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Research article

Glacier retreat decreases mutualistic network robustness over spacetime

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Glaciers are retreating worldwide at an ever-increasing rate, exposing new ice-free areas to ecological succession. This process leads to changes in biodiversity and potentially to novel species interactions. However, we still have a limited understanding of how glacier retreat influences species interaction networks, particularly the structure and robustness of mutualistic networks. After reconstructing plant–pollinator networks along a 140-years chronosequence on a glacier foreland, we address the effects of glacier retreat on network structure and robustness. Our results show that the prevalence of different network motifs changes over spacetime, leading to a decrease of network robustness. With glacier retreat, mutualistic networks shift from highly connected with diverse specialist interactions to loosely connected with few generalist interactions. Furthermore, despite the turnover of plant species, we find that species structural roles remain constant over spacetime while depending on species identity. Our findings suggest that glacier retreat reshuffles mutualistic networks with motifs posing low robustness, leading to increased fragility. Understanding the assembly and breaking down of species interaction networks provides novel insights into the development and stability of novel, post-glacial ecological systems facing glacier extinction.

Keywords: biodiversity change, climate change, food web, interaction diversity, pollination networks, species interactions

Introduction

Glacier retreat is one of the most emblematic symptoms of anthropogenic climate change (Marzeion et al. 2014, Roe et al. 2017). According to recent projections (Rounce et al. 2023), $49 \pm 9\%$ of the world's glaciers may disappear at the



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current global warming rate by 2100. Glacier retreat has a wide range of hydrological, geomorphological and ecological consequences on mountain ecosystems (Moore et al. 2009, Stibal et al. 2020). However, the effects of glacier retreat on species interactions are still poorly understood and difficult to predict.

As glaciers retreat, new ice-free areas are progressively exposed to colonisation by microbes, plants and animals (Whittaker 1993, Chapin et al. 1994, Bradley et al. 2014, Eichel 2019). This process leads to a primary succession, with the communities closest to the glacier front being the youngest and those further away the oldest (Matthews 1992, Walker et al. 2010). Following glacier retreat, there is an initial increase in plant and pollinator diversity, leading to a local increase in biodiversity (Cauvy-Fraunié and Dangles 2019, Ficetola et al. 2021, Losapio et al. 2021a, Tu et al. 2024). However, this is just a temporary, transient phase. Once glaciers disappear from the landscape, the entire glacier foreland will tend to an equilibrium state that reflects the later stages of succession, leading to a significant reduction in local and regional biodiversity (Whittaker 1993, Chapin et al. 1994, Stibal et al. 2020, Losapio et al. 2021a). This effect is particularly pronounced in subalpine ecosystems, where vegetation changes from bare ground to closed forest in a few hundred metres within one hundred years (Boggs et al. 2010, Tu et al. 2024). Yet, we still know very little about the impact of glacier retreat on the structure and assembly of food webs and ecological networks, and on their ability to withstand disturbances.

Mutualistic networks, including plant–pollinator networks, represent a formidable example of coevolution and species interactions involving both food webs (the feeding of pollinators on flowers) and pollination networks (the reproduction of plants). The structure of mutualistic networks is typically analysed by using indices that distil network architecture (Bascompte and Jordano 2014). Examining mutualistic network structure along a glacier foreland chronosequence would allow us not only assessing architectural differences, but also inferring its drivers in space and estimating its assembly over time (Losapio et al. 2015, Tu et al. 2024). Network structure at the meso-scale is represented by network motifs, which are recurring patterns of interactions in a sub-graph, between small groups of species (Milo et al. 2002, Bascompte and Melian 2005, Stouffer et al. 2007, Simmons et al. 2019, Losapio et al. 2021b, Lanuza et al. 2023). Motifs are the building blocks upon which the network is assembled (Milo et al. 2002, Bascompte and Melian 2005, Alon 2007, Simmons et al. 2019), they represent different functions within the network (Stouffer et al. 2007), and they influence species persistence (Losapio et al. 2021b). For example, a four-species motif could involve two plant species visited by two pollinator species in which one pollinator visits only one plant while a second pollinator visits both plants. This motif indicates that one pollinator is a generalist species visiting multiple plants, while the other pollinator is a specialist visiting only one plant. This simple four-species motif represents a pattern of interactions that can occur across the network

in response to changes in local interactions (Simmons et al. 2019, Cirtwill et al. 2024). As the number of species increases, the number of possible motifs also increases. Specifically, for motifs of up to six species, there are 44 possible types of network motifs (Simmons et al. 2019). Furthermore, structural roles within motifs influence the size and population dynamics of species (Cirtwill et al. 2018) and can help understanding network assembly over spacetime. However, as species interactions and ecological networks have been overlooked in glacier ecosystems (Albrecht et al. 2010, Losapio et al. 2015, Tu et al. 2024), we still do not know how network motifs change in response to glacier retreat over spacetime and their implications for biodiversity maintenance.

Recently deglaciated terrains are characterized by harsh environmental conditions and low species richness, which can promote specialized interactions as specialist species, posing strong coevolved dependencies, are better adapted to the limited availability of resources and low competition (Thompson 2005, Schleuning et al. 2012, Dalsgaard et al. 2017). As succession progresses with glacier retreat, species richness and interaction diversity increase, while the community composition becomes more dynamic due to high turnover rates (Albrecht et al. 2010, Tu et al. 2024). In later stages, facing the decrease in biodiversity, generalist species are favoured as they can exploit a broader range of resources and are more resistant to environmental perturbations and competition than specialist ones (Mello et al. 2011, Bascompte and Jordano 2014, Dalsgaard et al. 2017). This shift toward generalization reflects a loosening of network structure as generalists reduce dependency on specific partners. Consequently, it is reasonable to hypothesise that the prevalence of generalist interactions increases with glacier retreat, leading to a shift from specialized to generalized network motifs.

As different motifs reflect various degrees of specialisation or generalisation in the network, changes in their prevalence over spacetime are informative of assembly processes (Stouffer et al. 2007, Lanuza et al. 2023) and can influence network robustness. Network robustness is the ability of pollination networks to resist external disturbances that would lead to extinction cascades (Dunne et al. 2002, Memmot et al. 2004, Landi et al. 2018). Robustness is a fundamental feature of complex biological systems that reflects resistance to species loss (Olesen et al. 2006, Bascompte and Jordano 2014), a key systemic property for understanding, predicting and anticipating the consequences of climate change and glacier retreat on biodiversity. For example, specialised motifs result from strong pairwise coevolution between specific plant–pollinator pairs (Thompson 2005), which lead to networks with strong dependencies that can be more sensitive to disturbances, as the loss of a single species can lead to coextinction cascades given that a specialist plant (pollinator) loses its sole pollinator (plant) (Bascompte and Jordano 2014). On the opposite side of the spectrum, generalised motifs create more loosely connected networks that can be more robust given that if one plant or pollinator disappears, other species can fill their role, preventing an extinction cascade

(Mello et al. 2011). Yet, specialised motifs in plant–pollinator networks can provide more stable communities as mutually adapted species are less vulnerable to competition and invasion (Bascompte and Jordano 2014). However, to the best of our knowledge, there has been no prior investigation of the effects of glacier retreat on network robustness and how it depends on network motifs.

This case study aims to provide first empirical insights into how network motifs change over spacetime in response to glacier retreat, and their implications for network robustness against disturbance. We address the following questions and test the following hypotheses: 1) how does glacier retreat influence network motif patterns? We hypothesise that glacier retreat leads to a shift in motif prevalence from specialisation to generalisation. 2) How do network motifs mediate the impact of glacier retreat on network robustness? We hypothesise that network robustness decreases following glacier retreat and is linked to changes in motif prevalence. 3) How does glacier retreat influence plant species roles within network motifs? We hypothesise that structural roles change with glacier retreat depending on species identity and interaction diversity.

Material and methods

Study area

This case study was carried out at Mont Miné glacier foreland, Val d'Hérens, Switzerland (46°03'33.646'N, 07°32'54.550'E). Mont Miné together with the adjacent Ferpècle glacier covered an area of 26.9 km² by the maximum of the Little Ice Age (LIA, ca 1860) (Nicolussi et al. 2022). Since the end of the LIA, Mont Miné glacier retreated by 2.53 km. Through geochronology, it is possible to infer glacier dynamics and hence reconstruct a chronosequence, which provides different developmental stages of the ecosystem over space and over the years (Matthews 1992, Whittaker 1993, Chapin et al. 1994, Walker et al. 2010). Glacier forelands represent therefore a unique natural model system for studying the impacts of climate change on pollination networks (Albrecht et al. 2010, Losapio et al. 2021a, Tu et al. 2024). Yet, some caution must be exercised since time is not the only driver in shaping communities along successions (Raffl et al. 2006, Johnson and Miyanishi 2008, Walker et al. 2010, Ficetola et al. 2021). For instance, plant species richness is a strong predictor of plant–pollinator interaction diversity (Tu et al. 2024).

Based on existing geochronological records of the Mont Miné glacier (Lambiel et al. 2016, Nicolussi et al. 2022) and our reconstruction of historical cartography ('Journey through time' tool at <https://map.geo.admin.ch>), we reconstructed terrain age given the year when the glacier retreated and divided the chronosequences into four stages: stage 1, stage 2, stage 3 and stage 4, representing terrain deglaciated since at least 1989, 1925, 1900 and 1864, respectively (Fig. 1). We measured the average age (x) of each stage (1

as $x_i = x_s - \frac{x_{old} + x_{young}}{2}$, where x_s is the year of sampling, x_{old} and x_{young} are the ages of the two moraines delimiting the i -th stage (Losapio et al. 2021a, Tu et al. 2024). The results indicate that stage 1 is 17 years, stage 2 is 66 years, stage 3 is 111 years and stage 4 is 141 years.

In each stage, we randomly selected four sampling sites, for a total of 16 sites (Fig. 1). Sites were chosen only on the west side of the river, ensuring minimal variation in elevation gradient (1961–2000 m a.s.l) and avoiding as much as possible the impact of human construction activities related to the Barrage de Ferpècle on the east side of the river (Lambiel et al. 2016). In each site, we installed two orthogonal transects measuring 25 m in length and 1 m in width, corresponding to 50 m² per site (Gibson et al. 2011, Grange et al. 2021, Martínez-Núñez et al. 2022, Tu et al. 2024, Supporting information).

Sampling pollination interactions

Sampling took place during the flowering season of summer 2023, from 15 June to 15 July. Within each transect, we recorded plant–pollinator visitations. Flower visitors getting in direct contact with flower anthers or stigmas were considered as pollination interactions (Gibson et al. 2011). We recorded interactions by collecting flower-visiting insects using an entomological aspirator or by sweep netting, depending on insect size. Collected pollinators were stored in 70% ethanol for subsequent identification. For conservation and ethical reasons, we limited the collection of specimens to those necessary for species identification, and proceeded with recording visitations by direct observation. To standardise sampling time, we walked transects at a slow and steady pace for 15 min per branch, for a total of 30 min per transect (Martínez-Núñez et al. 2022). Sampling sessions were carried out on sunny and low-wind days from 08:30 to 17:30 h. We sampled each site eight times, for a total of $n = 128$ temporal replicates. To minimise the impact of sampling time on pollinator activity, we randomised the sampling order of sites within a day and within a round of replicates such that every site was sampled at all possible times of the day (Martínez-Núñez et al. 2022).

Plant species were identified according to Flora Helvetica (Lauber and Wagner 1998, updated ver. on <https://www.infoflora.ch>). We observed a total of 108 blooming plant species belonging to 28 families. Pollinator specimens were identified at species or morphospecies level whenever possible using taxonomic keys (Rognes 1991, Cerretti et al. 2012, Gregor et al. 2016, Cappellari et al. 2018, Falk 2019, Michez et al. 2019, Rasmont et al. 2021). We identified 65 families belonging to 8 orders; 29.0% of specimens are identified at species level, 42.3% as morphospecies, 1.7% as genus, 27% at family or higher level. Specimens were photographed using a stereomicroscope and updated on iNaturalist platform to gather further taxonomic information, for data sharing and for public outreach (<https://www.inaturalist.org/projects/pollinator-diversity-at-ferpecle-glacier-ecosystems>).

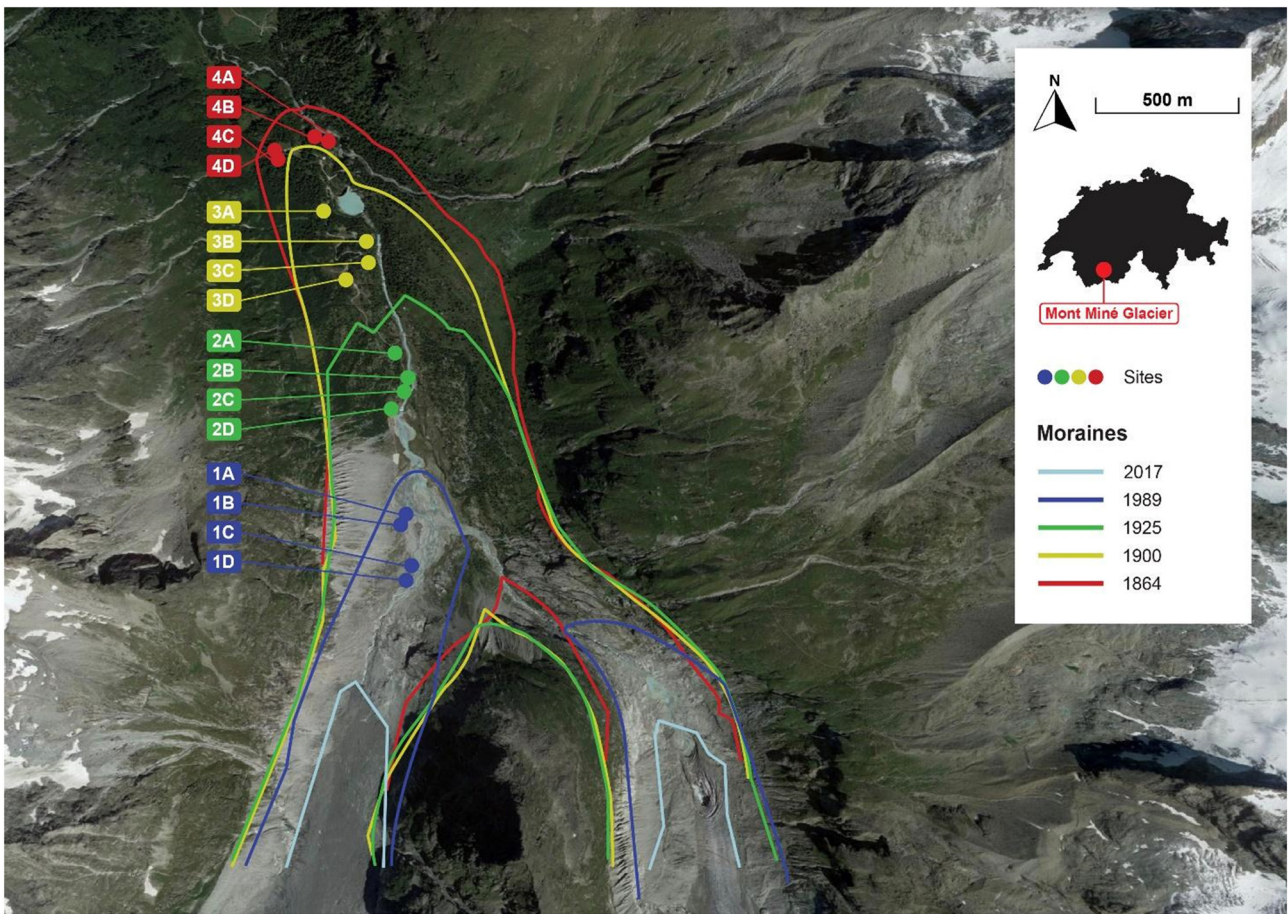


Figure 1. Satellite image (Google Earth 10.48.0.2, 08/07/2016) of Mont Mine glacier ecosystem (left) with its chronosequence, stages and sampling sites.

Data analysis

All statistical analyses were performed in R software (ver. 4.3.1, www.r-project.org). We constructed networks by pooling interactions at the site level ($n=16$) using the ‘bipartite’ package (Dormann et al. 2023). We adopted a null model approach to identify network motifs and to compare robustness among networks differing in matrix size and filling (Vázquez and Aizen 2003, Dormann et al. 2023).

Motif prevalence

To detect network motifs, we randomised the observed networks 100 times using four different null models that vary in their level of conservatism: ‘r00_ind’, ‘r0_ind’, ‘c0_ind’ (Oksanen et al. 2022), and ‘vaznull’ (Dormann et al. 2023). As results converge among the null models (Supporting information), here, we present findings from the standard r00_ind null model, which is based on a shuffling method that randomises individuals among cells of the matrix while preserving row and column grand sums. We calculated the occurrence frequency of all 41 possible motifs composed of four to six species (Supporting information) using the ‘bmotif’ package (Bramon-Mora et al. 2018, Simmons et al. 2019). Motif occurrence was converted to relative frequency

by expressing counts as a proportion of the total number of motifs in the network (Bramon-Mora et al. 2018, Simmons et al. 2019). To test whether observed motif frequency significantly differs from that of null model networks, we performed a one-sample Z-test for each motif within each site. For motifs above the 1.96 threshold, we can reject the null hypothesis and accept the alternative hypothesis that observed motifs are informative of specific interaction patterns. To test how network motifs change with glacier retreat, we used a linear regression model (Bingham and Fry 2010) with motif prevalence as response variable and the statistical interaction between motif id and glacier retreat (stage age) as predictor variables.

To understand how different stages differ in terms of motif prevalence and to detect the most important motifs, we performed a sparse partial least squares discriminant analysis (sPLS-DA) using the ‘mixOmics’ package (Lê Cao et al. 2024). This technique is designed for the analysis of high dimensional data that is aimed to provide a dimensionality reduction and to relate multicollinear response variables to a set of predictor variables. We performed dimensionality reduction maximising the separation between successional stages depending on motif prevalence in the first three components for the first eight terms. We tested the significance of

the separation analysis by means of linear regression model with stage as predictor variable of site coordinates on each component. We tested the variance explained by this model with type-II ANOVA (F-test).

Network robustness

To calculate network robustness, we built a function in R software that: 1) simulates a secondary extinction cascade on a pollination network, 2) interpolates an extinction function based on this cascade, and 3) calculates robustness as the area under the extinction curve. The secondary extinction cascade occurs by removing plant species from the network and counting how many pollinators are left with at least one link. This process is iterated until all plants and pollinators are left with no links. The number of extinct plants and number of remaining pollinators at each iteration are scaled to 1 and a function is interpolated to fit the resulting curve. This extinction curve is the attack tolerance curve (ATC) (Supporting information) as devised by Memmot et al. (2004) and Burgos et al. (2007). Robustness (R) is defined as the area under the ATC and is calculated via the definite integral from 0 to 1 (Dunne et al. 2002, Burgos et al. 2007) as $R = \int_0^1 f(x) dx$, where $f(x)$ is the fitted ATC. A robustness value close to 1 is associated with a very robust network, while a value close to 0 is associated with a very fragile network (Burgos et al. 2007). Although this model relies on static assumptions such as lack of rewiring, it provides a solid estimation of the ability of communities to resist disturbance (Dunne et al. 2002).

We built two robustness models: the test model and the random model. In the test model, plant species go extinct with a probabilistic approach. Plant species have different extinction probabilities based on their distribution, which accounts both for coverage and habitat specificity along the glacier foreland. The narrower and more centred around the early stages a species distribution is, the lower its mean coverage, the higher the chances that the robustness function extracts the corresponding species. In the random model, all plant species have the same probability of going extinct regardless of their distribution and coverage. We iterated each model 1000 times and calculated robustness as the mean robustness value. To test whether the test model was significantly different from the random model, for each site we performed two-sample Z -tests. Z -score calculation was excluded for sites 4A and 4B as the averages of the two models are identical and the standard deviation for both is zero because these networks comprise solely *Rhododendron ferrugineum* as plant species and their extinction cascade always repeats itself across iterations.

To test the effects of glacier retreat on robustness, we fitted beta regression models (one model for observed robustness and one for random model robustness) with stage as predictor (factor with four levels) of mean robustness value. Furthermore, to test whether differences in motif prevalence among stages correspond to changes in network robustness,

we used site coordinates of the first two sPLS-DA components as predictor of robustness. We tested the variance explained by model predictors with type-II ANOVA (F-test). The significance of predictors was estimated via t -test of the regression beta coefficients (hereafter, indicated with b). Two separate models were run to avoid collinearity and issues with degrees of freedom using the 'betareg' package (Zeileis et al. 2022).

We emphasise that the model used here is solely based on a static topological approach that does not account for rewiring (i.e. changes in the interactions between species without changes in species composition), an assumption that may change depending on species adaptability (Valdovinos et al. 2009). Future studies should examine potential implications of network rewiring, especially for generalist species, and examine the influence of network motifs. Furthermore, pollination networks are known to exhibit significant variability across years driven by species turnover (i.e. changes in the presence or abundance of plant and pollinator species) and interaction rewiring (Petanidou et al. 2008). This inter-annual variability may lead to changes in network robustness. As our case study represents a single year of sampling, further longitudinal, long-term studies would be necessary to account for the natural temporal variability in these networks and assess the extent to which observed patterns persist. As rewiring can stabilise networks, long-term measures are necessary to capture the full picture.

Structural role of plant species

To investigate the structural role of plant species within network motifs, first we calculated plant species contributions to motif node positions as the sum of interactions of the focal species divided by the sum of all interactions in that motif (Simmons et al. 2019). We considered all possible positions within motifs up to five species (Supporting information). Second, to test for difference between observed and random (null model) networks we performed one sample Z -tests calculating Z -scores for each possible node position up to five species in each stage.

Third, we used non-metric multidimensional scaling ('vegan' package, Oksanen et al. 2022) to identify dissimilarities among plant species roles in observed networks. We chose this type of ordination technique because it is very efficient in handling many variables at once and does not rely on parametric assumptions. We considered Bray–Curtis dissimilarity to construct the distance matrix and to map distances among structural roles in the low-dimensional space. We tested multivariate homogeneity of group dispersion among stages and species identities using PERMANOVA. Fourth, we calculated interaction diversity for all plant species via the Shannon index (H') as $H' = -\sum_{i=1}^n p_i \ln p_i$, where p_i is the proportional abundance of the i th interaction and n is the total number of interactions (Bersier et al. 2002). Finally, we performed PERMANOVA and dispersion analyses using stage, species and plant species interaction diversity as grouping factors of species roles.

Results

Motif prevalence

We found that 36% of network motifs were significantly over-represented in observed networks compared to null model networks overall (Supporting information). The prevalence of 19 motifs changes with time since deglaciation (Fig. 2). The prevalence of poorly interconnected motifs (i.e. with lower mean node degree) characterised by generalist plant interactions significantly increases with glacier retreat ($p_{M7} = 0.006$, $p_{M17} < 0.001$, $p_{M25} = 0.002$, $p_{M27} < 0.001$, $p_{M38} < 0.001$, $p_{M39} < 0.001$, $p_{M44} < 0.001$). On the other hand, motifs characterised by the highest levels of connection (i.e.

highest mean node degree) significantly decrease with glacier retreat ($p_{M6} = 0.04$, $p_{M12} = 0.04$, $p_{M16} = 0.04$, $p_{M22} = 0.05$, $p_{M23} = 0.05$, $p_{M24} = 0.05$, $p_{M33} = 0.04$, $p_{M34} = 0.04$, $p_{M35} = 0.04$, $p_{M36} = 0.04$, $p_{M37} = 0.04$, $p_{M43} = 0.04$).

The sPLS-DA analysis identified significant groups of motifs characterising glacier retreat stages (Fig. 3). The first component (variance explained=28%, $F_{3,12} = 7.56$, $p = 0.004$) separates stage 1 and 2 from stage 3 and 4. Motifs ID 22, 26 and 28 characterise stage 1 and 2 while they are underrepresented in stage 3 and 4 (Fig. 4). The second component (variance explained=11%, $F_{3,12} = 9.02$, $p = 0.002$) separates stage 1 and 4 from stage 2 and 3. Motifs ID 36, 38 and 42 characterise stage 2 and 3 while they are underrepresented in stage 1 and 4 (Fig. 4).

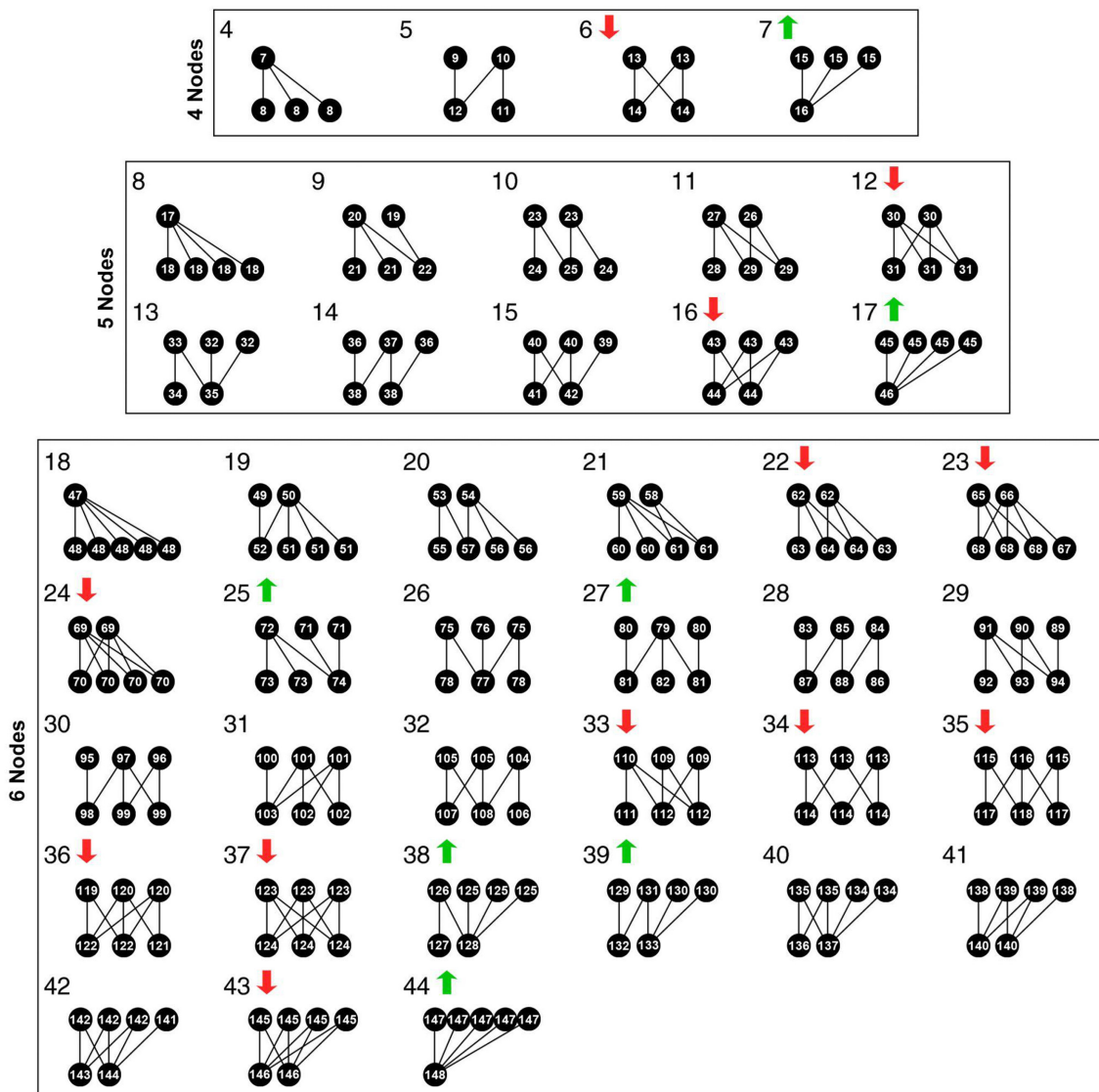


Figure 2. All possible network motifs from four to six nodes. Large numbers identify each motif ID. Small numbers inside nodes represent the unique positions species can occupy within motifs. Lines between nodes indicate undirected species interactions. Lower nodes represent plant species, upper nodes are pollinators. The red arrows mark motifs whose prevalence within the networks decreases significantly following glacier retreat, while the green arrow indicates those whose prevalence increases.

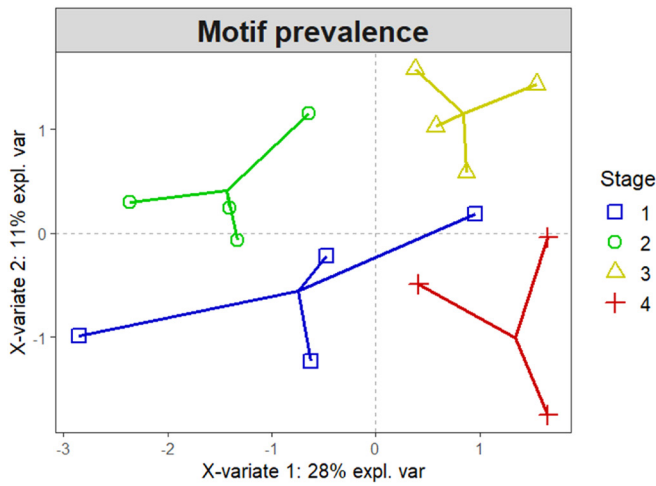


Figure 3. Biplot ordination (sPLS-DA) of motif prevalence. Each point represents a site. The closer the sites on the ordination plot, the more similar their pattern of motif prevalence.

Network robustness

Two-sample Z-tests revealed significant differences between observed and null model networks in terms of network robustness for all sites (Z-test: $|Z| > 1.96$, $p < 0.05$) except 3C (Supporting information). Furthermore, observed networks were more robust to targeted extinction than random extinctions in all sites ($Z > 1.96$, $p < 0.05$) but 4C ($Z < -1.96$, $p < 0.05$). Notably, glacier retreat influenced observed network robustness in both scenarios (target extinction: $F_{3,12} = 9.75$,

$p = 0.02$; random extinction: $F_{3,12} = 21.40$, $p < 0.001$). In the target extinction scenario, in which plant distribution determines plant extinction risk, robustness significantly decreased from stage 2 ($b = 0.18 \pm 0.16$, $p = 0.02$) and from stage 3 ($b = 0.37 \pm 0.16$, $p = 0.005$) to stage 4 (Fig. 5a). In the random extinction model, robustness significantly decreased from stage 1 ($b = 0.12 \pm 0.06$, $p = 0.03$), from stage 2 ($b = 0.22 \pm 0.06$, $p < 0.001$) and from stage 3 ($b = 0.24 \pm 0.06$, $p < 0.001$) to stage 4.

We found a significant relationship between robustness and motif prevalence in both the target extinction scenario (first component: $F_{1,14} = 2.8$, $p = 0.09$; second component: $F_{1,14} = 14.0$, $p < 0.001$) and the random extinction model (first component: $F_{1,14} = 13.7$, $p < 0.001$; second component: $F_{1,14} = 45.2$, $p < 0.001$). Robustness decreases along the first component ($b = -0.06 \pm 0.03$, $p = 0.09$; $b = -0.04 \pm 0.01$, $p < 0.001$) (Fig. 5a), while it increases along the second component in both cases ($b = 0.19 \pm 0.05$, $p < 0.001$; $b = 0.09 \pm 0.01$, $p < 0.001$) (Fig. 5b).

Structural role of plant species

We found that 19% of node positions across all stages and species had a significant contribution in terms of structural roles by comparing observed networks with null model networks (Supporting information). With PERMANOVA analyses we observed that structural roles change among species ($p < 0.001$), whereas no significant differences were found among stages ($p > 0.1$). Interaction diversity proved to be a strong grouping factor explaining differences in species structural roles within motifs ($p < 0.001$).

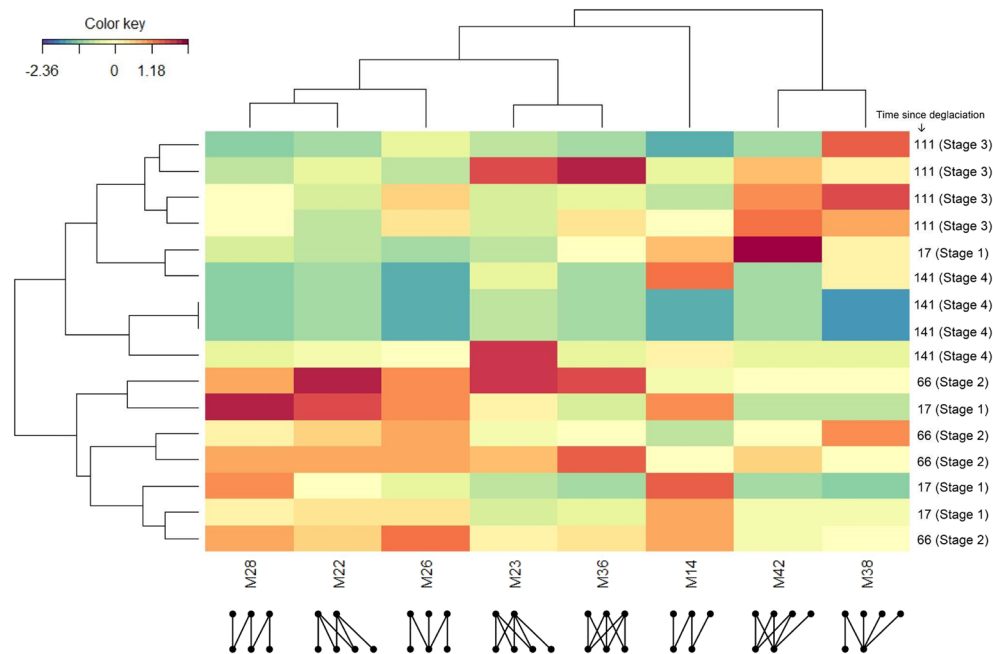


Figure 4. A heat map with clustering representing how different stages of glacier retreat cluster according to the prevalence of motifs, and which motifs are important in determining their separation.

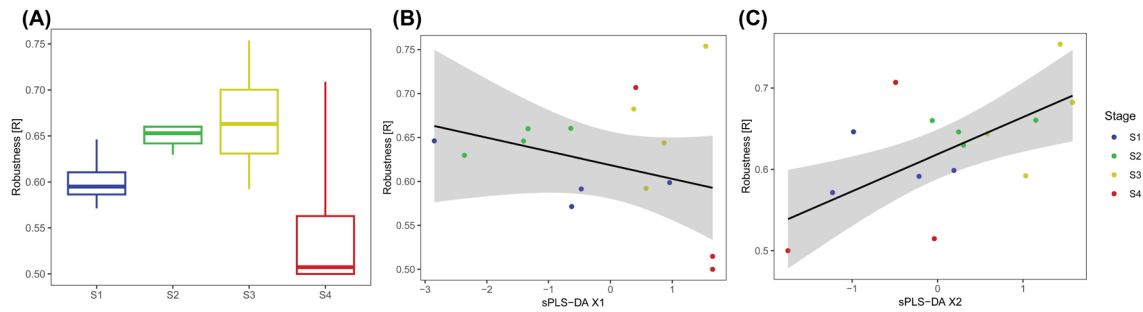


Figure 5. (A) Effects of glacier retreat on network robustness considering a target scenario. (B) Relationship between the first axis of the sPLS-DA of motif prevalence and network robustness. (C) Relationship between the second axis of the sPLS-DA of motif prevalence and network robustness. Displayed are fitted lines with 95% CI.

Discussion

The impact of glacier retreat on biodiversity and species interactions is increasingly documented, but still poorly investigated. Understanding how food webs and ecological networks develop and break down after glacier retreat is key for understanding biodiversity maintenance in novel, post-glacial ecosystems. In this study, we aimed to explore how glacier retreat impacts plant–pollinator networks, examining specifically the Mont Miné glacier foreland. While our findings provide interesting insights into the changes in network motifs and robustness over spacetime, we recognize that this is a case study conducted at a single glacier foreland over one year. Therefore, caution must be exercised in extending these findings to other glacier forelands without further spatial and temporal replication. This limitation is inherent to many studies of network ecology due to the challenges of accessing multiple sites in high-altitude environments over multiple years.

Our results show that the prevalence of different network motifs changes along the foreland of the Mont Miné glacier, reflecting how plant–pollinator interactions evolve in response to glacial retreat within this subalpine ecosystem. About half of the examined motifs (19) were affected by glacier retreat, with contrasting responses depending on their degree of connectivity and specialisation. Loosely connected motifs characterised by generalist plant interactions, such as M7, M17 and M44, increase with glacier retreat over spacetime. Those motifs particularly characterise the latest stage in the larch forest where interactions are dominated by *R. ferrugineum*, a ‘super generalist’ plant. In contrast, highly interconnected and symmetrical motifs such as M22, M34 and M37 decrease with glacier retreat. This shift suggests that network motif assembly begins with strong specialised interactions in which plants and pollinators coevolved mutual dependencies (Thompson 2005). With glacier retreat, network development proceeds towards plant generalisation and asymmetrical, loosened interactions. This shift confirms our first hypothesis, while bringing along novel questions on the evolution of specialised interactions (Brosi et al. 2016) and their relationship with species range dynamics (Alexander et al. 2015) and ecological stability. This tendency has also proven to have serious implications for the robustness of the examined mutualistic networks.

Network robustness initially increases after glacier retreat, probably as a result of increasing interaction diversity (Tu et al. 2024). However, in the long term, robustness sharply decreases in forest communities where the current influence of glaciers is limited. These results confirm our hypothesis that glacier retreat, but particularly glacier extinction, decreases network robustness over spacetime. It has been shown that glacier retreat impact has two distinct phases: initially, the disappearance of the ice makes space for species colonisation and the development of open grasslands (Whittaker 1993, Chapin et al. 1994, Bradley et al. 2014, Cauvy-Fraunié and Dangles 2019, Eichel 2019, Ficetola et al. 2021), which increases pollinator diversity (Albrecht et al. 2010, Tu et al. 2024) and supports complex food webs (Losapio et al. 2015). Yet, the continuing disappearance of glaciers and the establishment of woodlands and forests decreases biodiversity locally (Losapio et al. 2021a, Tu et al. 2024). Here, we show that this process favours plant generalisation at the expense of specialised species. Since differences in network robustness among stages can be explained in terms of motif prevalence and attributed to specific motifs, our case study indicates that detecting motif patterns in pollination networks may provide valuable insights into biodiversity maintenance and the ability of ecological systems to withstand disturbances.

Notably, we found evidence that structural roles plant species play within network motifs are mostly driven by their intrinsic differences, especially interaction diversity, rather than by stages. These findings partially confirm our third hypothesis, indicating that species occurring in different stages can perform similar roles, whereas glacier retreat per se does not have a strong influence on species-specific roles. They also indicate that structural roles are decoupled from species (taxonomic) turnover, since key roles are consistently filled by different species. We suggest that the emergence of structural turnover occurs within networks, while structural consistency emerges between networks.

To illustrate this structural turnover and structural consistency we provide a simple example (Fig. 6). *Epilobium fleischeri* is a pioneer species with high interaction diversity that plays a crucial role in early-stage networks. *Linaria alpina* is another pioneer species, but it is characterised by low interaction diversity. *Rhododendron ferrugineum* is the dominant species in the latest stage and is characterised by

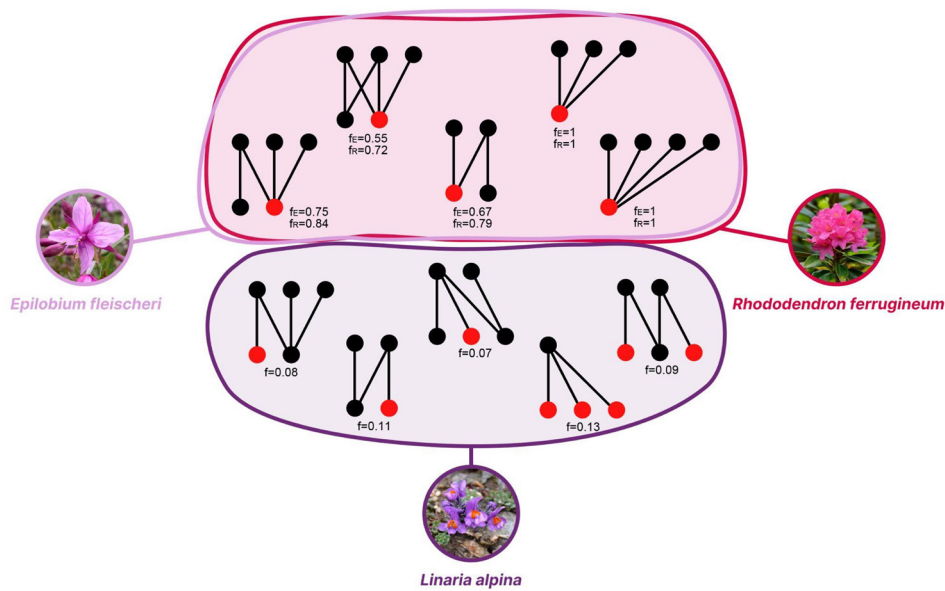


Figure 6. Most representative node positions for *Epilobium fleischeri*, *Linaria alpina* and *Rhododendron ferrugineum*. Lower nodes represent plants, upper nodes pollinators. Red dots represent the positions occupied by plant species within motifs. If there is more than one highlighted position within a motif, it means that they are topologically the same position. We report the frequency (f) and the contribution of plant species to node position on average over the four stages. Picture rights belong to Barbara Studer (*E. fleischeri*), Bernard Dupont (*R. ferrugineum*) and Muriel Bendel (*L. alpina*) (©<https://creativecommons.org/licenses/by-sa/4.0/>).

high interaction diversity. The five most representative node positions within motifs for these three species indicate that *E. fleischeri* and *R. ferrugineum* have the same roles within their networks, despite occurring in contrasting environments. In contrast, *E. fleischeri* and *L. alpina* show contrasting roles while occurring in the same pioneer communities. In summary, this example highlights that structural roles may vary regardless of species turnover.

Given the challenges associated with replicating network studies across multiple glacier forelands, this work represents a detailed case study of how glacier retreat influences mutualistic networks at one subalpine ecosystem. Further research is necessary to confirm whether similar patterns emerge in other forelands under different environmental conditions. Furthermore, temporal variability is a critical factor in understanding mutualistic networks (Petanidou et al. 2008), and the long-term robustness of these networks may depend not only on species turnover but also on the ability of species to rewire their interactions (Valdovinos et al. 2009). Although our findings indicate a decrease in robustness with glacier retreat, it is important to note that interaction rewiring could mitigate some of the negative impacts of species loss, a factor that would need to be addressed in multi-year studies. Future studies should aim to capture these temporal dynamics by conducting longitudinal studies that span multiple years and consider the potential for interaction rewiring as an adaptive response to environmental changes and disturbance.

In conclusion, glacier retreat has a strong impact on species interactions, pushing network motifs towards an architectural reshuffling that is associated with a loss of robustness. As motifs mediate the impacts of glacier retreat

on mutualistic network robustness, we propose that studying the species functional roles in network motifs could provide novel insights into the development and stability of novel post-glacial ecological systems.

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Author contributions

Matteo Conti: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Writing – original draft (lead). **Pierfilippo Cerretti:** Investigation (equal); Resources (equal). **Andrea Ferrari:** Investigation (equal). **Paolo Gabrieli:** Investigation (equal); Resources (equal). **Francesco Paone:** Investigation (equal). **Carlo Polidori:** Investigation (equal); Resources (equal). **Daniele Sommaggio:** Investigation (equal); Validation (equal); **Gianalberto Losapio:** Conceptualization (lead); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Resources (lead); Supervision (lead); Writing – original draft (supporting).

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.xksn02vr3> (Conti et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

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