universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Mound density and distribution changes of *Formica* ants in an era of rapid environmental change in the Swiss Alps"

verfasst von / submitted by

Miriam Simma, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2023 / Vienna, 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 833
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Ecology and Ecosystems
Betreut von / Supervisor:	PD Dr. Anita C. Risch

Table of contents

Abstract

In mountain ecosystems, ambient temperatures are strongly related to elevation, and, thus, studies along elevational gradients are often used to detect climate warming-induced alterations in species distributions over time. One insect taxon with several species occurring in mountainous areas are the mound-building ants of the genus *Formica*. However, little is known about distributional responses of *Formica* species to climate change. In a mountainous area in Switzerland, ant mound occurrences were systematically mapped in 1982. In this study, I resurveyed the same area for *Formica* mounds in 2022 to analyse potential changes in mound distributions along an elevational gradient. I hypothesised to find a climate warming-induced increase in mound densities at high altitudes relative to the historical survey. Additionally, I investigated potential environmental predictors of the recent mound occurrences and mound volumes. I found a 3.5-fold increase in mound numbers in 2022 compared to 1982. However, this increase was independent from elevation, indicating that climate warming had no direct effect on the mound distributions. Further, I found a positive relationship between mound volume and elevation as well as canopy closure, indicating that ants can adapt to changing temperatures by adjusting their mound size and nest site choice. I suggest that this ability of the mound-building *Formica* species is advantageous to a successful adaptation to ongoing global warming. Whilst warmer temperatures can directly or indirectly benefit the ants, human land use changes, such as land abandonment, are other supposable factors, which might have facilitated a mound density increase during the last decades.

Zusammenfassung

In Gebirgsökosystemen hängen die vorherrschenden Umgebungstemperaturen stark mit der Seehöhe zusammen, weshalb Studien entlang von Höhengradienten häufig durchgeführt werden, um Klimawandel bedingte Veränderungen in Artverbreitungen zu untersuchen. Eine Insektengruppe mit hochgelegenen Artvorkommen sind die hügelbauenden Ameisen der Gattung *Formica*. Dabei ist über potentielle Klimawandeleffekte auf die Verbreitungsmuster dieser Ameisenarten sehr wenig bekannt. In einem Schweizer Gebirgstal wurden die Haufenvorkommen von *Formica* Arten im Jahr 1982 systematisch kartiert. Im Zuge der vorliegenden Arbeit habe ich diese Kartierungen im Sommer 2022 wiederholt, um potentielle Veränderungen der Haufenvorkommen entlang eines Höhengradienten zu untersuchen. Ich nahm an eine durch die Klimaerwärmung bedingte erhöhte Haufendichte in den höheren Lagen vorzufinden. Zudem habe ich potentielle Umweltvariablen als Determinanten der aktuellen Artverbreitungen sowie der Haufenvolumen untersucht. Im Jahr 2022 fand ich die 3,5-fache Dichte an Ameisenhaufen im Vergleich zu 1982 vor. Allerdings veränderte sich die Haufenanzahl unabhängig von der Höhenlage (Seehöhe), was darauf hindeutet, dass die klimatische Erwärmung keinen direkten Effekt auf die Haufenverbreitung hatte. Darüber hinaus zeigte sich ein positiver Zusammenhang zwischen Haufenvolumen und Höhenlage sowie Kronenschluss, was darauf hindeutet, dass sich die Ameisen durch Anpassung der Haufengrößen und Wahl des Neststandorts an sich verändernde Temperaturen anpassen können. Ich nehme an, dass diese Fähigkeit eine vorteilhafte Eigenschaft der hügelbauenden *Formica* Arten für eine erfolgreiche Anpassung an die fortschreitende globale Erwärmung ist. Während wärmere Temperaturen direkte oder indirekte positive Effekte auf die Ameisen haben können, sind lokale Veränderungen in der Landnutzung während der vergangenen Jahrzehnte weitere Einflussfaktoren, die eine Zunahme an Ameisenhaufen begünstigt haben könnten.

Acknowledgements

First, I want to thank Anne Kempel and Anita C. Risch for all their efforts and support as supervisors during this project. I thank the staff at the WSL Institute for Snow and Avalanche Research SLF, particularly Giulia Mazzotti for providing the transmissivity data and Kevin Patrick Helzel as well as Andreas Stoffel for their valuable advice on GIS. I thank Hannes Jenny, one of the observers in the survey in 1982, for providing the historical maps and for the personal exchange about the field sampling in 1982.

Many thanks to Univ.-Prof. Mag. Dr. Konrad Fiedler at the University of Vienna for all support, particularly for sharing his expertise and for making a genetical species analysis possible, which filled an important knowledge gap in the data. In this regard, I further thank Brigitte Gottsberger for conducting the DNA barcoding of the red wood ant samples in the genetic laboratory of the Department of Botany and Biodiversity Research of the University of Vienna. The DNA barcoding was funded by the Department of Botany and Biodiversity Research at the University of Vienna.

I thank Céline Stoffel at the Musée cantonal de zoologie in Lausanne for spontaneously performing a more specific molecular analysis of a few red wood ant samples, which assured the coexistence of the two presented red wood ant species in the study area.

A warm thanks goes to Kimberly Y. Brosche, for whose invaluable help, collaboration and friendship during my studies I am deeply grateful.

Lastly, I want to sincerely thank my family and Mamadou for all encouragement, their patience and understanding during the last years.

Introduction

The understanding of global warming effects on ecosystems, biodiversity and individual species evolved to one of the main research focuses in the field of ecology within the last decades. At the same time, ecological responses to ongoing anthropogenic climate change are rather complex to address but are suggested to threaten biodiversity and lead to extraordinary changes in biological systems (IPCC, 2007; Walther et al., 2002). In principle, species have two possibilities to respond to drastic long-term environmental changes: adaptation or migration (Donoghue, 2008; Parmesan, 2006). With the assumption of unrestrained dispersal capabilities, the latter is considered as easier (Donoghue, 2008).

Elevational gradients are ideal to investigate biotic responses to a rapidly warming environment, as ambient temperatures change distinctively within short distances along mountain slopes (Körner, 2007). An upward migration has already been shown for several taxonomic groups and species in the European Alps (reviewed by Vitasse et al., 2021) and other mountainous areas (e.g., Engler et al., 2011; Parmesan, 2006; Wilson et al., 2007) during the last decades. Nonetheless, species' responses are variable and non-detected shifts or even downward migrations can occur (McCain & Garfinkel, 2021). However, some taxonomic groups are still underrepresented in studies about climate change related range shifts, as historical data about species distributions are rarely at hand and mostly restricted to plants (e.g., see Steinbauer et al., 2018). One such understudied taxonomic group with high ecological significance are the ants (Parr & Bishop, 2022).

A study about warming tolerances of ants predicted tropical species to be most vulnerable to climate warming. Conversely, ant species in temperate regions might be 'climate change winners', as they are currently below their thermal tolerance (Diamond et al., 2012). In warming experiments in temperate latitudes, ants showed rather complex responses in community composition and species abundances to elevated temperatures (Diamond et al., 2016; Menke et al., 2014; Pelini et al., 2012). In general, these studies underlined the thermal responsiveness of ants (Parr & Bishop, 2022) and the individuality of species' responses to a warmer environment. Thus, a focus on lower taxonomic levels is required to specifically evaluate effects of global warming on groups of concern (Balzani et al., 2022).

Among all ant species in temperate to cold regions of Europe, the probably most eye-catching and famous ones are the mound-building ants of the genus *Formica* (LINNAEUS 1758). Particularly, the subgenus *Formica rufa senu stricto*, the red wood ants, are known for their ecological importance in forest ecosystems and have a remarkable research history (e.g., Adlung, 1966; Gösswald, 1941, 1951; Steiner, 1924). As keystone species, red wood ants substantially impact the abundance, distribution, and diversity of other animal species, including other insects within their territory (Çakır, 2019; Fowler & Macgarvin, 1985; Hawes et al., 2002; Punttila et al., 2004; Skinner & Whittaker, 1981). Besides their role as predators and

scavengers, they also form mutualistic interactions with aphids (Hemiptera: Aphidina) (Billick et al., 2007; Erős et al., 2009; Skinner & Whittaker, 1981; Stadler & Dixon, 2005). Some *Formica* species are known to be important for pest control (Karhu, 1998; Trigos-Peral et al., 2021; Youngs & Campbell, 1984) or as bioindicators for land use change (Ellison, 2012). Furthermore, *Formica* species are characterised as ecosystem engineers, as they transfer organic material (e.g., seeds, litter, prey) from the surrounding environment to their nest. Thereby, they also impact carbon and nutrient pools and fluxes in forest ecosystems at small spatial scales (Domisch et al., 2009; Jurgensen et al., 2008; Ohashi et al., 2007; Risch et al., 2005; Wellenstein, 1952). Moreover, to name an example beyond the *Formica rufa* group, *Formica exsecta* (subgenus *Coptoformica*) substantially alters the seedbank and vegetation patterns in grassland ecosystems, most likely leading to an increased habitat heterogeneity in the landscape (Schütz et al., 2008). Given the ecological significance of *Formica* ants in terrestrial ecosystems, understanding their response to climate warming is important.

Being ectothermic and thermally responsive organisms, the *Formica* ants are likely to strongly react to temperature rises. Although *Formica* ants can actively regulate the temperatures within their mounds to create optimal conditions for their brood (Horstmann & Schmid, 1986; Kadochovà & Frouz, 2014; Steiner, 1924), the mound temperatures are also influenced by the ambient temperature and solar insolation (Frouz & Finer, 2007; Parr & Bishop, 2022; Steiner, 1924; Stukalyuk et al., 2020). Thus, mound size and location impact thermal conditions in the nest (Chen & Robinson, 2014; Steiner, 1924; Stukalyuk et al., 2020). Slope aspect was shown as one relevant factor determining *Formica* species' distribution (Vandegehuchte et al., 2017) as well as their mound size. More precisely, mounds at northern-faced slopes tend to be larger than mounds at other aspects (Gösswald, 2012; Risch et al., 2005). Moreover, the mound size of *F. lugubris*, for example, was shown to be significantly larger when the canopy closure increased, whereas in forest locations with closer canopies the ambient temperatures at the nest site were colder but more stable (Chen & Robinson, 2014). Whilst red wood ants can additionally increase the nest temperature by metabolic heat production (Steiner, 1924), other *Formica* species, such as *F. exsecta*, cannot do this and, hence, they depend more on direct insolation at their nest sites (Seifert, 2000).

Mound-building *Formica* ants appear in the Red list of the IUCN as "near threatened" and several European countries protect *Formica* species by law (Balzani et al., 2022). Thus, knowledge about the effects of global climate change on their distribution and abundance is highly pertinent. Given the relevance of temperature for nest site choice of mound-building *Formica* species, I hypothesise that the effects of global warming on the ants' distribution along an elevational gradient should be detectable over longer time frames.

To shed light on species distribution responses to environmental change, historical datasets provide valuable opportunities for investigations of developments over time (McCain &

Garfinkel, 2021). In my thesis, I took advantage of systematically collected, historical data of the *Formica* mound distribution in a subalpine area in Switzerland from the 1980s. In combination with a resurvey of the same area in 2022, these data enabled a comparison of mound occurrences and density along an elevational gradient between 1982 and 2022. As the mound size is suggested to be closely related to ambient temperature conditions (Chen & Robinson, 2014; Gösswald, 2012), I was further interested in a potential relationship between elevation and mound volume of *Formica* ants.

Thus, in my thesis I addressed the following research questions:

1) How did the distribution of *Formica* mounds change along an elevational gradient in a subalpine environment over the last four decades?
2) How do elevation, slope aspect and canopy closure affect the current mound distribution of *Formica* species?
3) How do elevation, slope aspect and canopy closure affect the mound volume of the *Formica* species?

Due to the predicted benefits of warmer temperatures for ants in temperate regions (Kaspari et al., 2019; Parr & Bishop, 2022) and the generally low temperatures in subalpine areas, I expected to find an increase in mound densities at higher altitudes in 2022 compared to 1982. Further, I expected to detect a positive relationship between mound volume and elevation. However, I assumed the relationship to be stronger for ant species of open habitats (e.g., *Formica exsecta*), whereas forest species, such as red wood ants, might compensate for lower temperatures at higher elevations by choosing nest sites with lower canopy closure.

Material and methods

Study area

The study area was located in the Dischma Valley, Davos, Switzerland (Figure 1). The area is part of the Western Central Alps and characterised by a continental climate (Günter, 1986) with a mean annual temperature of 4 °C and a mean annual precipitation of 1046 mm measured at the meteorological station in Davos for the 1991-2020 period (DAV, 1594 m asl; geographic coordinates: 46.812969 / 9.843558; MeteoSwiss, 2022). In Switzerland, the average temperature trend per decade was + 0.135 °C in the 20th century, whereas a trend of

+ 0.57 °C was reported for the last three decades at the end of the century (Rebetez & Reinhard, 2008). The warmest years since 1864, however, have all been measured after 2010, and by the year 2019, the average temperature in the country had increased by around 2 °C relative to the pre-industrial age (FOEN, 2020). These values measured in Switzerland were double as high as the mean temperature trends in the Northern Hemisphere in the same time periods (FOEN, 2020; Rebetez & Reinhard, 2008). The study area was of approximately 500 ha in size and ranged from 1600 to 2150 m asl (meters above sea level). The main slope aspect of the area was south-west to west. Large parts of the study area were covered by mixed subalpine forests, which have been utilised and altered in structure by the settlers since centuries (Hefti, 1986). Whilst intensively used grasslands can be found below the lower forest edge, there is a more continuous change from forest to a mixture of dwarf-shrub and grassland habitats at the treeline ecotone. The latter is extensively used by grazing cattle during summertime. The dominating tree species in the forest is the European spruce (*Picea abies*), followed by the European larch (*Larix decidua*). At the upper edge, also the Cembran pine (*Pinus cembra*) is found (Leibold, 2012).

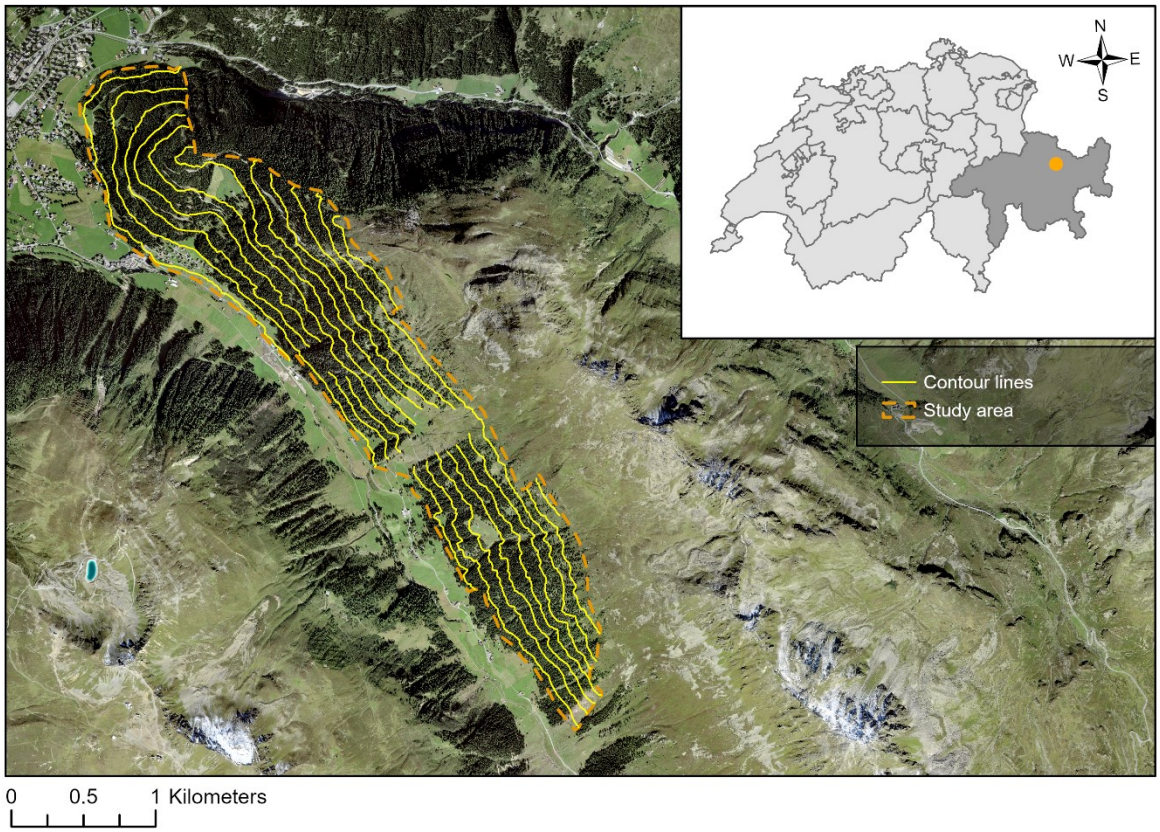

Figure 1: Study area and surveyed contour lines in the Dischma Valley in Switzerland, canton Grisons. Map created in the ArcGIS Pro software by Miriam Simma, 07.12.2022. Basemap (SWISSIMAGE) and country boarders provided by the Swiss Federal Office of Topography (swisstopo).

Historical and recent field sampling

The historical mound presence data were collected within a multi-species survey in 1982. The aim of the historical survey was to map the distributions of ungulates and grouse by walking along contour lines. In addition to those vertebrate species, the researchers also recorded *Formica* mounds on an analogous topographic map. The considered contour lines were separated by an altitudinal difference of 50 m (Müller et al., 1988, see Figure 1). The start and end points of the lines were additionally marked, and the total length of contour lines was about 47 km. To transfer the historical data into a digital format, the maps were georeferenced and digitised in spring 2022.

I resurveyed the mound presences by walking along the same contour lines in July and August 2022. In contrast to the field sampling in 1982, I only focused on *Formica* mounds and did not record vertebrates. I recorded each mound with the ArcGIS Survey 123 data collection app. To remain on a specific contour line, I oriented myself using the GPS and a digital swisstopo map on a tablet device. I recorded the GPS coordinates of each mound using an external GNSS receiver (STONEX S500) to enhance GPS precision.

In addition, I measured the mean diameter and the height of each mound to estimate mound volume. The mean diameter was derived from measurements of the longest basal diameter and the perpendicular diameter (Chen & Robinson, 2013). Furthermore, I collected 10 individual ants from each nest to identify the species. Specimens were stored in 95% ethanol.

Species identification

Morphological identification: Due to the large intraspecific variability and interspecific similarity in morphology among species of the *Formica rufa* group, morphological identification is challenging (Bernasconi et al., 2010; Seifert, 2021). Therefore, I only morphologically identified *F. exsecta*, and used DNA metabarcoding to distinguish between the *F. rufa* group species (see below). Due to financial constraints this was only possible for 50 red wood ant samples, which I randomly selected from the pool of all red wood ant samples.

DNA metabarcoding: A 658 bp long fraction of the COI gene, termed the “barcode region” (Hebert et al., 2003), was obtained by extracting DNA from the entire ant specimen pursuant to Analytik Jena innuPREP DNA Micro Kit (https://www.analytik-jena.de/). A 100µL buffer was used for the DNA elution. The ant individual was homogenised with ceramic beads using a Precellys 24 homogeniser (Bertin Technologies) set to 6000/min for 2 × 10 s. PCR reactions were done with the Thermo Scientific PCR system along these lines: 1 µl of 10 × $(NH_4)_2SO4$ PCR buffer, 1 µl $MgCl_2$ (25 mM/l), 0.2 µl dNTPs (10 mM/µl), 0.2 µl of each primer (10 pg/µl), 0.1 µl BSA, 1 µL RediLoad™ (Invitrogen),1 µl DNA, 0.2 µl Taq DNA polymerase and filled up

to 10 µl with PCR-grade H_2O (https://www.thermofisher.com). As primers, LepF1 (TTCAACCAATCATAAAGATATTGG) and LepR1 (AACTTCTGGATGTCCAAAAAATCA) were used (Hebert et al., 2004; Wilson, 2012). The cycles performed as a split program in the PCR were as follows: 1x: 94 °C for 4 minutes; 5x: 94 °C for 1 minute, 44 °C for 1.5 minutes, 72 °C for 1.5 minutes; 35x: 94 °C for 1 minute, 49 °C for 1.25 minutes, 72 °C for 1.25 minutes; 1x: 72 °C for 7 minutes. The PCR reactions were refined by digestion with shrimp alkaline phosphatase and exonuclease for 45 minutes at 37°C and, subsequently, for 15 minutes at 80°C. Further, the sequencing reactions were arranged with 0.4 µl primer, 1.75 µl sequencing buffer, 0.5 µl BigDye™ Terminator v3.1 (ThermoFisher, MA, USA), 1 µl template DNA, 2 µl Trehalose and filled up to 10 µl with PCR grade H_2O. Finally, Sanger sequencing was conducted by the usage of an ABI PRISM 3730 Genetic Analyzer. In the sequencing, both directions of the DNA fragments were considered, edited and assembled with a DNASTAR Lasergene SeqMan 7.1.0 (DNASTAR Inc.). The raw sequence data were further assembled using a SeqMan 7.1.0 program (Burland, 1999). FASTA files were aligned using a multiple sequence alignment web tool MAFFT version 7 (Katoh et al., 2019; https://mafft.cbrc.jp/alignment/software/), and the FASTA file containing the result sequences were sent to an identification engine on BOLD (Ratnasingham & Hebert, 2007; http://boldsystems.org/).

Data preparation and environmental variables

For the first part of data preparation, I used ArcGIS Pro version 3.0.3 (ESRI, 2022) and the extensions “3D Analyst” and “Spatial Analyst”. As the datasets of both timepoints consisted of presence-only data, I generated 1000 random points throughout the study area, which were considered as “pseudo-absences” in further analyses (Barbet-Massin et al., 2012).

I derived the environmental variables elevation and slope aspect from the SwissAlti3D digital elevation model that is provided by the Swiss Federal Office of Topography (swisstopo). I extracted the values for the point data (mound presences and pseudo-absences) from a 0.5 x 0.5 m resolution raster for elevation and from a 2 x 2 m resolution raster for slope aspect. To allow for the inclusion of slope aspect as a predictor in the statistical models, I used the cosine of aspect in radians, measured clockwise from north (Vandegehuchte et al., 2017). Therefore, the variable is hereafter referred to as “northness” (index). To analyse the effect of canopy closure on current mound distribution and volume, I used the calculated transmissivity for diffuse shortwave radiation (hereafter referred to as “sky view fraction”) in the forest as a proxy. The values were obtained from synthetical images derived from enhanced LiDAR data (see Webster et al., 2020) and represent a ratio of sky vs. total number of pixels (see Jonas et al., 2020). Synthetic images were evaluated at 5 m spacing and provided in a 5 x 5 m resolution

raster by the Snow Hydrology group at the WSL Institute for Snow and Avalanche Research SLF. A sky view fraction value of “0” indicates 100% canopy closure (no sky visible), a value of “1” indicates 0% canopy closure (open habitat).

In addition, I derived the approximative lengths (m) of the investigated contours in the study area from the SwissAlti3D digital elevation model, to enable a calculation of mound density per 100 m contour line. Although a consequent walking along the contour lines is precluded in the rocky and steep terrain of subalpine forests, the contour lines are still representative for the approximate length of the effectively surveyed line transects.

Statistical analysis

The statistical analysis was performed in the R programming software (v.4.2.1; R Core Team, 2022). P-values of ≤ 0.05 were considered as significant. I used the package “dplyr” (v.1.0.10; Wickham & Wickham, 2020) to format the data and the packages “ggplot2” (v.3.4.0; Wickham et al., 2016) and “ggeffects” (v.1.1.4; Lüdecke et al., 2020) for visualisations. To visualise mound densities along the elevational gradient, the presence points were subdivided into elevational bands. By doing so, all mound records up to 25 m below and above each contour line were summarised in the corresponding elevational band.

Changes in mound distribution in relation to elevation over time

To test for a difference in mound density distribution along the elevational gradient between 1982 and 2022, I fitted logistic regression models with year (i.e., survey) and elevation, and their interaction, as predictor variables. Further, I included a quadratic term for elevation and its interaction with year, as elevation showed a non-linear relationship to the mound occurrences. The presence data of each survey and the randomly generated pseudo-absences were used as binary response variables. To account for the high number of zeros, I weighted presences and pseudo-absences equally in the models, which is recommended for good model performance (Barbet-Massin et al., 2012). I standardised the predictors to a mean of zero and a standard deviation of one to allow for a direct comparison of the coefficients and their relative effect size. To account for uncertainty in the model coefficients due to a lack of ‘true’ absences, I applied a bootstrapping procedure and fitted 1000 models on resampled data (Brun et al., 2020).

In the bootstrapping, the same set of pseudo-absences (n = 1000) was applied for both time points (1982 and 2022). To preserve the original ratio of presence points, the presence and pseudo-absence data of both years were resampled separately. Accordingly, I resampled with replacement from 100% of the four original datasets (the presence data and the pseudo-

absences of both time points). Subsequently, the four sample datasets were merged to one set of sample data, which served as input data for the logistic regression model. The bootstrapping procedure was repeated 1000 times. Finally, the 95% confidence intervals and the medians of the coefficients of all bootstrap models (n = 1000) were visualised to illustrate the significance of the relationships between the predictors and the mound occurrence probabilities (Brun et al., 2020).

Current mound distributions and volumes in relation to environmental variables

To analyse the effects of environmental variables on the recent mound distribution, I fitted logistic regression models for each species group separately. I used the variables elevation, northness and sky view fraction and, as elevation and sky view fraction showed non-linear relationships to the mound occurrences, also the quadratic terms of those, as predictors. For each species group, their presence data of the recent survey and the randomly generated pseudo-absences (n = 1000) were used as binary response variables. I standardised the predictors to a mean of zero and a standard deviation of one to allow for a direct comparison of the coefficients and their relative effect size. Finally, as described in the section about the analysis of changes in mound distributions over time (see above), I weighted the presences and pseudo-absences equally in the models and applied the same bootstrapping procedure to account for uncertainty in the model coefficients. For visualisations of the recent mound occurrence probabilities in relation to environmental variables, I additionally fitted a logistic regression model on the original dataset (without resampling) and illustrated the predicted values.

To estimate the mound volumes, I calculated a cone volume by using the mean diameter and the height of the mounds ($1/3*(diameter/2)^2 * \pi * height$). I fitted linear regression models to analyse the relationships between mound volume and the environmental variables elevation, northness, and sky view fraction. The mound volume was log transformed to increase variance homogeneity and normality. As in the GLMs, I standardised the predictors to a mean of zero and a standard deviation of one to allow for a direct comparison of the coefficients and their relative effect size.

Results

Mound distribution and density changes along the elevational gradient after 40 years

The total number of recorded mounds was 67 in 1982 and 236 in 2022. That is, the density of mounds per 100 m contour line increased from a mean density of 0.16 mounds/100 m in 1982 to a mean density of 0.51 mounds/100 m in 2022 (Figure 2). However, against my expectation, the increase in mound occurrence probability was independent from elevation (no year*elevation interaction, Figure 3).

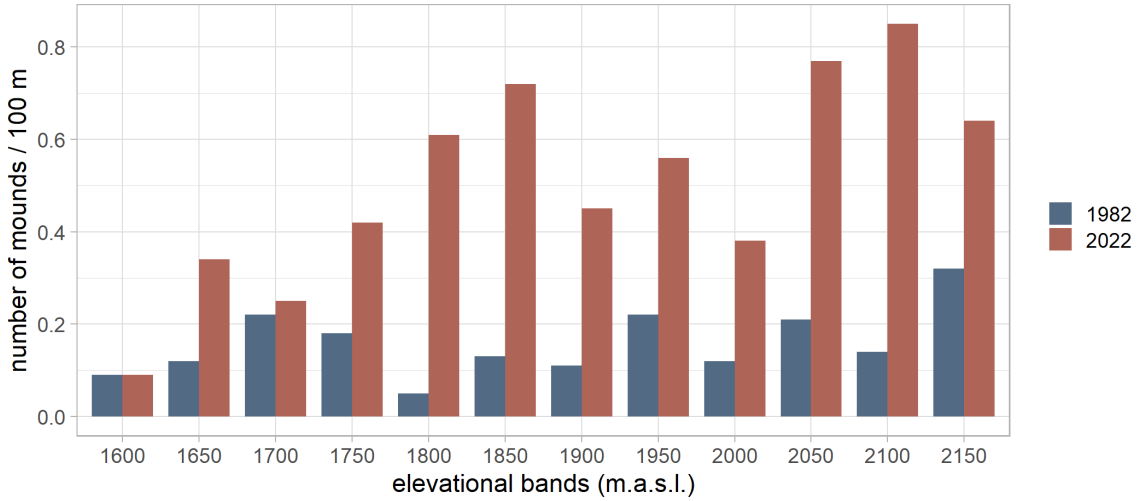

Figure 2: Mound density per 100 m contour line in 1982 and 2022. Mound records of each contour line were summarised for each elevational band. Note that the densities at 2150 m must be viewed with caution, as this elevational band was less than 500 m long and therefore underrepresented in the overall dataset (Figure A1 in Appendix). The illustrated data include all *Formica* species occurring in the study area.

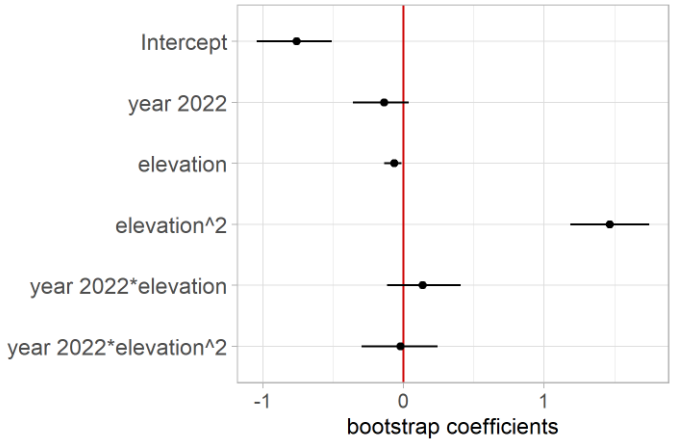

Figure 3: Medians of coefficients derived from logistic regression models (n = 1000) fitted on resampled data to test for a difference in the relationship of mound occurrence probability and elevation between the survey years. Error bars represent 95% confidence intervals. Effects are considered as significant if the confidence interval shows no overlap with zero (red line). Predictor variables are z-transformed. The analysis included all *Formica* species occurring in the study area.

Current mound distribution in relation to environmental variables

In 2022, 41 mounds (17%) were identified as *Formica exsecta* (NYLANDER 1846) mounds. The remaining 195 mounds (83%) were identified as either *Formica lugubris* (ZETTERSTEDT 1838) or *Formica paralugubris* (SEIFERT 1996) mounds. As an unambiguous differentiation between the two potential red wood ant species was infeasible in the barcoding procedure, I use the term "*Formica para-/lugubris*" in the following.

F. para-/lugubris occurred at all elevational bands but showed an unequal pattern along the elevational gradient (Figure 4) with the highest probability of occurrences at elevations between 1800 and 1900 m asl (significant elevation^2 effect, Figure 5a, 6a). In contrast, almost all *F. exsecta* mounds were found above 1975 m asl (Figure 4), and hence their probability of occurrence was positively affected by elevation (Figure 5b, Figure 6b). Unlike for *F. para-/lugubris*, the negative relationship between mound occurrences and northness was significant for *F. exsecta* (Figure 5a, b, Figure 6c, d).

Sky view fraction was significantly related to the occurrence of both species groups (Figure 5a, b). *F. para-/lugubris* mound occurrence probability showed a unimodal response with highest probabilities of occurrence at sky view fractions around 0.6 (i.e., 40% canopy closure, Figure 6e). *F. exsecta* showed a preference for open habitats and a high probability of absence of the species at sky view fractions below 0.4 (i.e., above 60% canopy closure, Figure 6f).

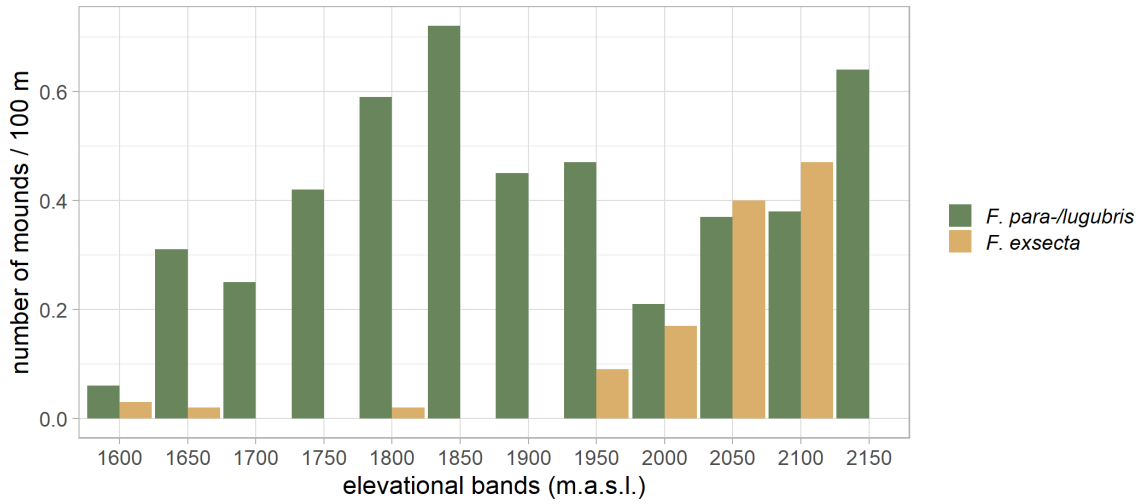

Figure 4: Mound density per 100 m contour line transect of each species group in 2022. Mound records of each contour line were summarised for each elevational band. Note that the densities at 2150 m must be viewed with caution, as this elevational band was less than 500 m long and therefore underrepresented in the overall dataset (Figure A1 in Appendix).

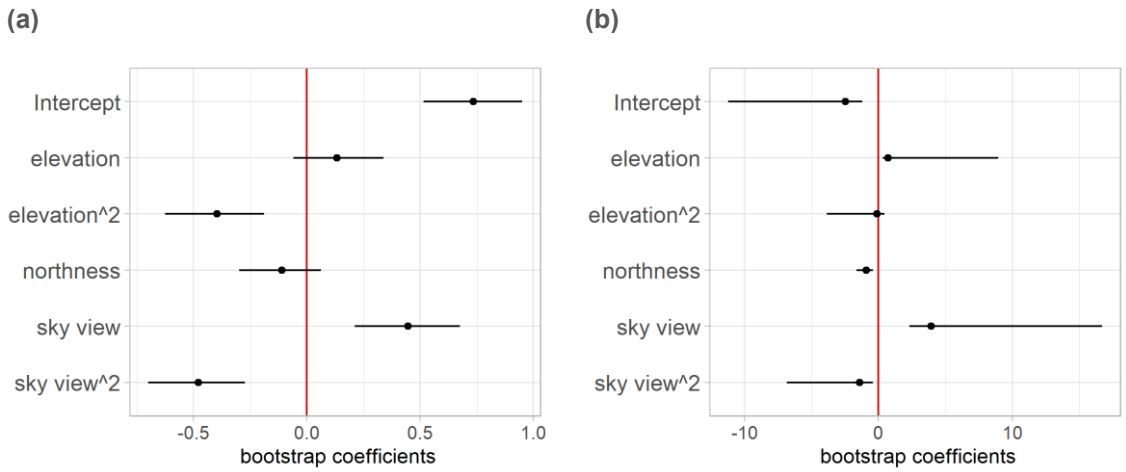

Figure 5: Medians of coefficients derived from logistic regression models (n = 1000) fitted on resampled data to test for relationships between environmental variables and the mound occurrence probability of *Formica para-/lugubris* **(a)** and *Formica exsecta* **(b)**. Error bars represent 95% confidence intervals. Effects are considered as significant if the confidence interval shows no overlap with zero (red line). Predictor variables were z-transformed.

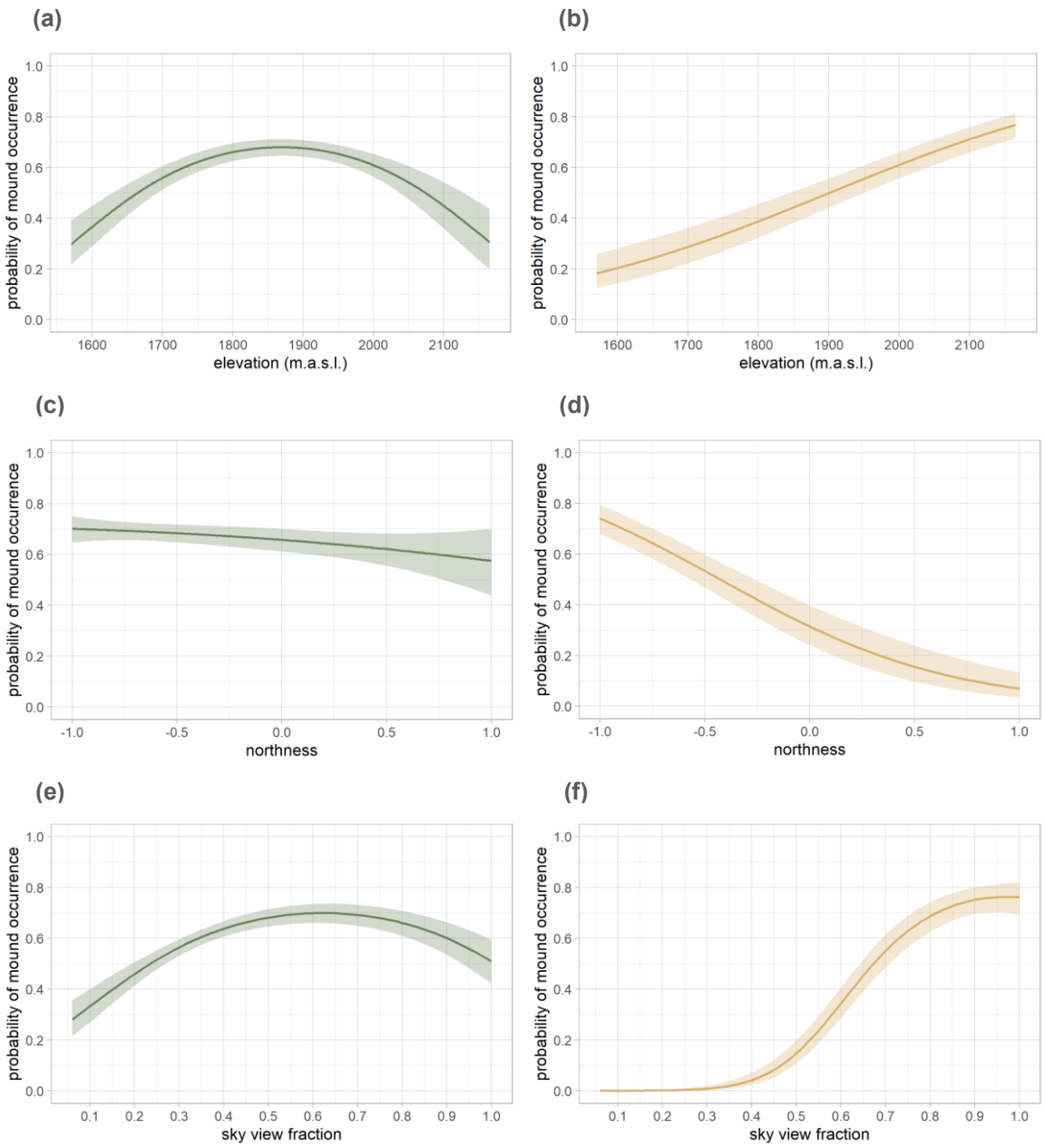

Figure 6: Predicted probability of mound occurrence in relation to environmental variables for *Formica para-/lugubris* **(a, c, e)** and *Formica exsecta* **(b, d, f)**. The northness index (c, d) ranges from 1 (= north) to -1 (= south). Lines are predicted values derived from a logistic regression model of the original data set without resampling, shaded areas represent 95% confidence intervals.

Mound volume in relation to environmental variables

The mound volumes of *F. para-/lugubris* ranged from 0.001 up to 5.4 m^3. However, large mound volumes (> 0.5 m^3) were rare, resulting in a median of 0.127 m^3 and a mean of 0.265 m^3 (Figure 7a). The mound volumes of *F. exsecta* ranged between 0.002 and 0.106 m^3, with a median of 0.020 m^3 and a mean of 0.026 m^3 (Figure 7b). For both species, mound volume increased significantly with increasing elevation (Table 1, Table 2; Figure 8a, b). Moreover, mound volume significantly decreased with an increase in sky view fraction (Figure 8c, d). No significant relationship was found between mound volume and northness (Table 1, 2).

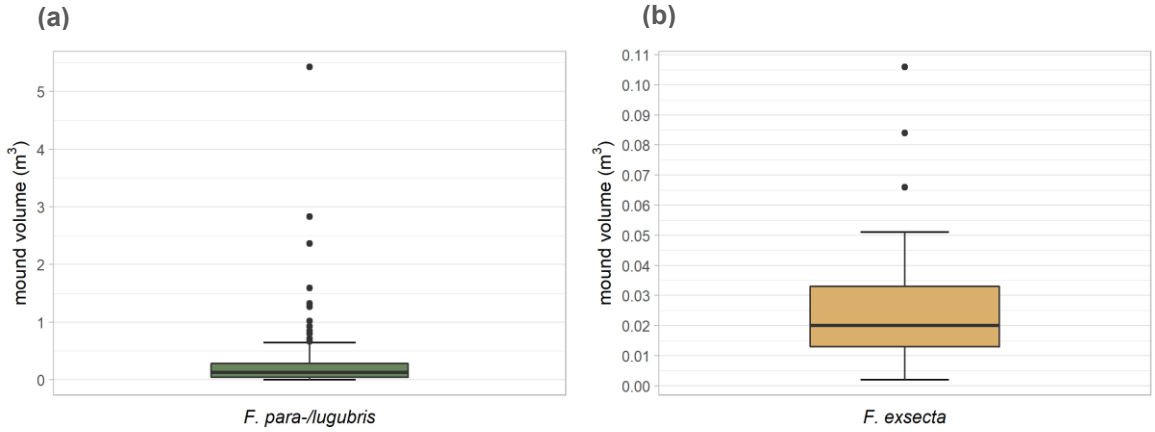

Figure 7: Mound volumes of *Formica para-/lugubris* **(a)** and *Formica exsecta* **(b)**. Note different y-axis ranges.

Table 1: Effects of environmental variables on the mound volume (m^3) of *Formica para-/lugubris* (r^2 = 0.11, adjusted r^2 = 0.09) using a linear regression model. Predictor variables are z-transformed. Significant values are highlighted in grey.

	estimate	std. error	t value	p value
(Intercept)	-2.270	0.103	-22.006	marginal
elevation	0.307	0.135	2.273	0.024
northness	0.062	0.104	0.599	0.550
sky view	-0.623	0.135	-4.605	< 0.0001

Table 2: Effects of environmental variables on the mound volume (m^3) *Formica exsecta* (r^2 = 0.24, adjusted r^2 = 0.18) using a linear regression model. Predictor variables are z-transformed. Significant values are highlighted in grey.

	estimate	std. error	t value	p value
(Intercept)	-3.967	0.121	-32.772	marginal
elevation	0.328	0.126	2.599	0.013
northness	0.149	0.124	1.197	0.239
sky view	-0.295	0.126	-2.336	0.025

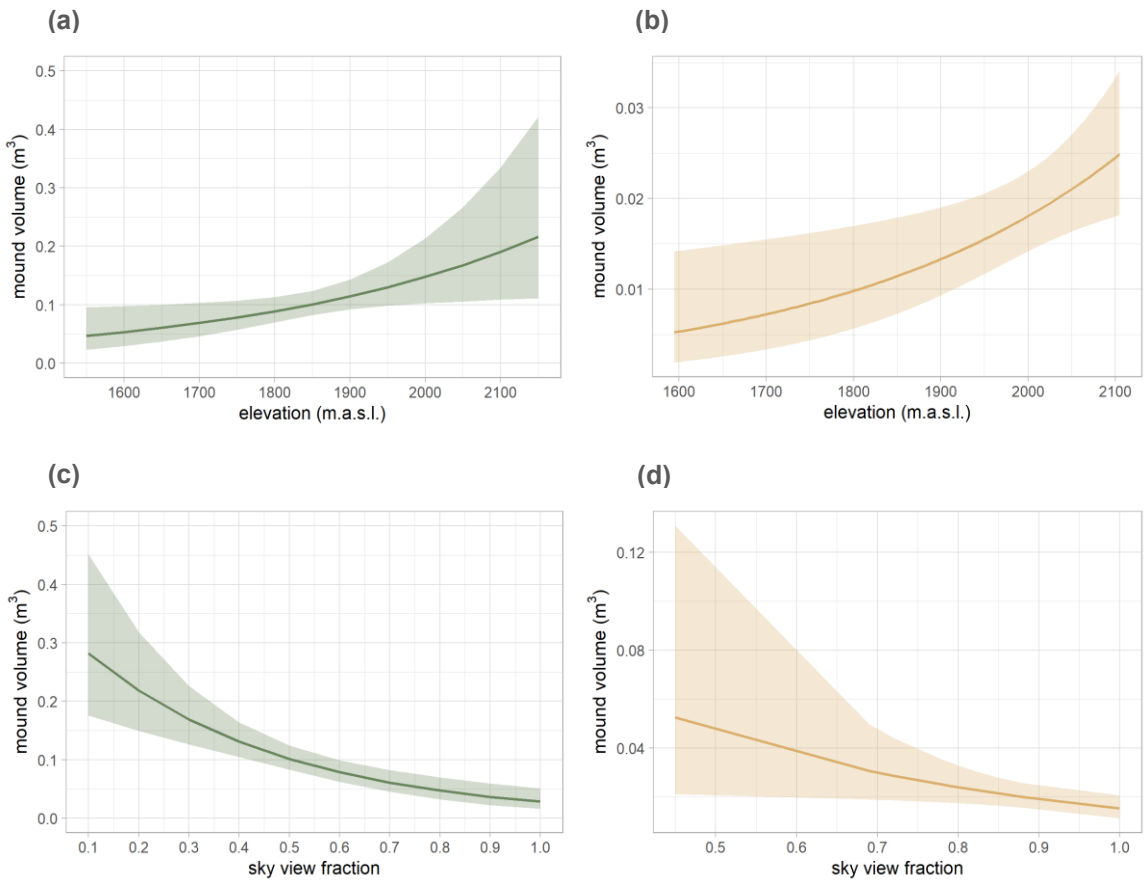

Figure 8: Relationships between mound volume and the environmental variables elevation and sky view fraction for *Formica para-/lugubris* **(a, c)** and *Formica exsecta* **(b, d).** Note different y-axis ranges. Lines are predicted values from the linear models, shaded areas represent 95% confidence intervals.

Discussion

Mound distribution and density changes along the elevational gradient after 40 years

The main aim in this thesis was to assess potential effects of a warming climate on the *Formica* mound density distribution in a subalpine environment in the Swiss Alps. I found a 3.5-fold increase in mound density within a 40-years period of climate warming. However, against my expectations, I could not link mound density changes in the Dischma Valley to elevation.

Comparable studies about recent elevational or latitudinal range-shifts of *Formica* species are lacking. However, investigations of other ant distribution changes in the Appalachian Mountains showed an upward movement of the lowland species *Aphaenogaster rudis* to higher elevations, where it replaced the more cold-adapted *Aphaenogaster picea* in the period between 1974 and 2012 (Warren & Chick 2013). The upward movement was related to the

rise of the minimum temperatures in that area and highlights the role of thermal tolerance in species' migration capabilities and competition strength in a warming climate (Warren & Chick, 2013). Similarly, in another study along an elevational gradient in the Rocky Mountains, ant communities shifted upwards with current higher elevation communities resembling lower elevation communities of the past (Menke et al., 2014). Nonetheless, the magnitude of change in community structure was uneven along the elevational gradient, with species turnover over time being most pronounced at mid-elevation sites (Menke et al., 2014).

One reason for the lack of evidence of an elevation dependent mound density increase in my study could be the limited survey area. The inclusion of the lower and upper range limits of the study species is known to strengthen inferences about elevational shifts (McCain & Garfinkel, 2021). However, the survey area in the Dischma Valley only covered elevations from 1600 to 2150 m asl, whereas the upper elevational range limits of the species identified in this study were previously reported to be at 2400 m asl for *F. lugubris*, at 2300 m asl for *F. paralugubris* and at 2250 m asl for *F. exsecta* (Seifert, 2007) Thus, the colonisation of higher altitudes of the Dischma Valley remains unknown and potential elevational shifts may not have been detected.

Despite being elevation independent, the 3.5-fold increase in mound density across time is remarkable and suggests that the ongoing temperature change might be either advantageous, or easy for the ants to compensate. This is in accordance with the predicted beneficial effects of a warmer climate on ant species in temperate regions (Kaspari et al., 2019; Parr & Bishop, 2022). It is likely that many ant species, such as the mound-building *Formica* ants, can compensate rising temperatures by relocation of their nests, by adapting their brood movement cycles or even their foraging activities (Domisch et al., 2009; Parr & Bishop, 2022).

Like in most other European countries, information about general population developments of *Formica species* is currently lacking in Switzerland, as the first systematic countrywide red wood ant survey was conducted only a few years back (Vandegehuchte et al., 2017). Other positive population trends were documented for some lowland wood ant species in parts of Germany (Steinhoff & Roch, 2008). Conversely, a resurvey of wood ant occurrences was conducted in a large area in Belgium in 2008, in which the modern records were compared to a similar mapping at the beginning of the 1990s. Interestingly, they reported a drastic halving in colony numbers within two decades. The most probable reason proposed for the negative trend was a loss in habitat quality, for instance by changes in the forest structures and intensification of agriculture (Dekoninck et al., 2010).

Although no species-specific information is available for the historical records in the Dischma Valley, the species identification in 2022 allows for some assumptions about species group-specific density changes. I attribute the increased mound density below 2050 m asl to F. para-/lugubris, as *F. exsecta* was almost absent in those areas in the recent survey. Whether both species multiplied their mound numbers at the tree line ecotone remains unknown in this study,

as they coexist in this zone of the study area. However, although red wood ants are known to be strong competitors even outside the closed forest at the tree line ecotone (Guariento & Fiedler, 2021), I still assume an increased mound density in both species. In the period between 1997 and 2021, a remarkable increase in *F. exsecta* mound numbers was observed on the Alp Stabelchold in the Swiss National Park (Morger, 2022). It was found that the mound number almost doubled within ten years at the beginning of this century, and, thus, similar trends might be possible in other close alpine regions.

Potential reasons for the increase in mound number

In the Alps, *F. paralugubris and F. exsecta* are mainly described as polygyn - polydomous (Seifert, 2007). *F. lugubris* can be polygynous and polydomous, however, can also be monogynous and monodomous if needed. Being polygynous and polydomous means that nest foundation can by achieved by nest budding (dispersal over short distances) and is often related to a high density of nests (Bernasconi et al., 2005). Budding could potentially explain the large increase in ant mounds in my resurvey of the Dischma valley (Figure A2 in Appendix).

Human land use change is one possible indirect driver of wood ant population dynamics (Sorvari, 2016). In the Alps, forest management decreased by the end of the 20th century, which resulted in a development to generally denser forests. Furthermore, land use change has led to forest extension along steep mountain slopes across the Alps during the last century (Bebi et al., 2017; Brändli, 2000) and tree line upward-shifts are reported in the Swiss Alps (Gehrig-Fasel et al., 2007). In the Dischma Valley, tree line advances were detected between 1972 and 2012 (Erdle, 2013) in addition to a forest extension at the lower elevations (Bebi et al., 2017). Hence, the transition from alpine livestock grazing to secondary forests at higher elevations might have increased the area of suitable habitats for wood ants within a few decades. Therefore, the mound density increase found in this study could be related to land abandonment and reforestation. Nonetheless, these land use change effects are just as relevant for the habitat availability of *F. exsecta* and would be insufficient to explain a potential positive trend in its mound abundance.

Another reason for the increase in mound densities might be a larger availability of resources for ants. Honeydew derived from aphid tending is an important food source for many *Formica* species (Stadler & Dixon, 2005; Wellenstein, 1952). Positive effects of climate change on ant – plant sucker mutualisms have been shown in several studies. For instance, experimentally elevated CO_2 concentrations positively affected honeydew production and ant tending frequency on aphids (Kremer et al., 2018). Another experimental study revealed increased mealybug colony growth, honeydew production rates and ant tending activities at warmer temperatures (Zhou et al., 2017). However, in a review about climate change impacts on

aphids and their mutualistic ants, Blanchard et al. (2019) emphasised the strong dependence on the included species and selected environmental conditions in the study. Noteworthy, positive relationships, particularly with temperature, were non-linear and turned into negative when optimum temperatures were exceeded. Thus, in subalpine areas of the Alps, positive effects of warmer temperatures on honeydew production might potentially have led to higher resource availability for wood ants and explain the increase in mound numbers during the last 40 years.

Formica species additionally depend on protein rich food, supplied by insect prey, to raise their brood (Domisch et al., 2016; Gösswald, 2012; Wellenstein, 1952). Hence, a change in insect biomass could also affect ant abundance. However, it is often challenging to disentangle climatic effects from other factors causing population fluctuations of forest insects. Consequently, responses of forest insects to global warming are difficult to interpret and vary largely between regions and species (Rouault et al., 2006). For example, for the grey larch budmoth (*Zeiraphera griseana*) in Switzerland, current fluctuations in population densities and changes in development cycles could not be linked to climate change (Büntgen et al., 2020; Esper et al., 2007; Myers & Cory, 2013). Whether changes in population dynamics of the insect community in my study area have beneficially changed for the opportunistic *Formica* ants, is currently unknown.

In addition, wood ant populations were critically threatened in Central Europe in the 20th century (Gösswald, 1951), as humans made use of their pupae, the formic acid and nest material (including the resin, Gösswald, 1951). Therefore, as the first insect group, wood ants received a countrywide protection status in Switzerland in 1966 (NHV, 1991; Wermelinger et al., 2018). Also, *F. exsecta* is considered as “critically endangered” in the Red lists of Switzerland (Bundesamt für Umwelt, Wald und Landschaft, 1994). A population recovery of the two species groups due to a restriction of human exploitation and damage is hence another possible explanation for the increase in mound numbers between the historical and the recent survey. However, evidence of an intensive usage of ants or nest material is lacking for the Dischma Valley. Therefore, and due to a variety of potential mound density drivers, the ant’s conservation only remains one out of several possible reasons for an increase in *Formica* mound density.

Finally, methodical issues might have contributed to a higher number of sighted *Formica* mounds in the recent survey. First, the historical survey might have overlooked some ant mounds, as the focus was on ungulate and grouse evidence. Second, due to the sampling of ant individuals from each nest and the measurements of mound size in 2022, I had to move off the transect line to approach each mound. It is known that this can potentially lead to additional `secondary` detections, as further mounds in the vicinity of the original mound are likely to be found (Buckland et al., 2001). Nevertheless, this would not explain why increases

in mound numbers are absent in some elevational bands (e.g., at the lowest bands). Moreover, the large difference in mound density between the two surveys is clearly indicative of an increased mound abundance in 2022, independent from differences in survey intensity.

Current mound distributions and relations to environmental variables

F. para-/lugubris occurrence probability peaked at mid-elevation in 2022. Although, in the Alps, *F. paralugubris* and *F. lugubris* mainly occur above 1500 m asl (Risch et al., 2016), I assume this relationship to mainly be induced by unknown variables correlated with elevation, rather than by elevation itself, particularly in terms of ambient temperatures. The *F. para-/lugubris* mound occurrences were not related to northness in my study, although clear preferences in slope aspect have been found for most forest wood ant species in a previous study in Switzerland (Vandegehuchte et al., 2017). This discrepancy could be related to the small variation of aspects in my study area, which was mainly restricted to south-west to west facing slopes. *F. para-/lugubris* occurrence probability also depended on canopy closure and peaked at moderate to open canopies in my study, which matches the findings of former studies (Chen & Robinson, 2014; Vandegehuchte et al., 2017).

The occurrence of *F. exsecta* was highly related to an open canopy. This is in line with previous knowledge on the species' dependency on open to low shaded habitats (Seifert, 2000). In contrast to red wood ant species, *F. exsecta* is incapable of regulating the nest temperature by metabolic heat production, and, hence, this species depends on direct insolation at the nest sites (Seifert, 2000). Consequently, the distribution of this species in Switzerland and Austria, which ranges between 300 and 2250 m asl, is bimodal (Seifert, 2000). In my study, open habitats were mainly located at the upper edge of the area and to a small proportion at the lower edge. Indeed, this pattern is reflected in the *F. exsecta* mound densities along the elevational gradient (Figure A3 in Appendix) and might have contributed to a positive relationship between mound occurrences and elevation. Mound densities were in general low at the lowest elevational band (1600 m). At this elevation open habitats are intensely managed agricultural grasslands with high disturbance by mowing, which could have kept the densities of *F. exsecta* low. In contrast to *F. para-/lugubris*, a negative relationship between *F. exsecta* mound occurrences and northness was detectable. Besides the high relevance of solar insolation for the species, this could also be due to the large forest cover at west-facing slopes in the study area (Figure A3 in Appendix). Generally, in my study, conclusions about the species' general habitat preferences regarding elevation and slope aspect are limited, as the species occupy a broader variety of those environmental conditions in the Alps and beyond (Seifert, 2007). However, my results highlight the importance of canopy closure for the occurrence of these species groups.

Mound volumes and relations to environmental variables

The significant increase in mound volume with an increase in canopy closure for both species groups (i.e., decrease in sky view fraction) is in line with previous findings particularly for wood ants (Chen & Robinson, 2014; Vandegehuchte et al., 2017). As the variance in mound volumes, as well as the range in occupied sky view fractions was small for *F. exsecta*, the positive relationship once again emphasises the importance of solar insolation for the mound temperatures (Seifert, 2000). Likewise, mound volume also increased with elevation for both species groups, which highlights the relevance of ambient temperature conditions as drivers of mound size, even for forest species such as *F. para/-lugubris*, which are able to choose their nest sites within a broad variability of microclimatic conditions. Interestingly, in a similar study in the Yellowstone National Park, no relationship was found between the size of *Formica* mounds and elevation or any other potentially relevant environmental variable (Risch et al., 2008). Nonetheless, my findings clearly indicate that the *Formica* ants can flexibly adjust their mound size under a range of environmental conditions.

Conclusions and limitations

In cold environments, such as subalpine areas in the Alps, the ongoing climate warming could indirectly benefit *Formica* species in the medium term. The *Formica* mound density increased in the Dischma Valley within the last four decades of environmental change, but I could not relate this change to elevation. However, I detected a positive relationship between elevation and mound volumes for both species groups in the recent survey. Whilst this likely underlines the direct influence of ambient temperatures on the ants' nest properties, it may also highlight their adaptation capabilities to spatial temperature changes. Moreover, the limited evidence of a relationship between elevation and mound distribution within the investigated elevational range demonstrates their flexibility in nest site choice along microclimatic gradients within their habitat. Accordingly, I reasonably assume that these flexibilities in terms of mound size and nest site choice are just as beneficial for an adaptation to temporal temperature changes and might contribute to a successful adaptation of the ants to ongoing climate warming.

The factors discussed above are just a small selection of multiple possible drivers of the observed mound density change in this study. However, considering the complexity of ecological systems and the lack of knowledge about parallel changes in the study area, these points of discussion remain speculative. Most likely, the current findings are a result of multiple factors and interactions.

Overall, one should note that the data represent two snapshots of mound occurrences with a gap of knowledge about patterns in the years between 1982 and 2022. Thus, conclusions about the actual abundance trends and potential fluctuations, related or unrelated to environmental change, are limited. Moreover, the small study area additionally restricts the validity of potential inferences based on this study. As the drivers of mound density increase are unknown and even local changes in the study area can play a major role, general conclusions about a conservational success or universal positive population trends of the investigated species in times of environmental change have to be treated with caution. Thus, the remaining complexities in the distributional response and increased mound density calls for much further research on the fascinating mound-building *Formica* ants.

References

Adlung, K. G. (1966). A critical evaluation of the European research on use of red wood ants (*Formica rufa* group) for the protection of forests against harmful insects. *Zeitschrift für angewandte Entomologie*, *57*(1-4), 167–189.

Balzani, P., Dekoninck, W., Feldhaar, H., Freitag, A., Frizzi, F., Frouz, J., Masoni, A., Robinson, E., Sorvari, J., & Santini, G. (2022). Challenges and a call to action for protecting European red wood ants. *Conservation Biology*, *36*(6). https://doi.org/10.1111/cobi.13959

Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: How, where and how many? *Methods in Ecology and Evolution*, *3*(2), 327–338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Bebi, P., Seidl, R., Motta, R., Fuhr, M., Firm, D., Krumm, F., Conedera, M., Ginzler, C., Wohlgemuth, T., & Kulakowski, D. (2017). Changes of forest cover and disturbance regimes in the mountain forests of the Alps. *Forest Ecology and Management*, *388*, 43–56. https://doi.org/10.1016/j.foreco.2016.10.028

Bernasconi, C., Maeder, A., Cherix, D., & Pamilo, P. (2005). Diversity and genetic structure of the wood ant *Formica lugubris* in unmanaged forests. *Annales Zoologici Fennici*, *42*(3), 189–199. JSTOR.

Bernasconi, C., Pamilo, P., & Cherix, D. (2010). Molecular markers allow sibling species identification in red wood ants (*Formica rufa* group). *Systematic Entomology*, *35*(2), 243–249. https://doi.org/10.1111/j.1365-3113.2009.00503.x

Billick, I., Hammer, S., Reithel, J. S., & Abbot, P. (2007). Ant–aphid interactions: Are ants friends, enemies, or both? *Annals of the Entomological Society of America*, *100*(6), 887–892.

Blanchard, S., Lognay, G., Verheggen, F., & Detrain, C. (2019). Today and tomorrow: Impact of climate change on aphid biology and potential consequences on their mutualism with ants: Aphid biology under climate change. *Physiological Entomology*, *44*(2), 77–86. https://doi.org/10.1111/phen.12275

Brändli, U.-B. (2000). Waldzunahme in der Schweiz - gestern und morgen. *Forschungsanstalt WSL*.

Brun, P., Psomas, A., Ginzler, C., Thuiller, W., Zappa, M., & Zimmermann, N. E. (2020). Large-scale early-wilting response of Central European forests to the 2018 extreme drought. *Global Change Biology*, *26*(12), 7021–7035. https://doi.org/10.1111/gcb.15360

Buckland, T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., & Thomas, L. (2001). *Introduction to distance sampling: Estimating abundance of biological populations*. Oxford University Press.

Bundesamt für Umwelt, Wald und Landschaft. (1994). Rote Liste der gefährdeten Tierarten der Schweiz.

Büntgen, U., Liebhold, A., Nievergelt, D., Wermelinger, B., Roques, A., Reinig, F., Krusic, P. J., Piermattei, A., Egli, S., Cherubini, P., & Esper, J. (2020). Return of the moth: Rethinking the effect of climate on insect outbreaks. *Oecologia*, *192*(2), 543–552. https://doi.org/10.1007/s00442-019-04585-9

Burland, T. G. (1999). DNASTAR's Lasergene Sequence Analysis Software. In S. Misener & S. A. Krawetz, *Bioinformatics Methods and Protocols* (Bd. 132, S. 71–91). Humana Press. https://doi.org/10.1385/1-59259-192-2:71

Çakır, M. (2019). The negative effect of wood ants (*Formica rufa*) on microarthropod density and soil biological quality in a semi-arid pine forest. *Pedobiologia*, *77*, 150593. https://doi.org/10.1016/j.pedobi.2019.150593

Chen, Y.-H., & Robinson, E. J. H. (2013). A comparison of mark–release–recapture methods for estimating colony size in the wood ant *Formica lugubris*. *Insectes Sociaux*, *60*(3), 351–359. https://doi.org/10.1007/s00040-013-0300-z

Chen, Y.-H., & Robinson, E. J. H. (2014). The Relationship between Canopy Cover and Colony Size of the Wood Ant *Formica lugubris*—Implications for the Thermal Effects on a Keystone Ant Species. *PLoS ONE*, *9*(12), e116113. https://doi.org/10.1371/journal.pone.0116113

Dekoninck, W., Hendrickx, F., Grootaert, P., & Maelfait, J.-P. (2010). Present conservation status of red wood ants in north-western Belgium: Worse than previously, but not a lost cause. *European Journal of Entomology*, *107*(2).

Diamond, S. E., Nichols, L. M., Pelini, S. L., Penick, C. A., Barber, G. W., Cahan, S. H., Dunn, R. R., Ellison, A. M., Sanders, N. J., & Gotelli, N. J. (2016). Climatic warming destabilizes forest ant communities. *Science Advances*, *2*(10), e1600842. https://doi.org/10.1126/sciadv.1600842

Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Toro, I. D., Hirsch, C., Oberg, E., & Dunn, R. R. (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology*, *18*(2), 448–456. https://doi.org/10.1111/j.1365-2486.2011.02542.x

Domisch, T., Finér, L., Neuvonen, S., Niemelä, P., Risch, A. C., Kilpeläinen, J., Ohashi, M., & Jurgensen, M. F. (2009). Foraging activity and dietary spectrum of wood ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. *Ecological Entomology*, *34*(3), 369–377. https://doi.org/10.1111/j.1365-2311.2009.01086.x

Domisch, T., Risch, A. C., & Robinson, E. J. (2016). Wood ant foraging and mutualism with aphids. *Wood ant ecology and conservation*, 145.

Donoghue, M. J. (2008). A phylogenetic perspective on the distribution of plant diversity. *Proceedings of the National Academy of Sciences*, *105*(supplement_1), 11549–11555. https://doi.org/10.1073/pnas.0801962105

Ellison, A. M. (2012). Out of Oz: Opportunities and challenges for using ants (Hymenoptera: Formicidae) as biological indicators in north-temperate cold biomes. *Myrmecological News*.

Engler, R., Randin, C. F., Thuiller, W., Dullinger, S., Zimmermann, N. E., Araújo, M. B., Pearman, P. B., Le Lay, G., Piedallu, C., Albert, C. H., Choler, P., Coldea, G., De LAMO, X., Dirnböck, T., Gégout, J.-C., Gómez-García, D., Grytnes, J.-A., Heegaard, E., Høistad, F., … Guisan, A. (2011). 21st century climate change threatens mountain flora unequally across Europe: Climate change impacts on mountain florae. *Global Change Biology*, *17*(7), 2330–2341. https://doi.org/10.1111/j.1365-2486.2010.02393.x

Erdle, L. (2013). 40 Years of Alpine Treeline Shift in the Dischma Valley, Switzerland. *Master's thesis, University of Eastern Finland*.

Erős, K., Markó, B., Gál, C., Czekes, Z., & Csata, E. (2009). Sharing versus monopolizing: Distribution of aphid sources among nests within a *Formica exsecta* Nylander (Hymenoptera: Formicidae) supercolony. *Israel Journal Entomology*, *39*, 105–127.

Esper, J., Büntgen, U., Frank, D. C., Nievergelt, D., & Liebhold, A. (2007). 1200 years of regular outbreaks in alpine insects. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1610), 671–679. https://doi.org/10.1098/rspb.2006.0191

FOEN, et al. (2020). Management summary: Climate change in Switzerland. Indicators of driving forces, impact and response. *State of the environment, no. 2013*, 11.

Fowler, S. V., & Macgarvin, M. (1985). The Impact of Hairy Wood Ants, *Formica lugubris*, on the Guild Structure of Herbivorous Insects on Birch, Betula pubescens. *Journal of Animal Ecology*, *54*(3), 847–855. JSTOR. https://doi.org/10.2307/4382

Frouz, J., & Finer, L. (2007). Diurnal and seasonal fluctuations in wood ant (*Formica polyctena*) nest temperature in two geographically distant populations along a south – north gradient. *Insectes Sociaux*, *54*(3), 251–259. https://doi.org/10.1007/s00040-007-0939-4

Gehrig-Fasel, J., Guisan, A., & Zimmermann, N. E. (2007). Tree line shifts in the Swiss Alps: Climate change or land abandonment? *Journal of Vegetation Science*, *18*(4), 571–582. https://doi.org/10.1111/j.1654-1103.2007.tb02571.x

Gösswald, K. (1941). Rassenstudien an der roten Waldameise *Formica rufa* L. auf systematischer, ökologischer, physiologischer und biologischer Grundlage. *Zeitschrift für angewandte Entomologie*, *28*(1), 62–124.

Gösswald, K. (1951). *Die rote Waldameise im Dienste der Waldhygiene: Forstwirtschaftliche Bedeutung, Nutzung, Lebensweise, Zucht, Vermehrung und Schutz*. Metta Kinau.

Gösswald, K. (2012). *Die Waldameise: Biologie, Ökologie und forstliche Nutzung* (gekürzte Sonderausg. in einem Band). Aula-Verlag.

Guariento, E., & Fiedler, K. (2021). Ant Diversity and Community Composition in Alpine Tree Line Ecotones. *Insects*, *12*(3), 219. https://doi.org/10.3390/insects12030219

Günter, T. (1986). Das Testgebiet Davos. *Eidg. Anstalt für das forstliche Versuchswesen, Ber. 289*, 23.

Hawes, C., Stewart, A., & Evans, H. (2002). The impact of wood ants (*Formica rufa*) on the distribution and abundance of ground beetles (Coleoptera: Carabidae) in a Scots pine plantation. *Oecologia*, *131*(4), 612–619. https://doi.org/10.1007/s00442-002-0916-6

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, *101*(41), 14812–14817. https://doi.org/10.1073/pnas.0406166101

Hefti, R. (1986). Zustand und Gefährdung des Waldes. *Eidg. Anstalt für das forstliche Versuchswesen, Ber. 289*, 175–190.

Horstmann, K., & Schmid, H. (1986). Temperature Regulation in Nests of the Wood Ant, *Formica polyctena* (Hymenoptera: Formicidae). *Entomologia Generalis*, *11*(3–4), 229–236. https://doi.org/10.1127/entom.gen/11/1986/229

Intergovernmental Panel on Climate Change. Working Group 1. (2007). *Summary for policymakers*. Cambridge University Press.

Jonas, T., Webster, C., Mazzotti, G., & Malle, J. (2020). HPEval: A canopy shortwave radiation transmission model using high-resolution hemispherical images. *Agricultural and Forest Meteorology*, *284*, 107903. https://doi.org/10.1016/j.agrformet.2020.107903

Jurgensen, M. F., Finér, L., Domisch, T., Kilpeläinen, J., Punttila, P., Ohashi, M., Niemelä, P., Sundström, L., Neuvonen, S., & Risch, A. C. (2008). Organic mound-building ants: Their impact on soil properties in temperate and boreal forests. *Journal of Applied Entomology*, *132*(4), 266–275. https://doi.org/10.1111/j.1439-0418.2008.01280.x

Kadochová, Š., & Frouz, J. (2014). Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (*Formica rufa* group). *F1000Research*, *2*, 280. https://doi.org/10.12688/f1000research.2-280.v2

Karhu, K. J. (1998). Effects of ant exclusion during outbreaks of a defoliator and a sap-sucker on birch. *Ecological Entomology*, 23(2), 185–194. https://doi.org/10.1046/j.1365-2311.1998.00116.x

Kaspari, M., Bujan, J., Roeder, K. A., Beurs, K., & Weiser, M. D. (2019). Species energy and Thermal Performance Theory predict 20-yr changes in ant community abundance and richness. *Ecology*, *100*(12). https://doi.org/10.1002/ecy.2888

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*(4), 1160–1166. https://doi.org/10.1093/bib/bbx108

Körner, C. (2007). The use of ‘altitude’ in ecological research. *Trends in Ecology & Evolution*, *22*(11), 569–574. https://doi.org/10.1016/j.tree.2007.09.006

Kremer, J. M. M., Nooten, S. S., Cook, J. M., Ryalls, J. M. W., Barton, C. V. M., & Johnson, S. N. (2018). Elevated atmospheric carbon dioxide concentrations promote ant tending of aphids. *Journal of Animal Ecology*, *87*(5), 1475–1483. https://doi.org/10.1111/1365-2656.12842

Leibold, E. (2012). Räumlich-zeitliche Analyse der alpinen Waldgrenze während der letzten 40 Jahre im Dischmatal (Davos). *Master's thesis, University of Natural Resources and Applied Life Sciences (BOKU), Vienna.*

Lüdecke, D., Aust, F., Crawley, S., & Ben-Shachar, M. (2020). Package 'ggeffects'. *Create tidy data frames of marginal effects for "ggplot" from model outputs*, *23*.

McCain, C. M., & Garfinkel, C. F. (2021). Climate change and elevational range shifts in insects. *Current Opinion in Insect Science*, *47*, 111–118. https://doi.org/10.1016/j.cois.2021.06.003

Menke, S. B., Harte, J., & Dunn, R. R. (2014). Changes in ant community composition caused by 20 years of experimental warming vs. 13 years of natural climate shift. *Ecosphere*, *5*(1), art6. https://doi.org/10.1890/ES13-00234.1

MeteoSwiss. (2022). https://www.meteoswiss.admin.ch/.

Morger, A. (2022). *Formica exsecta* increases heterogeneity in the grassland ecosystem Alp Stabelchod in the Swiss National Park. *Master's thesis, ETH Zürich*.

Müller, J. P., Ackermann, G., & Jenny, H. (1988). *Schlussber. Schweiz. MAB-Programm Nr. 34*. 10.

Myers, J. H., & Cory, J. S. (2013). Population cycles in forest Lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 565–592.

NHV. (1991). *Verordnung über den Natur- und Heimatschutz, Schweiz*.

Ohashi, M., Kilpeläinen, J., Finér, L., Risch, A. C., Domisch, T., Neuvonen, S., & Niemelä, P. (2007). The effect of red wood ant (*Formica rufa* group) mounds on root biomass, density, and nutrient concentrations in boreal managed forests. *Journal of Forest Research*, *12*(2), 113–119. https://doi.org/10.1007/s10310-006-0258-z

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669. JSTOR.

Parr, C. L., & Bishop, T. R. (2022). The response of ants to climate change. *Global Change Biology*, *28*(10), 3188–3205. https://doi.org/10.1111/gcb.16140

Pelini, S. L., Diamond, S. E., MacLean, H., Ellison, A. M., Gotelli, N. J., Sanders, N. J., & Dunn, R. R. (2012). Common garden experiments reveal uncommon responses across temperatures, locations, and species of ants. *Ecology and Evolution*, *2*(12), 3009–3015. https://doi.org/10.1002/ece3.407

Punttila, P., Niemelä, P., & Karhu, K. (2004). The impact of wood ants (Hymenoptera: Formicidae) on the structure of invertebrate community on mountain birch (Betula pubescens ssp. Czerepanovii). *Annales Zoologici Fennici*, *41*(2), 429–446. JSTOR.

R Core Team. (2022). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. http://www.R-project.org/.

Ratnasingham, S., & Hebert, P. D. N. (2007). BARCODING: Bold: The Barcode of Life Data System (http://www.barcodinglife.org): BARCODING. *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rebetez, M., & Reinhard, M. (2008). Monthly air temperature trends in Switzerland 1901–2000 and 1975–2004. *Theoretical and Applied Climatology*, *91*(1–4), 27–34. https://doi.org/10.1007/s00704-007-0296-2

Risch, A. C., Ellis, S., & Wiswell, H. (2016). Where and why? Wood ant population ecology. *Wood ant ecology and conservation*, 81.

Risch, A. C., Jurgensen, M. F., Schütz, M., & Page-Dumroese, D. S. (2005). The contribution of Red wood ants to soil C and N pools and CO_2 emissions in subalpine forests. *Ecology*, *86*(2), 419–430. https://doi.org/10.1890/04-0159

Risch, A. C., Jurgensen, M. F., Storer, A. J., Hyslop, M. D., & Schütz, M. (2008). Abundance and distribution of organic mound-building ants of the *Formica rufa* group in Yellowstone National Park. *Journal of Applied Entomology*, *132*(4), 326–336. https://doi.org/10.1111/j.1439-0418.2007.01243.x

Rouault, G., Candau, J.-N., Lieutier, F., Nageleisen, L.-M., Martin, J.-C., & Warzée, N. (2006). Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. *Annals of Forest Science*, *63*(6), 613–624. https://doi.org/10.1051/forest:2006044

Schütz, M., Kretz, C., Dekoninck, L., Iravani, M., & Risch, A. C. (2008). Impact of *Formica exsecta* Nyl. On seed bank and vegetation patterns in a subalpine grassland ecosystem. *Journal of Applied Entomology*, *132*(4), 295–305. https://doi.org/10.1111/j.1439-0418.2008.01293.x

Seifert, B. (2000). A taxonomic revision of the ant subgenus *Coptoformica* (Müller, 1923) (Hymenoptera, Formicidae)

Seifert, B. (2007). *Die Ameisen Mittel- und Nordeuropas*. Lutra-Verlags-und-Vertriebsgesesellschaft.

Seifert, B. (2021). A taxonomic revision of the Palaearctic members of the *Formica rufa* group (Hymenoptera: Formicidae) – the famous mound-building red wood ants. https://doi.org/10.25849/MYRMECOL.NEWS_031:133

Skinner, G. J., & Whittaker, J. B. (1981). An Experimental Investigation of Inter-Relationships Between the Wood-Ant (*Formica rufa*) and Some Tree-Canopy Herbivores. *Journal of Animal Ecology*, *50*(1), 313–326. JSTOR. https://doi.org/10.2307/4047

Sorvari, J. (2016). Threats, conservation and management. *Wood ant ecology and conservation*, 264.

Stadler, B., & Dixon, A. F. G. (2005). Ecology and Evolution of Aphid-Ant Interactions. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 345–372. https://doi.org/10.1146/annurev.ecolsys.36.091704.175531

Steinbauer, M. J., Grytnes, J.-A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., Rixen, C., Winkler, M., Bardy-Durchhalter, M., Barni, E., Bjorkman, A. D., Breiner, F. T., Burg, S., Czortek, P., Dawes, M. A., Delimat, A., Dullinger, S., Erschbamer, B., Felde, V. A., … Wipf, S. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature*, *556*(7700), 231–234. https://doi.org/10.1038/s41586-018-0005-6

Steiner, A. (1924). Über den sozialen Wärmehaushalt der Waldameise (*Formica rufa* Var. *Rufo-pratensis* For.). *Zeitschrift für vergleichende Physiologie*, *2*(1), 23–56.

Steinhoff, S., & Roch, T. (2008). Auf neuen Wegen zur Flächendeckenden Waldameisenkartierung – erste Ergebnisse der Erhebungen im Landkreis Bautzen (Freistaat Sachsen). *Ameisenschutz aktuell*, *22*.

Stukalyuk, S., Radchenko, Y., Netsvetov, M., & Gilev, A. (2020). Effect of mound size on intranest thermoregulation in the red wood ants *Formica rufa* and *F. polyctena* (Hymenoptera, Formicidae). *Turkish Journal of Zoology*, *44*(3), 266–280. https://doi.org/10.3906/zoo-1912-26

swisstopo, Swiss Federal Office of Topography. https://www.swisstopo.admin.ch/de/geodata.html.

Trigos-Peral, G., Juhász, O., Kiss, P. J., Módra, G., Tenyér, A., & Maák, I. (2021). Wood ants as biological control of the forest pest beetles *Ips spp. Scientific Reports*, *11*(1), 17931. https://doi.org/10.1038/s41598-021-96990-5

Vandegehuchte, M. L., Wermelinger, B., Fraefel, M., Baltensweiler, A., Düggelin, C., Brändli, U.-B., Freitag, A., Bernasconi, C., Cherix, D., & Risch, A. C. (2017). Distribution and habitat requirements of red wood ants in Switzerland: Implications for conservation. *Biological Conservation*, *212*, 366–375. https://doi.org/10.1016/j.biocon.2017.06.008

Vitasse, Y., Ursenbacher, S., Klein, G., Bohnenstengel, T., Chittaro, Y., Delestrade, A., Monnerat, C., Rebetez, M., Rixen, C., Strebel, N., Schmidt, B. R., Wipf, S., Wohlgemuth, T., Yoccoz, N. G., & Lenoir, J. (2021). Phenological and elevational shifts of plants, animals and fungi under climate change in the European Alps. *Biological Reviews*, *96*(5), 1816–1835. https://doi.org/10.1111/brv.12727

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O., & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389–395. https://doi.org/10.1038/416389a

Warren, R. J., & Chick, L. (2013). Upward ant distribution shift corresponds with minimum, not maximum, temperature tolerance. *Global Change Biology*, *19*(7), 2082–2088. https://doi.org/10.1111/gcb.12169

Webster, C., Mazzotti, G., Essery, R., & Jonas, T. (2020). Enhancing airborne LiDAR data for improved forest structure representation in shortwave transmission models. *Remote Sensing of Environment*, *249*, 112017. https://doi.org/10.1016/j.rse.2020.112017

Wellenstein, G. (1952). Zur Ernährungsbiologie der Roten Waldameise. (*Formica rufa* L.). *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz*, 430–451.

Wermelinger, B., Düggelin, C., Freitag, A., Fitzpatrick, B., & Risch, A. C. (2018). Die Roten Waldameisen – Biologie und Verbreitung in der Schweiz. *Merkbl. Prax. 63*.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics. *Version*, *2*(1), 1–189.

Wickham, H., & Wickham, M. H. (2020). Package 'plyr'.

Wilson, J. J. (2012). DNA Barcodes for Insects. In W. J. Kress & D. L. Erickson (Hrsg.), *DNA Barcodes*. Humana Press. https://doi.org/10.1007/978-1-61779-591-6_3

Wilson, R. J., Gutiérrez, D., Gutiérrez, J., & Monserrat, V. J. (2007). An elevational shift in butterfly species richness and composition accompanying recent climate change. *Global Change Biology*, *13*(9), 1873–1887. https://doi.org/10.1111/j.1365-2486.2007.01418.x

Youngs, L. C., & Campbell, R. W. (1984). Ants preying on pupae of the western spruce budworm, *Choristoneura occidentalis* (Lepidoptera: Torticidae), in Eastern Oregon and Western Montana. *The Canadian Entomologist*, 116(12), 1665–1669. https://doi.org/10.4039/Ent1161665-12

Zhou, A., Qu, X., Shan, L., & Wang, X. (2017). Temperature warming strengthens the mutualism between ghost ants and invasive mealybugs. *Scientific Reports*, *7*(1), 959. https://doi.org/10.1038/s41598-017-01137-0

Appendix

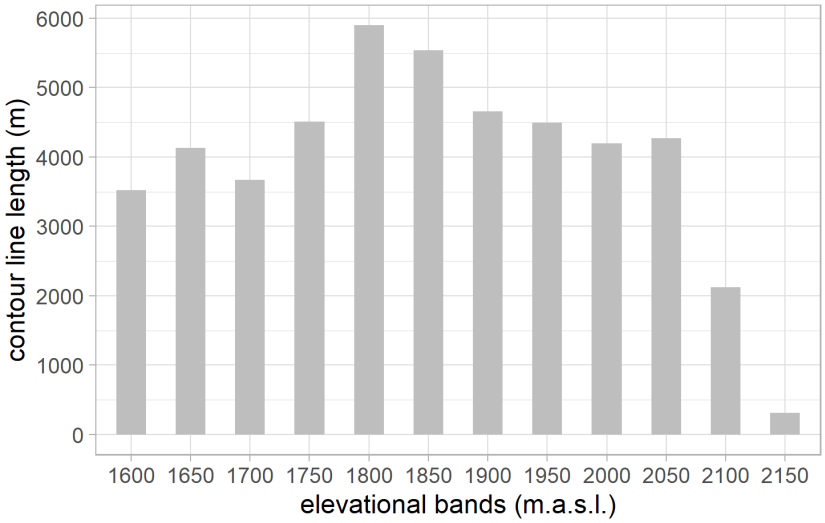

Figure A1: Considered contour lines and their total lengths in the surveys. Total length of contour lines = 47314 m.

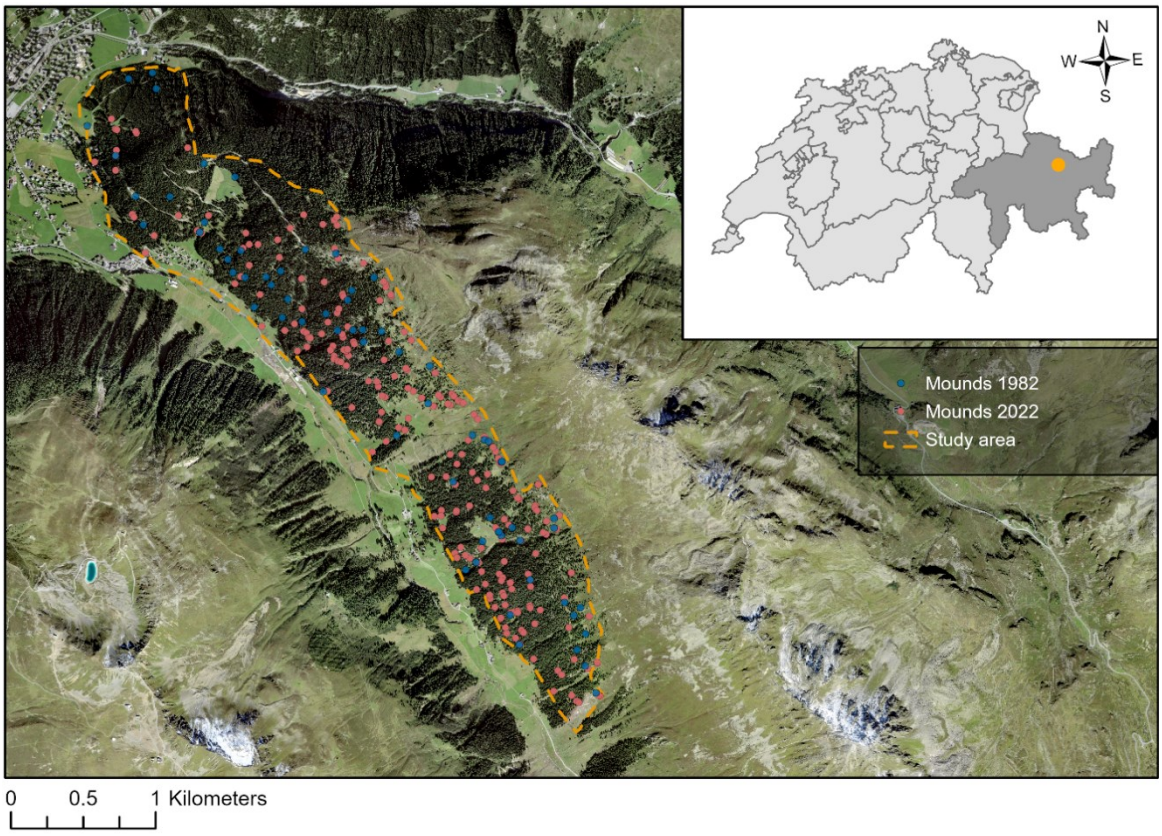

Figure A2: Mound presences recorded in the systematic surveys in 1982 and in 2022. Map created in the ArcGIS Pro software by Miriam Simma, 07.12.2022. Basemap (SWISSIMAGE) and country boarders provided by the Swiss Federal Office of Topography (swisstopo).

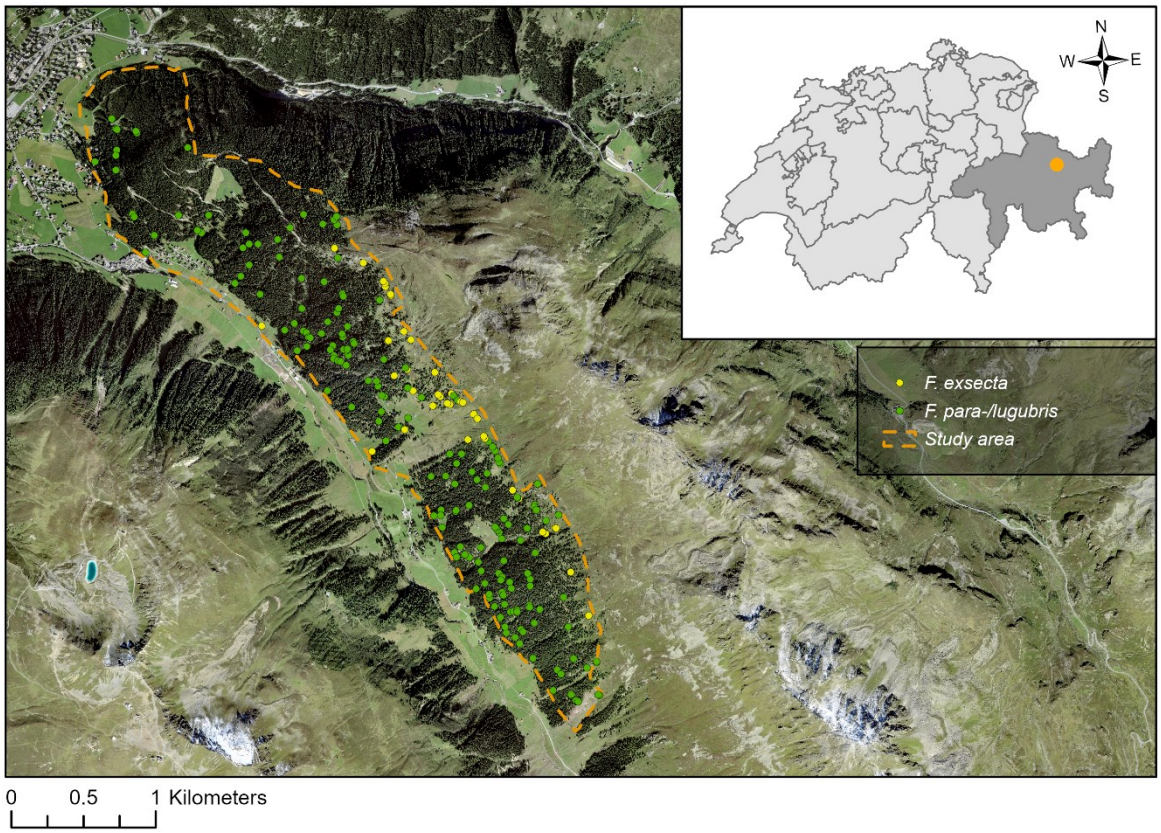

Figure A3: Mound presences recorded in the systematic resurvey in 2022. Map created in the ArcGIS Pro software by Miriam Simma, 07.12.2022. Basemap (SWISSIMAGE) and country boarders provided by the Swiss Federal Office of Topography (swisstopo).