* [*NINA Brage: Overvåking av effekter av tiltak for truet natur. Feltmetodikk, analyser og resultater for sju arter og en naturtype*]

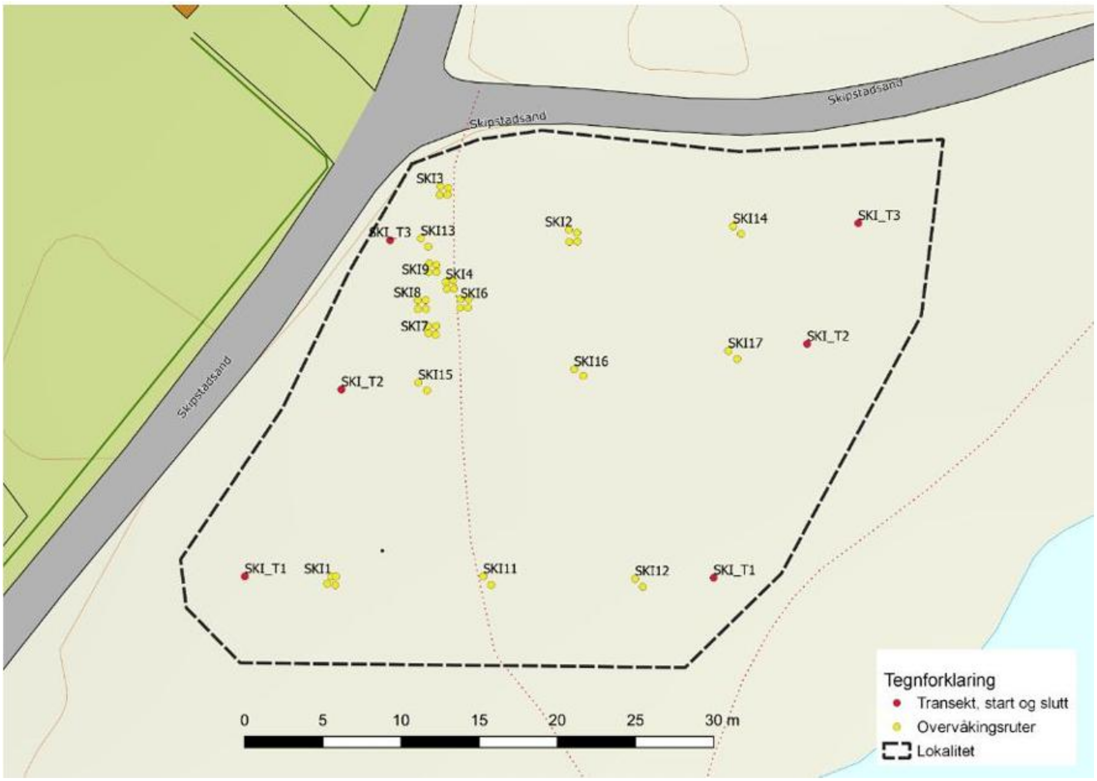


*Figure A2: A map over the transects at Skipstadsand. The red dots symbolize the start and end of each transect, while the yellow dots illustrate the GPS-points. The black dotted lines outline the area of the study location. Map taken from* [*NINA Brage: Overvåking av effekter av tiltak for truet natur. Feltmetodikk, analyser og resultater for sju arter og en naturtype*]

# Appendix B: Species composition and field form

Fieldform

| Site: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
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|---|---|---|---|
| Vascular plants (%) | | Bare ground (%) | |
| Vegetation height (cm) | | Bryophytes (%) | |
| Dead plant material (%) | | | |
| Comment | | | |

*Figure B1: A copy of the field form used during the field work. Showing how the species were mapped and additional variables. One field from were used for each plot. Species names were written in the first collum with presence marked in each quadrat.*

*Table B1: The table shows the species' composition at each location. X indicates presence.*

| Species | Skipstadsand | Skjellvik |
|---|---|---|
| *Achillea millefolium* | X | X |
| *Achillea ptarmica* | | X |
| *Alnus glutinosa* | | X |
| *Angelica sylvestris* | X | X |
| *Antennaria diocia* | | X |
| *Anthoxanthum odoratum* | | X |
| *Anthyllis vulneraria* | | X |
| *Artemisia campestris* | | X |
| *Avenula pratensis* | | X |
| *Avenula pubescens* | X | |
| *Bolboschoenus maritimus* | | X |
| *Briza media* | X | X |
| *Calluna vulgaris* | | X |
| *Caltha palustris* | | X |
| *Calystegia sepium* | | X |
| *Cardamine pratensis* | X | X |
| *Carex dioica* | | X |
| *Carex distans* | X | |
| *Carex echinata* | | X |
| *Carex nigra* | X | X |
| *Carex panicea* | X | X |
| *Carex paupercula* | | X |
| *Carex sp.* | | X |
| *Carex vaginata* | X | X |
| *Carum carvi* | X | X |
| *Centaurea jacea* | X | X |
| *Centaurium littorale* | X | |
| *Cirsium palustre* | | X |
| *Comarum palustre* | | X |
| *Deschampsia cespitosa subsp. Cespitosa* | | X |
| *Epilobium palustre* | | X |
| *Equisetum arvense* | X | |
| *Festuca ovina* | X | X |
| *Festuca rubra* | X | X |
| *Filipendula ulmaria* | X | X |
| *Galium palustre* | X | X |
| *Galium trifidum* | X | |
| *Galium uliginosum* | | X |
| *Galium verum* | X | X |
| *Gentianella campestris* | X | X |
| *Geranium sanguineum* | | X |
| *Geum rivale* | | X |
| *Herminium monorchis* | X | X |
| *Iris pseudacorus* | | X |
| *Juncus gerardii* | X | X |

| | | |
|---|---|---|
| *Juniperus communis* | | X |
| *Lathyrus pratensis* | | X |
| *Linum catharticum* | X | X |
| *Lotus corniculatus* | X | X |
| *Lycopus europaeus* | | X |
| *Lythrum salicaria* | X | X |
| *Medicago lupulina* | | X |
| *Melilotus latissimus* | X | |
| *Mentha arvensis* | | X |
| *Menyanthes trifoliata* | | X |
| *Molinia caerulea* | X | X |
| *Myrica gale* | | X |
| *Ononis arvensis* | | X |
| *Ophioglossum vulgatum* | X | |
| *Orchidaceae sp* | | X |
| *Pedicularis palustris* | | X |
| *Pentanema salicinum* | | X |
| *Pilosella officinarum* | | X |
| *Pimpinella saxifraga* | | X |
| *Pinus sylvestris* | X | |
| *Plantago maritima* | X | X |
| *Poa sp.* | | X |
| *Potentilla anserina* | | X |
| *Potentilla anserina subsp. Anserina* | X | |
| *Potentilla erecta* | | X |
| *Prunella vulgaris* | | X |
| *Ranunculus acris* | X | X |
| *Rhinanthus minor* | X | X |
| *Rubus idaeus* | | X |
| *Rubus saxatilis* | | X |
| *Sagina nodosa* | X | X |
| *Scorzoneroides autumnalis* | | X |
| *Selinum carvifolium* | | X |
| *Senecio sylvaticus* | | X |
| *Sonchus arvensis subsp. Arvensis* | X | |
| *Succisa pratensis* | | X |
| *Thalictrum flavum* | | X |
| *Thalictrum minus* | | X |
| *Trifolium fragiferum* | X | |
| *Trifolium pratense* | X | X |
| *Triglochin maritima* | X | |
| *Unknown 1* | | X |
| *Unknown 2* | | X |
| *Unknown 3* | | X |
| *Unknown 4* | | X |
| *Unknown 5* | X | X |
| *Veronica officinalis* | | X |

| | | |
|---|---|---|
| *Vicia cracca* | X | X |
| *Vicia sepium* | X | |
| *Vicia Sp.* | X | X |
| | | |
| **Total species** | 42 | 82 |

## Appendix C: Supplementary statistics and figures

*Table C1: Results from the Shapiro–Wilk tests for normality. The W – value (ranging from 0 to 1) indicates how well each variable fits a normal distribution, but it is the P – value that shows whether any deviation is statistically significant.*

| Variable | W-value | P-value |
|---|---|---|
| Individuals of *H. monorchis* | 0.68 | **< 0.001** |
| Soil moisture | 0.92 | **< 0.001** |
| Soil depth | 0.95 | **0.049** |
| Cover of vascular plants | 0.54 | **< 0.001** |
| Vegetation height | 0.93 | **0.016** |
| Species richness | 0.99 | 0.9 |

*Table C2: The results from an Akaike information criterion to determine whether cover of vascular plants or cover of litter should be included. Df shows the numbers of parameters included. The AIC shows the relative quality of the statistical method used.*

| Variable | Df | AIC |
|---|---|---|
| With cover of vascular plants | 8 | 247.5 |
| With cover of litter | 8 | 248.3 |

*Table C3: The results from three correlation tests comparing soil moisture values from three years. Tau values, ranging from -1 to 1, show the strength of the correlation. Values closer to 1 indicate high correlation, and -1 indicates less correlation, with a following P-value.*

| Year | Tau | P-value |
|---|---|---|
| 2014 vs 2016 | 0.73 | **< 0.001** |
| 2014 vs 2017 | 0.77 | **< 0.001** |
| 2016 vs 2017 | 0.68 | **< 0.001** |

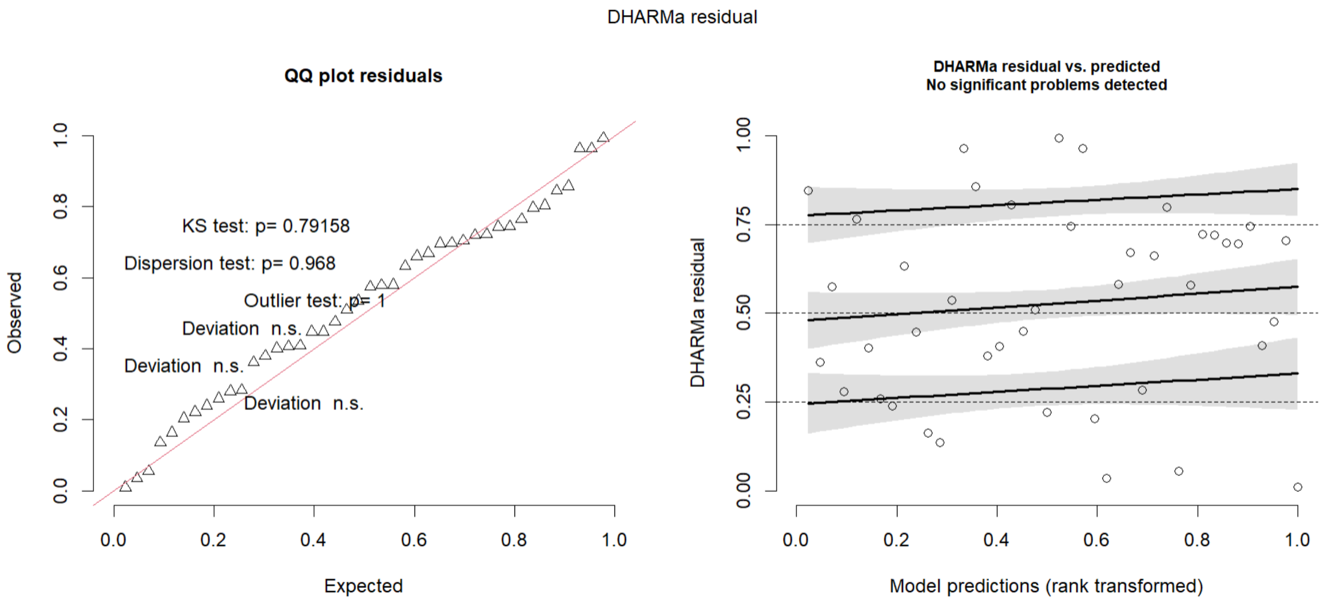


*Figure C1: DHARMa residual diagnostics for the generalized linear mixed model. The QQ plot (left) shows that residuals follow a uniform distribution (KS test: p = 0.792), with no significant outliers or deviations. The residuals vs. predicted plot (right) shows no systematic patterns, indicating that the model fits the data well and that residuals behave as expected.*

*Table C4: Results from Detrended Correspondence Analysis (DCA) of species composition data. Since the first axis length exceeds 4, indicating a unimodal species response, Canonical Correspondence Analysis (CCA) was used for further constrained ordination.*

| | DCA1 | DCA2 | DCA3 | DCA4 |
|---|---|---|---|---|
| Axis lengths | 4.7889 | 2.7610 | 1.9541 | 1.6116 |

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