The importance of species addition *versus* replacement varies over succession in plant communities after glacier retreat

Definitive version published as:
Cantera, I., Carteron, A., Guerrieri, A., Marta, S., Bonin, A., Ambrosini, R., Anthelme, F., Azzoni, R.S., Almond, P., Gazitúa, P.A., Cauvy-Fraunié, S., Lievano, J.L.C., Chand, P., Sharma, M.C., Clague, J., Rapre, J.A.C., Compostella, C., Encarnación, R.C., Dangles, O., Eger, A., Erokhin, S., Franzetti, A., Gielly, L., Gili, F., Gobbi, M., Hagvar, S., Khedim, N., Meneses, R.I., Peyre, G., Pittino, F., Rabatel, A., Urseitova, N., Yang, Y., Zaginaev, V., Zerboni, A., Zimmer, A., Taberlet, P., Diolaiuti, G., Poulenard, J., Thuiller, W., Caccianiga, M., Ficetola, G.F., 2024. The importance of species addition versus replacement varies over succession in plant communities after glacial retreat. *Nature Plants*.

https://doi.org/10.1038/s41477-41023-01609-41474.

Abstract

The mechanisms underlying plant succession remain highly debated. Due to the local scope of most studies, we lack a global quantification of the relative importance of species addition versus replacement. We assessed the role of these processes on the variation (β-diversity) of plant communities colonizing the forelands of 46 retreating glaciers worldwide, using both environmental DNA and traditional surveys. Our findings indicate that addition and replacement concur in determining community changes in deglaciated sites, but their relative importance varied over time. Taxa addition dominated immediately after glacier retreat, as expected in harsh environments, while replacement became more important for late-successional communities. These changes were aligned with total β-diversity changes, which were more pronounced between early-successional communities than between late-successional communities (>50 years since glacier retreat). Despite the complexity of community assembly during plant succession, the observed global pattern suggests a generalized shift from the dominance of facilitation and/or stochastic processes in early-successional communities to a predominance of competition later on.

MAIN TEXT

Ecological successions - how communities change or replace one another over time - have been a cornerstone of ecology since its inception[1,2]. Primary successions, *i.e.*, the development of ecosystems where a severe disturbance opens up large areas lacking most of lifeforms[3], start when a given species or a set of species colonize a newly exposed surface, which would then be further colonized by other species to reach complex communities[3]. Plant communities have been a major focus of primary succession studies for over a century since Clements's[4] work. Still, despite decades of work, the mechanisms that drive plant primary succession remain not fully understood[2,5]. In the pioneering Clementsian deterministic view, succession occurs in a progressive, directional, and homogenous manner, with a relatively stable and predictable community structure (the climax stage) reached after some time without punctuated changes[4,5]. This deterministic predictability was rapidly questioned and debated[6]. Over the last century, some studies showed homogenous responses among plant successions[7,8] while others showed that successions are not always convergent[9,10], barely reach an equilibrium[3], and are largely determined by stochastic processes[11].

While there is no clear concession about their determinism or stochasticity, successions are generally characterized by an increase in the number of plant species over time, accompanied by changes in community composition[2,3] (but see[12]). Those changes can be the result of two non-exclusive mechanisms: changes in species richness (due to species addition) and species replacement. Under a mechanism of species addition during succession, a community at time *t* is a subset of the species assemblage at *t+1*, because of the persistence of early colonizers (*i.e.*, pioneer species) and the addition of new species. In contrast, replacement involves the substitution over time of early colonizers by other species[1,13]. The relative importance of these two key mechanisms on plant succession is difficult to quantify as it may vary over the succession time, but also as a function of the of the local topographic and

environmental characteristics[2,8,12,14]. Thus, addressing the importance of species addition *versus* replacement requires the assessment of plant successions in multiple environmental settings.

Ongoing climate change is dramatically accelerating the retreat of glaciers worldwide[15,16], exposing new terrains to the development of plant successions[13]. As such, glacier forelands provide opportunities to study plant primary successions. Recently deglaciated terrains are typically isolated and characterized by harsh conditions, including a cold climate, unstable substrate and limited nutrient availability[17]. Thus, those emerging ecosystems typically show sparse vegetation[18] dominated by few species that exhibit a high degree of specialization to live in those conditions[19]. In this context, successions are generally dominated by a gradual addition of species during ecosystem development[20,21], while replacement is often hypothesized to be weak or absent[2,12,14]. It is, nonetheless, possible that the relative importance of species addition *versus* replacement changes over time, and the short time window covered by most of the studies[20,21] could have overemphasized the importance of addition.

In principle, species accumulation could result from the joint effect of dispersal-related processes (i.e. species require time to disperse in a new area) and facilitative interactions[7,12,22–24], which have a key role under severe environmental conditions[25–27]. Plant species that initially colonize recently deglaciated terrains can modify the environment through the accumulation of nutrients and organic matter and can create new micro-environmental conditions[25,26,28–30] that are more suitable to the establishment and development of subsequent colonizers[1]. Nevertheless, competitive exclusions and facilitation were found to jointly affect biodiversity in alpine plant communities[31]. Their relative contributions vary along environmental gradients[12] and are expected to change over time along the glacier foreland. For instance, the increase in nutrient availability and the reduction of physical constraints over succession may progressively reduce limiting conditions, stimulating growth and reproduction[3,19,32]. In the long term, this can intensify competition for space and light[24,33,34]. Moreover, nutrient availability generally

increases during the first stages of succession, but can reach saturation in late stages[25,34,35]. The resulting nutrient limitation can favour late successional species that are efficient nutrient users[25,34,36]. Therefore, species addition is expected to play a dominant role in plant succession soon after glacier retreat, with its importance decreasing as replacement becomes more important over time. However, a proper quantification of these expectations has never been carried out so far, as it requires a global dataset that spans large temporal and spatial scales.

In this study, we addressed this challenge by measuring and decomposing β-diversity (*i.e.* compositional variation between assemblages[37]) of plant communities colonizing 46 glacier forelands distributed worldwide (Fig. 1a). Glacier forelands include chronosequences of progressively older terrains at growing distances from the glacier forefront[38]. We used this space-for-time substitution to encompass communities covering a wide range of time since glacier retreat (sites deglaciated from 1 to >400 years); each site represents the past position of the glacier at known dates (Fig. 1b). Along these chronosequences, we collected environmental DNA (eDNA) from soil to reconstruct 266 communities of vascular plants, *i.e*., assemblages of taxa inhabiting a deglaciated site with a specific age since glacier retreat. eDNA metabarcoding allows producing inventories consistent with traditional inventories of aboveground plants[39–41] and enables the rapid assessment of communities over broad geographic scales and from remote areas, yielding data that would have been challenging to assemble with traditional methods[42,43]. Although soil eDNA is increasingly used to analyze plant communities, some uncertainty persists on the correspondence between patterns obtained with this approach and with traditional sampling[33,36,37]. Thus, we compared eDNA-based patterns with the ones obtained through morphological identification of species ("Traditional sampling") for a subset of forelands (Fig. 1a).

To understand how compositional variation changes over plant succession after glacier retreat, first, we quantified the total β-diversity (β-total) between communities within each

foreland and assessed if it decreases over the succession[22]. Second, we tested the hypothesis that taxa addition prevails over taxa replacement soon after glacier retreat, but its importance decreases along the succession. To this end, β-total was decomposed into its β-richness (*i.e.*, richness differences between communities due to species gain) and β-replacement components (*i.e.*, the substitution of one taxon by another, without affecting species richness) and the temporal patterns of these components was assessed. Finally, we assessed if the detected patterns are progressive or show breakpoints over successions[22].

Diversity of communities

After data filtering and removal of likely contaminants, 519 molecular taxonomic units (MOTUs) of vascular plants were detected with eDNA metabarcoding across the dated sites of the 46 glacier forelands (Tables S1 and S2). eDNA metabarcoding detected 0-60 MOTUs per site (mean ± SE = 11±0.6, Fig. S1a) and 5-150 MOTUs per foreland (mean = 36±4.4). No plant MOTUs were detected at 17 sites, all of which were <32 years old (Table S1). Using traditional floristic surveys, 365 vascular plant taxa were detected across 57 dated sites from 13 glacier forelands (Tables S1 and S3), with 0-133 (mean = 24±3) taxa per site (Fig. S1a) and 15-162 taxa (mean = 48±10) per foreland. Traditional sampling did not observe any vascular plant species only in one site, which was deglaciated since just one year (Carihuairazo glacier; Table S1).

To assess the variation in community composition over time, as well as the relative contribution of taxa addition *versus* replacement on successions, we quantified and partitioned the β-total between pairs of communities. We compared communities within each foreland (excluding comparisons between communities belonging to different forelands), also including comparisons that do not correspond to directly subsequent age classes (Fig. 1b). This resulted

in 771 and 102 comparisons of communities for the eDNA and the traditional data, respectively. β-total was partitioned into taxa replacement (β-replacement) and richness differences (β-richness) following ref.[46] (see Methods). Overall, the contributions of β-richness and β-replacement to β-total were comparable (Fig. 2; eDNA data: mean contribution of β-richness and β-replacement was 54% and 46%, respectively; traditional data: mean contribution of β-richness and β-replacement was 49.8% and 50.2%, respectively). β-richness values tended to be higher than β-replacement values for eDNA data (randomization test for paired samples: $P < 0.001$), but not for traditional data ($P = 0.773$).

Variation of β-diversity components over successions

Bayesian Generalized Linear Mixed Models (GLMMs) were used to assess how the β-diversity components varied over successions, considering two independent variables: "age differences" between communities, and their "mean age" (Fig. 1b). The age differences indicate how different the communities are from each other, in terms of age since deglaciation. Low values represent comparisons between communities in similar successional stages (*e.g.*, early *versus* early or late *versus* late), while high values represent comparisons between communities at very different successional stages (*e.g.*, early *versus* late). The mean age is the averaged time since glacier retreat between the compared communities; low values represent comparisons between young communities, while high values represent comparisons between late successional communities.

For both eDNA and traditional data, β-total increased with age differences (the 95% credible intervals (CIs) of this effect were consistently positive; Fig. 3a-c; Table 1). Furthermore, the dissimilarity between old communities was generally smaller than the dissimilarity between young communities with similar age differences (the mean age of

compared communities showed effects with 95% CIs consistently negative for eDNA data, and slightly overlapping zero for traditional data, Fig. 3b-d; Table 1).

β-richness and β-replacement showed distinct responses to mean age and age differences. β-richness increased with age differences between compared communities (consistently positive CIs; Fig. 3a-c; Table 1), while it decreased with the mean age of compared communities (Fig. 3b-d; Table 1), with similar patterns between eDNA and traditional data. The relationship between β-replacement and age differences was negative but generally weak, with CIs overlapping zero for eDNA and, marginally, for traditional data (Fig. 3a-c; Table 1). Finally, β-replacement tended to increase with the mean age of communities (CIs consistently positive for traditional data and slightly overlapping zero for eDNA; Fig. 3b-d; Table 1). Despite some differences across forelands, the relationships remained consistent when we restricted the analyses to forelands with more sites (Fig. S2-S3). The pattern also remained consistent when we repeated the analyses using the Sørensen's instead of Jaccard's index to calculate dissimilarities (Fig. S4) and when we excluded rare MOTUs (Fig. S5).

β-total, β-richness and β-replacement changed steadily over time, given that segmented regressions did not reveal significant breakpoints for the relationships between those variables and mean age (all $P > 0.05$). Models with breakpoints also showed higher Bayesian information criterion values, compared to the linear ones (Table S4).

Discussion

Predicting ecosystem responses to disturbance events and environmental changes requires understanding the mechanisms that govern community assembly during primary successions and, thus, modulate biodiversity. According to our results, compositional changes during successions after the retreat of glaciers are shaped by both the addition and the

replacement of taxa. Within 400 years after deglaciation, both mechanisms provided an overall similar contribution to compositional differences in plant communities (Fig. 2). Nevertheless, their contribution to total β-diversity varied over time, supporting the hypothesis that the mechanisms driving succession after glacial retreat change over time[22,47–49]. Immediately after glacier retreat, richness differences contributed more to β-total than replacement, as expected in harsh environments[27]. This suggests an overall predominant role of taxa addition in early plant primary succession, while replacement becomes dominant after more than 50 years following glacier retreat.

The more the communities differed in age, the more dissimilar they were in terms of composition. Furthermore, the dissimilarity between communities with strong age differences was mostly driven by β-richness (Fig. 3a-c, Table 1). This pattern matches the observed taxonomic accumulation from recently deglaciated terrains to late-successional stages[13,47,50]. Our results question studies advocating that severe environments are characterized by a constant initial floristic composition without changes in species composition over time resulting from the lack of the establishment of additional species (autosuccession)[12,14]. In fact, only 16% of the taxa detected with eDNA and 35% of taxa detected with traditional sampling persisted after 50 years of succession (Fig. 4). Such apparent incongruity could be explained by the ambiguity of the concept of "severe" or harsh" environment. These terms apply to limiting conditions both linked to climate (high altitude and/or latitude conditions) and specific to micro-habitats within recently-deglaciated terrains, where soils are nutrient-poor and geomorphological disturbances are frequent[51]. All these conditions are typical of recently deglaciated terrains but can have distinct effects on communities. While the climatic and edaphic constraints faced by plants in arctic and alpine environments are reported to reduce species replacement, sometimes leading to autosuccession[12,14], the retreat of glaciers also results in changing physical conditions over time. The initial absence of soil and biota would constrain colonization to relatively fast-

205 growing and opportunistic species adapted to alpine environments[36,52], with the gradual
206 addition of more dispersal-limited species[32,53]. Later, these species are followed by mountain
207 specialists that are more competitive because they are shade-resistant and/or exploit nutrients
208 efficiently[24,34,36,51,54].

209 Total community dissimilarity was influenced not only by the differences in ages
210 between communities[13,47,50] but also by their mean age. In early successional stages, β-total
211 between communities was generally larger than between communities with similar age
212 differences but being in late successional stages. Thus, dissimilarity between sites decreases
213 over time during succession. Considering that stochastically structured communities should
214 exhibit divergent taxonomic compositions, our results suggest that community composition in
215 early stages is strongly affected by initial conditions and/or stochastic processes (*e.g.*, priority
216 effects, probabilistic dispersal, and local extinction[55]), in agreement with temporal observations
217 from studies using permanent plots[9,22,56]. Then deterministic processes (*e.g.*, habitat filtering,
218 competitive interactions) may drive more convergent community structures in late successional
219 stages

220 When we compared communities in early successional stages (having on average <50
221 years), richness differences contributed more than replacement in determining the dissimilarity
222 between communities (Fig. 3a-d). Immediately after glacial retreat, soils are generally nutrient-
223 poor and affected by surface instability, but early colonizers do not inhibit the establishment of
224 new colonizing taxa[1]. This may be explained either by neutral interactions (due to the
225 predominant role of the environment[57] or of stochastic processes[9,10]) in these species-poor early
226 stages, or by facilitative interactions[1], where the beneficiary species are not constraining the
227 already established ones[58]. However, the importance of taxa addition quickly decreased over
228 time, and replacement becomes the dominant pattern for late-successional stages, suggesting
229 an increasing competition, as expected when resources, species richness and cover increase[27].

In late successional stages, the stabilization of nutrients and terrains can allow new and more competitive alpine taxa to establish and replace pioneer species[34]. Such substitutions may occur either because early arrivers modify the environment, making the conditions less suitable for themselves compared to other colonizers[1], or because later successional species outcompete the already established early species[34,58].

Threshold dynamics have been proposed during the biotic colonization of glacier forelands, with a fast increase in alpha richness during the first 60 years followed by a plateau and a decline in total β-diversity[22,59,60]. However, in our study, the trends of β-diversity and its components during succession did not exhibit significant breakpoints (Table S4), in agreement with Clements's view of successions as continuous trajectories without abrupt changes. Differences between this finding and previous results[22] might occur because temporal patterns obtained by focusing on one specific taxon (*e.g.*, plants) can differ from successional trajectories aggregating multiple diverse taxa[35] (such as plants, animals, and microbes). It should be noted that our plant communities did not reach a stable point within the considered time frame. Even in our late successional communities, β-total remained substantial, and β-richness remained well above zero. Our sampling focused on terrains deglaciated since the Little Ice Age (mostly after 1850), whose ages may remain too young for a stabilization of community composition. Longer time series (thousand years) would be required for a complete understanding of β-diversity changes and their drivers[24,61], especially to identify whether and when β-diversity changes decelerate.

Our conclusions were highly consistent between eDNA and traditional sampling (Fig. S1b), despite eDNA generally detecting fewer taxa per site. Like all sampling approaches, eDNA has its limitations. For instance, some taxa can remain undetected, it does not provide estimates of absolute biomass, and taxonomic resolution is limited by the lack of complete reference databases[43]. Furthermore, marker amplification strongly depends on the amount of

255 DNA present in environmental samples[43], potentially favoring abundant taxa[62–64]. Amplifying
256 rare taxa in soil samples can be particularly challenging, given the low diffusion rates of DNA
257 in the soil and the presence of inhibitors[44,65]. Increasing the number of subplots per site, PCR
258 replicates, and sequencing depth can improve the detection of rare species. In most forelands,
259 very recent sites without visible vegetation were not sampled by traditional methods whereas
260 they were using eDNA. This might also contribute to the lower number of plants detected, in
261 average, by eDNA. Another possible issue is that soil eDNA can detect past plant species[44].
262 However, the signal of those species is weaker than that of current plant communities[40,41,44],
263 and eDNA inventories generally match better traditional inventories of current plant
264 communities than those of older ones[39]. Despite these potential limitations, eDNA yielded
265 temporal patterns extremely similar to the ones of traditional methods, confirming that it
266 provides reliable diversity estimates, particularly for β-diversity[66].

267 Some studies have questioned the robustness of the conclusions obtained from the
268 analysis of chronosequences. Sites placed at similar distances from the glacier margin can differ
269 from each other due to microhabitat conditions or the identity of first colonizers (priority
270 effects), potentially following different trajectories[38,67]. Nevertheless, the analysis of temporal
271 data from permanent plots yielded patterns of β-richness and β-replacement highly consistent
272 with our results (See Methods and Extended data Figure 1), confirming the robustness of
273 conclusions drawn from the chronosequence approach. Moreover, the chronosequence
274 approach assumes that there are no disturbances over succession and that terrain age is the
275 community age, which is not always the case (see[17]). Even if we avoided sites clearly affected
276 by geomorphological disturbances, glacier forelands are dynamic landscapes, where
277 disturbances might interact with temporal patterns according to the frequency and magnitude
278 of the disturbance[17]. Indeed, despite retrieving general patterns of β-diversity changes, our data
279 exhibited high variability, suggesting that other site-level environmental conditions can affect

the dissimilarity between sites, such as micro-climate, soil properties and perturbations[13,17,47]. This calls for studies assessing how local drivers influence the contribution of taxa addition *versus* replacement, which will help to establish a general theory of succession that is lacking[2,3].

The debate on processes shaping succession has persisted since the onset of community ecology, with both stochastic[6] and deterministic[4] processes pinpointed as key successional drivers during the last century[5]. Our broad-scale study suggests that both processes play a fundamental role in community composition changes, with neutral and/or positive interactions dominating compositional variations in early successional communities, while competition becomes more important in late successional communities. Today, glaciers are retreating at an unprecedented rate, and plant communities play a keystone role in ecosystems developing after deglaciation[13]. The temporal changes of compositional drivers are expected to go beyond plant communities, affecting taxa interacting with plants through pollination, mutualism, herbivory, or parasitism[13,68]. Understanding how β-diversity measures co-vary across different components of communities will be a key challenge in predicting the long-term consequences of climate change on ecosystems[69].

Methods

eDNA sampling

In 46 glacier forelands (Fig. 1a), 1255 soil samples were collected from 2014 to 2020 to capture eDNA. In the sampled forelands, information on the dates of glacier retreat is available from the literature, remote sensing images, and field surveys[70]. For each glacier foreland, the chronosequence approach[38] was used to select 3-17 sites along deglaciated terrains for which the date of each glacier retreat is known. Each site corresponded to a given age class; the number of sites depended on the number of documented positions of the glacier foreland available from the literature, and we tried to cover as much as possible the whole history of the retreat of each glacier[70]. We avoided sites clearly affected by geomorphological disturbance. Within each site,

we sampled 2-10 plots (mean = 5, SE = 0.05, Table S1). The plots within each site had similar distances to the glacier forefront and were, if possible, regularly spaced at distances of ~20 m (See Fig. 1b). At each plot, we collected five soil subsamples within one meter (Fig. 1b) at a depth of 0–20 cm and pooled them together to form a composite sample of ~200 g per plot. We did not include soil litter and avoided roots, leaves, and other large plant organs. Composite samples were homogenized; from each sample, we took 15 g of soil and desiccated it immediately in sterile boxes with 40 g of silica gel[71]. Before the collection of each sample, all the sampling equipment underwent strict decontamination protocols (burned at >1,000°C with a portable blow torch). In all countries, sampling was performed during the warmest season (*e.g.*, late July-early September in temperate areas of the Northern hemisphere and February for temperate areas of the Southern hemisphere).

Environmental DNA from the soil samples was extracted in a dedicated laboratory using the NucleoSpin Soil Mini Kit (Macherey-Nagel), adding a preliminary step where the soil was mixed with 20 ml of phosphate buffer for 15 min[72] and we eluted eDNA in 150 µl of elution buffer. To control for contamination in the extraction room, we included one extraction control every ~10 samples (total: 101 extraction controls)[43]. We used the Sper01 primer pair[73](Forward: GGGCAATCCTGAGCCAA; reverse: CCATTGAGTCTCTGCACCTATC), which targets the P6 loop of the *trnL* intron in chloroplast DNA of Spermatophyta (seed plants). Amplicon size generally ranged from 10 to 220 bp (excluding the primers). We used reverse and forward primers that included 8-nucleotide-long tags on the 5′ end. Each tag had at least five nucleotide differences from the others, thus allowing bioinformatic discrimination of PCR replicates after sequencing[74]. DNA extracts were randomized in 96-well plates together with extraction controls, bioinformatic blanks (*i.e.*, tagging-system controls), PCR negative and positive controls (total across all plates: 291 blanks, 90 negative and 53 positive controls). In eDNA metabarcoding-based analyses, extraction and PCR negative controls are pivotal to monitor

contaminations, blanks allow identification of tag-jump issues, and positive controls allow monitoring of potential cross-contamination of samples, amplification and sequencing performance[43]. Positive controls consisted of a mock community composed of 16 non-tropical plant species belonging to 15 families (Taxaceae, Lamiaceae, Salicaceae, Polygonaceae, Betulaceae, Oleaceae, Pinaceae, Caprifoliaceae, Pinaceae, Aceraceae, Poaceae, Rosaceae, Brassicaceae, Geraniaceae, Ericaceae). Prior to amplification, we used quantitative PCR (qPCR) in a subset of samples to determine the optimal number of PCR cycles. We randomly selected 48 DNA samples and used 2 µl of undiluted or 1:10 diluted DNA, and 1 µl of 1:1,000 diluted SYBR Green I nucleic acid gel stain (Invitrogen), with a real-time PCR thermal cycler set to standard mode. Based on qPCR results and for all samples, we performed 45 amplification cycles of 2 µl undiluted DNA in a 20-µl reaction volume with 10 µl of AmpliTaq Gold 360 Master Mix 2X (Applied Biosystems), 2 µl of primers mix (5 µM of each primer) and 0.16 µl of bovine serum albumin (Roche Diagnostic).

PCR amplifications of samples were performed in 384-well plates and consisted of an initial step of 10 min at 95°C, followed by 45 cycles including 30 s denaturation at 95°C, 30 s annealing at 52°C, 60 s elongation at 72°C, and 7 min final elongation at 72°C. All samples and controls underwent four PCR replicates[75]. PCRs were performed in four distinct batches. All amplicons with a unique combination of forward and reverse tags within each batch were pooled. We used 5-µl aliquots of pooled amplicons to monitor the amplified fragment length and check for primer dimers using high-resolution capillary electrophoresis (QIAxcel Advanced System, Qiagen). Then, we purified six subsamples of the pooled amplicons using the MinElute PCR Purification Kit (Qiagen) following the manufacturer's protocol. Finally, we combined subsamples and sent them to Fasteris (Switzerland), where library preparation and sequencing were performed using the MetaFast protocol[74] and Illumina HiSeq platforms (paired-end approach, 2x150 bp), respectively.

The OBITools software suite[76] was used to perform the bioinformatic analyses of sequence data. First, forward and reverse reads were assembled with the *illuminapairedend* program and the *ngsfilter* program was used to assign sequences with an alignment score > 40 to the corresponding PCR replicate. Two mismatches on primers and zero mismatches on tags were allowed for this step. Then, we dereplicated sequences using the *obiuniq* program and filtered out those containing "N" and/or with an unexpected sequence length (*e.g.*, <10 bp) and singletons. Subsequently, the *obiclean* program was used to keep sequences present in at least one PCR and that were at least twice as abundant as other related sequences differing by one base (hereafter "head sequences"). This step permitted to remove PCR and sequencing errors. At this point, sequences from different experiments were concatenated into one file and clustered at a threshold of 97% sequence similarity using the SUMACLUST program (https://git.metabarcoding.org/obitools/sumaclust/wikis/home). This threshold was selected based on preliminary bioinformatics analyses as it represents the threshold minimizing the risk of merging different species in the same MOTU while avoiding splitting different sequences of the same species in different MOTUs[77]. Finally, we performed a taxonomic assignment of cluster heads based on the EMBL reference database (version 140). The reference database was built by carrying out an *in-silico* PCR with the *ecopcr* program[78]. Next, we assigned detected sequences to molecular operational taxonomic units (MOTUs) using the *ecotag* program, following the procedure described in Boyer *et al*.[76]. This program matches each sequence in the dataset against the reference database and then uses the lowest common ancestor algorithm to identify the taxonomic level of the assignment (*e.g.*, genus, family, order)[76]. To remove sequences detected at a low frequency that can be artefacts produced by PCR, contaminants, and sequencing errors[43,66], we performed additional filtering in R (version 4.0). Specifically, we discarded MOTUs with best identity < 90% and detected less than eight times in all samples, which corresponds to the minimum number of reads that removed ≥99.99% of sequences

detected in the blanks (*i.e.*, tag-jump errors). Then, we discarded MOTUs detected in only one sample, as they might represent singletons[79], MOTUs detected in <2 PCR replicates of the same sample, as they can represent false positives[75] and MOTUs detected in more than one extraction or PCR-negative control, as they might represent contaminants[43]. The complete codes and functions for bio-informatics and MOTU filtering are provided in Supplementary Scripts 1-3. See Table S5 for the number of sequences and MOTUs kept at each step of the procedure. Eleven of those MOTUs were removed because they were probably food contamination, as the corresponding families do not exist in the studied ecosystems and include species used as food (Table S2).

Traditional sampling

Even if the eDNA approach is emerging as a viable and reliable tool for sampling plant communities in soils[40,45,62,64], many features of the method remain poorly understood (*e.g*, the spatiotemporal scale[44]). To confirm the reliability of the obtained eDNA patterns, we thus compared them with the ones obtained from morphological plant surveys. To this aim, we gathered observational inventories of plant communities from 13 of the 46 glacier forelands that were sampled with eDNA (Fig. 1a). In three cases (Carihuairazo, Pasquale and Rutor, Table S1), floristic surveys were obtained from published studies performed in the same forelands where we collected eDNA; each of these studies sampled 4-5 dated sites per foreland. See refs.[20,54,80] for complete methodological aspects in these forelands. Literature data were complemented with inventories (Table S2) collected along the chronosequences of 10 additional forelands (Table S1). Along these chronosequences, 3 to 6 sampling sites corresponding to a given terrain age were sampled. Within each site, vascular plants were recorded in multiple plots within each site (1-9; mean ± SE: 3.4 ± 0.4 plots per site; depending on site surface and geomorphological heterogeneity; Table S1). Plots showed homogeneous altitudes, slopes, and aspects throughout their surface. All the plots within the same foreland

had the same size. In most of the forelands, the plot surface was 25 m^2 (Table S1), but in two forelands (Exploradores and Flaajokull), larger plots were used to better cover the geomorphological variability of the foreland (50-200 m^2; Table S1). Nevertheless, all results remain identical if the two forelands with larger plots were removed from the dataset (Table S6). Plots were located in dated sites, in the central portion of the foreland, *i.e.*, in front of the terminal part of the glacier tongue, avoiding disturbed areas (*e.g.*, those affected by glacial streams), as well as steep and unstable slopes. In each plot, we recorded every occurring vascular plant species; species that could not be identified on the field were collected and identified with the aid of identification keys for the local flora and with the aid of local experts, when necessary. Field sampling took into account all the vascular plants, *i.e.*, Angiosperms, Gymnosperms, and Pteridophytes (ferns, clubmosses, and horsetails) but, to enable comparison with eDNA data, Pteridophytes were not included into analyses.

Overall, by combining literature data with original data we gathered traditional data from forelands located in the Andes (n=2), the Alps (n=8), Iceland (n=1), Nepal (n=1), and Norway (n=1). The overall dataset included 57 dated sites (time since glacier retreat ranging 1-419 years), where vascular plant communities were traditionally inventoried.

β-diversity measures

For both methods, we combined the inventories obtained in all of the plots from the same dated site to recover comprehensive biodiversity inventories[81]. For eDNA and traditional data separately, we calculated the total dissimilarity (β-total) between communities within the same glacier foreland using the Jaccard's index based on presence/absence matrices. We then used the approach of Carvalho et al.[46] to decompose β-total into β-replacement and β-richness, and to quantify the relative importance of those two processes. β-richness represents the richness

differences between compared communities associated with taxa losses and gains, irrespective of nestedness[46,82]. β-diversity partitioning was performed through the *BAT* R package[86].

Baselga et al.[83] proposed an alternative approach to partition β-diversity, thus we also partitioned β-diversity into turnover (*i.e.,* replacement of some taxa by others between communities) and nestedness (*i.e.,* richness differences where a community is a strict subset of a broader community) following the Baselga et al.[83] approach, through the *betapart* package[84]. With this approach, most of dissimilarity was attributed to turnover for both eDNA and traditional data (average β-diversity attributed to turnover: 88% and 70% respectively, Fig. S7), while nestedness showed limited importance (12% and 30% with eDNA and traditional data, respectively, Fig. S7). Despite retrieving patterns similar to the ones obtained with the approach of Carvalho et al.[46] for traditional data (Fig. S6), for eDNA data the nestedness values were low and the total dissimilarity was mostly driven by the turnover component (Figs. S7 and S8). Thus, those measures were not considered because of the high collinearity between total β-diversity and turnover. Aligned with those results, it has been suggested that the approach of Baselga et al.[83]underestimates nestedness and overestimate the turnover component, especially when the number of shared taxa between the compared communities is very low and when richness differences between communities are large[46,82,85]. Specifically, when two compared communities have no species in common but still present richness differences, the nestedness will be equal to zero and all of the dissimilarity will be attributed to the turnover components, as they are additive. Such issues may be specially marked when assessing diversity with MOTUs (which is often the case for eDNA studies on a large geographic scale) and Carvalho's approach appeared to be less sensitive to these issues.

Seven of the 13 chronosequences sampled with traditional sampling showed an important variability in the number of sampled plots per site (Table S1). We accounted for this unbalanced sampling by using a subsampling procedure to calculate β-diversity and its

components between the sites of each chronosequence. First, we determined the minimum number of sampled plots per site '*N*'. Second, for sites having a number of plots > *N*, we randomly selected *N* plots. Third, we calculated β-diversity between sites, only considering the *N* randomly selected plots. We repeated this procedure 999 times and calculated the mean value of β-diversity and its components for each pair of compared communities. We run the main Bayesian models with these measures. Full details and the R code used are provided in Supplementary script 4.

Statistical analyses

First, we tested whether β-replacement and β-richness provide an overall different contribution to the β-diversity of pair of communities. Two-sample randomization tests for paired samples were used to asses if the observed β-replacement is significantly greater or lower than β-richness. To do this, we first calculated the mean difference between β-replacement and β-richness for each site, and then compared it to values expected under randomness[87]. Expected values were obtained by reshuffling the data 10,000 times across two random groups and calculating the mean difference between groups across permutations. For this analysis, we used the *EnvStats* package, considering a two-sided alternative hypothesis. We also used this approach to test the differences between turnover and nestedness (Fig. S7).

Subsequently, we used Bayesian GLMMs to assess how the different β-diversity measures varied over succession. We considered two predictor variables: the age differences between compared communities and the mean age between them. We ran three GLMMs per sampling method; each GLMM included a different measure of β-diversity as a dependent variable (*i.e.,* β-total, β-replacement, and β-richness differences). The glacier identity, and the identity of each site involved in the comparison, were included as random intercepts [β ~ age differences + mean age + (1|glacier) + (1|site1) + (1|site2)]. Age differences and mean ages

were log-transformed and then scaled (mean = 0, SD = 1) to allow comparison of their estimated effects. Models were run assuming a Beta distribution for all the response variables. β-diversity variables were rescaled to avoid fixed zeros and ones according to Smithson & Verkuilen[88] [(value * (N-1)+0.5)/ N; with N= number of observations]. Three Markov chain Monte Carlo chains using 10,000 iterations and a burn-in of 5,000 were run in the *brms* R package[89]. For all models, $\hat{c}$ was <1.01, indicating convergence. We interpreted a strong contribution of a predictor variable to the β-diversity measures if the 95% CIs of a parameter's posterior distribution did not overlap zero. The interaction between age difference and mean age was tested, but it was not relevant for any measure of β-diversity, so we kept the models without interaction. In the main results, we show the random intercept models, as models including glacier identity as both random intercepts and slopes did not show a substantial decrease in the WAIC (Widely Applicable Information Criterion) compared to models with random intercept only (except for the β-Richness and β-total models with traditional data, Table S7). All the effects remain identical if random slope models are used instead of random intercept models (Table S8). To illustrate the main results (Figure 3), we kept all the available comparisons but traditional data and eDNA data covered different temporal ranges. eDNA data ranged from 1-475 years and 2-350 for age differences and mean ages, respectively, while traditional data ranged from 4-397 years and 7.5-281.5 for age differences and mean ages, respectively. See Figure S9, where eDNA comparisons having age differences and mean ages outside of the ranges covered by traditional data were removed, for a visualization of the results of the main models within a common temporal range.

To assess threshold dynamics over time, we used segmented regressions[90,91]. Specifically, we checked for the existence of thresholds in the relationships of β-total, β-richness, and β-replacement with mean age. We used maximum likelihood to build linear mixed models with one breakpoint (*segmented*[90] package in R); glacier identity and cross-site identity

were included as random factors. Models with the breakpoint were compared with linear mixed models without the breakpoint based on the BIC. Simulations performed following the framework of Ficetola & Denoel[91] confirmed that this approach can detect different threshold typologies in datasets with features similar to the ones analyzed here (Figures S10 and S11). We used the packages ggplot2, cowplot, ggpubr and ggcorrplot to create figures and stringr reshape2 to format data.

Permanent plots to support conclusions drawn from the chronosequence approach

Two approaches have been most commonly used to study plant succession in glacier forelands: the chronosequence approach and temporal data obtained from permanent plots. The chronosequence approach represents a space-for-time substitution to infer temporal changes in vegetation dynamics through contemporary spatial patterns. This approach is the most widely used, as it is the only one enabling us to reconstruct long-term (>100 years) trends of ecosystem development[67,92]. In successions after glacier retreat, the chronosequence approach uses the assumption that communities established in terrains that are ice-free since longer times underwent a longer development time. However, the ability of chronosequences to reflect reliable temporal patterns has been questioned[38] (but see[67]). For instance, the method might misestimate the relative contribution of replacement and taxa addition if the landscape context strongly differs between sites along the chronosequences. Therefore, we used data obtained in permanent plots set up by Fickert & Grüninger[93] to validate the conclusions obtained through our analysis of chronosequences. Fickert & Grüninger[93] sampled 12 permanent plots during the first decade after the deglaciation of two glaciers in the Alps: Goldbergkees (Austria) and Lenksteinferner (Italy). Vascular plants in the plots were sampled with traditional methods every two years from 2005 to 2015. We used this compositional data from permanent plots to calculate and decompose β-diversity between communities using Carvalho et al.[46]'s approach.

On average, taxa addition contributed to 85% of total β-diversity, whereas replacement only to 15% (Extended data Figure 1a). Randomization tests for paired samples showed that β-richness and β-replacement were significantly different ($p<0.001$), with a pattern similar to the one observed with chronosequences. Furthermore, the importance of addition decreases over time, even if the trend is less marked than for our main results (Extended data Figure 1b). Overall, the analysis of permanent plots showed findings highly consistent with the one of chronosequences.

Data availability

Raw sequence data (Sper01 marker) generated using the protocols described in the "Methods" section is deposited in the "Sper01_raw_sequences.zip" folder available at the Zenodo link (https://zenodo.org/record/6620359#.Y8E1OP6ZO5d) . The data that support the findings of this study are provided as supplementary tables (Tables S1-S3).

Code availability

Codes for reproducing the results in this study are available as Supplementary scripts.

Supplementary script 1: Code reproducing bioinformatics steps.

Supplementary script 2: Code reproducing taxonomic assignation.

Supplementary script 3: R code for the MOTU filtering after bio-informatic analyses to remove sequences with best identity < 90% and detected at a low frequency that can be artefacts produced by PCR, contaminants, and sequencing errors.

Supplementary script 4: R code to calculate beta-diversity and its components, run the main models, and illustrate results.

Supplementary script 5: R code to test the ability of our sampling design to detect breakpoints in segmented regressions.

Acknowledgments

This study is funded by the European Research Council under the European Community's Horizon 2020 Programme, Grant Agreement no. 772284 (IceCommunities) to IC, A.C., A.G., S.M., A.B., R.A., R.S.A., F.G., L.G., N.K., G.A.D., J.P., W.T., M.C. and G.F.F. This research was also funded by Biodiversa+, the European Biodiversity Partnership under the 2021-2022 BiodivProtect joint call for research proposals, co-funded by the European Commission (grant agreement no. 101052342 'PrioritIce-Vanishing habitats: conservation priorities for glacier-related biodiversity threatened by climate change') to IC, R.A., W.T., M.C., M.G. and G.F.F. and with the funding organisations MUR and ANR. We are grateful to Rüdiger Kaufmann, Antoine Guisan, Katrin Sieron and Marco Aurelio Morales-Martínez for help and discussions at various phases of this project.

Author Contributions Statement

I.C., M.C. and F.F. conceived, developed and wrote the paper, with input from A.C., R. A., F.A., S.C.F., M.G., A.R., A. Ze., P.T., J.P., and W.T.; I.C. performed the statistical analyses; A.G., S.M., A.B., F.G., and G.F.F. contributed to data preparation and curation; A.G., A.B., L.G., performed laboratory analyses; A.G., S.M., A.B., R.A., F.A., R.S.A., P.A., P.A.G., S.C.F., J.L.C.L, P.C., M.C.S., J.C., J.A.C.R., C.C., R.C.E., O.D., A.E., S.E., A.F., L.G., F.G., M.G, S.H., N.K., R.I.M., G.P., F.P., A.R., N.U., Y.Y., V.Z., A.Ze., A.Zi., G.A.D., J.P. M.C., and G.F.F. participated to sampling and the initial development of the study. All authors reviewed and/or provided input on the manuscript.

Competing Interests Statement

The authors declare no competing interests.

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Fig. 1: Sampling design. a) Global distribution of the 46 glacier forelands where plant communities were sampled in dated sites along chronosequences with eDNA from the soil (all circles). In 13 of those glacier forelands, we also gathered traditional plant inventories (purple and yellow circles). The background blue grid represents the number of glaciers for each 1 x 1° cell (www.glims.org) and ranges from 1 (pale blue) to 3,500 glaciers (darkest blue). b) Sampling scheme used for the eDNA approach. For each of the 46 forelands, we identified 3-17 sites along the chronosequences. Each site represents the past position of the glacier at known dates (each color indicates a site and its corresponding age class). For each site, we established ~5 plots (diamonds). Within each plot, we collected 5 soil subsamples (circles) within 1 m^2, the distribution of subsamples is shown by the pink inset; subsamples were pooled into one composite sample per plot. The taxa detected in the different plots of the same site were combined to inventor plant communities in each of the 266 sites. For each pair of sites from the same glacier foreland, we calculated the total β-diversity and its β-replacement and β-richness components, as well as the mean age and the age differences between the compared communities. Comparisons between sites from different forelands were not assessed. We show examples of the calculation of age differences and mean age variables for a subset of sites. We considered all the pairwise comparisons, including comparisons that do not correspond to directly subsequent age classes in the chronosequence.

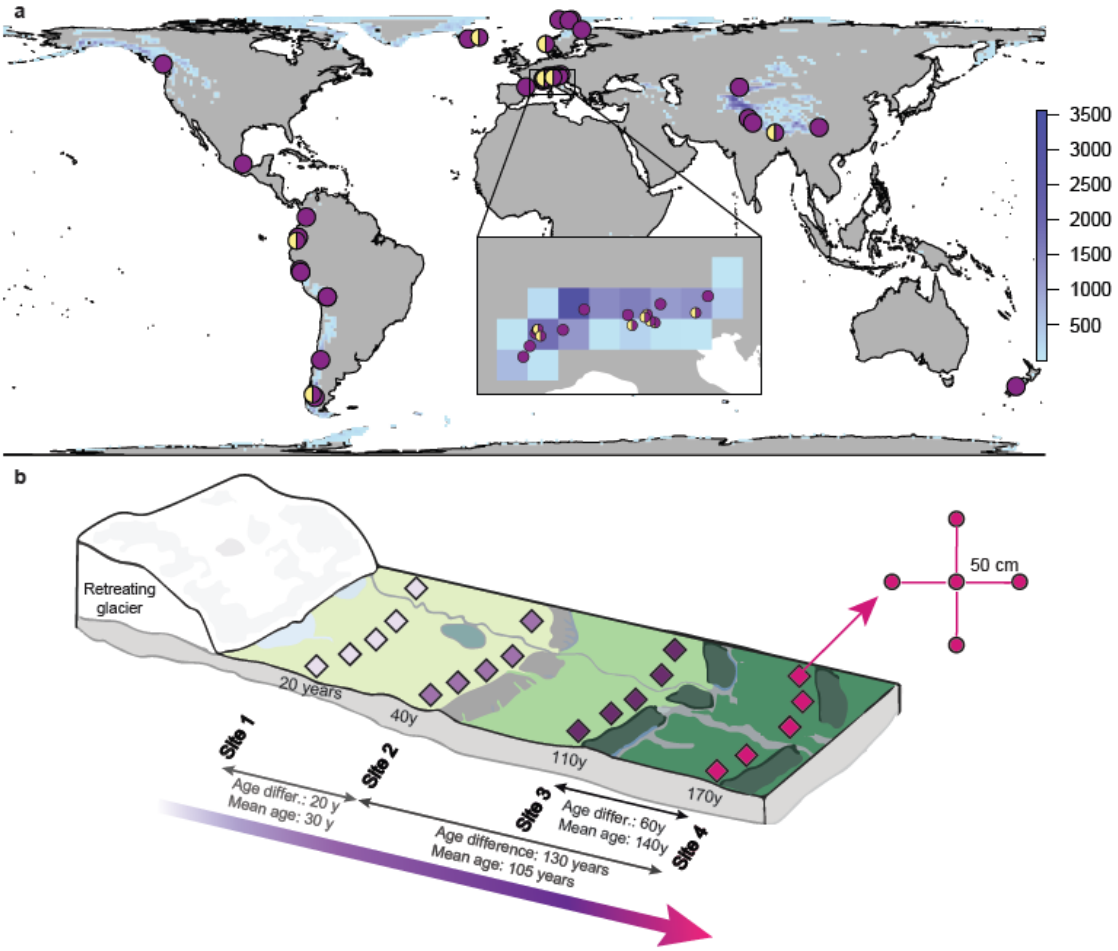

Fig. 2: β-diversity components obtained with eDNA (purple, 771 comparisons between communities within the same glacier foreland) and traditional methods (yellow, 102 comparisons). Boxplots indicate median (middle line), 25th, and 75th percentiles (box), as well as ranges of 1.5 * Interquartile range (whiskers) and outliers (dots). Diamonds indicate the mean values. *P* - values were obtained using two-sided randomization tests for paired samples assessing whether the differences between β-replacement and β-richness are significantly different.

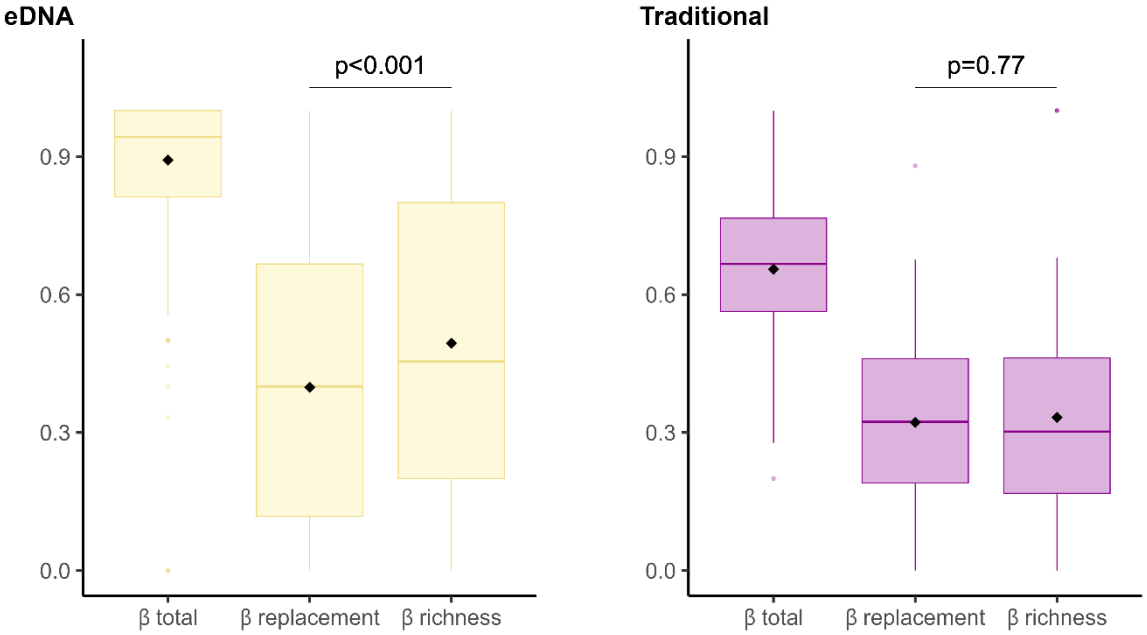

Fig. 3: Patterns of β-diversity components over plant succession following glacier retreat. Changes of total dissimilarity (β-total, grey), taxa replacement (β-replacement, dark green), and taxa addition (β-richness, light green) with age differences (a, c) and mean age (b, d) between compared communities. a-b patterns were obtained with eDNA data (771 comparisons); c-d patterns were obtained with traditional data (102 comparisons). Points represent the observed values and shaded areas are 95% CIs obtained from Bayesian GLMMs (see Methods). Parameters with 95% CIs non-overlapping and overlapping zero were represented with solid and dashed lines, respectively (see Table 1). The age differences between compared sites indicate how different the communities are, in terms of age, from each other. The mean age represents the average time since the glacier retreat of the compared communities.

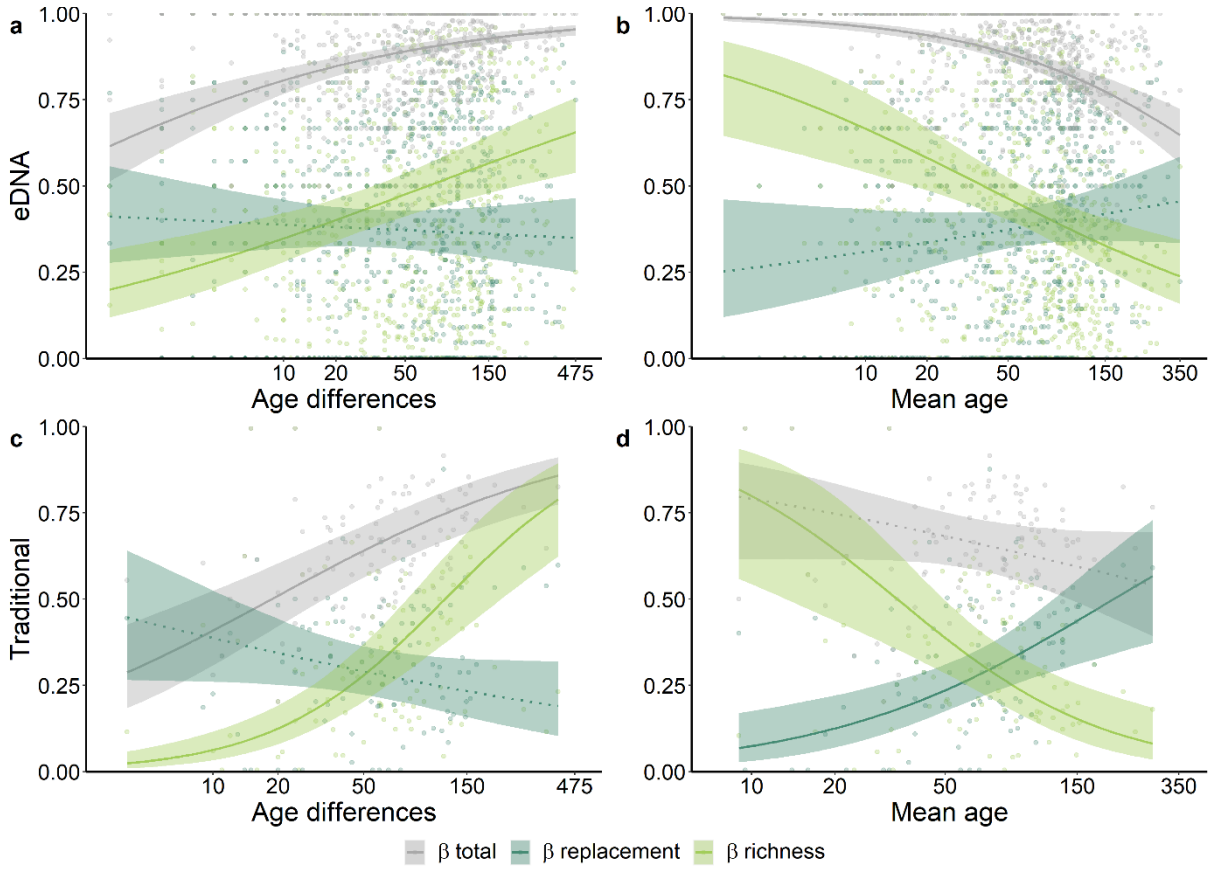

Fig. 4: Proportion of taxa present exclusively in communities with <50 years of succession ("Early colonizer"), taxa present exclusively in communities with > 50 years of succession ("Late colonizer"), and taxa present in both groups of communities ("Persister"). Taxa categorization was performed within the chronosequence of each glacier foreland.

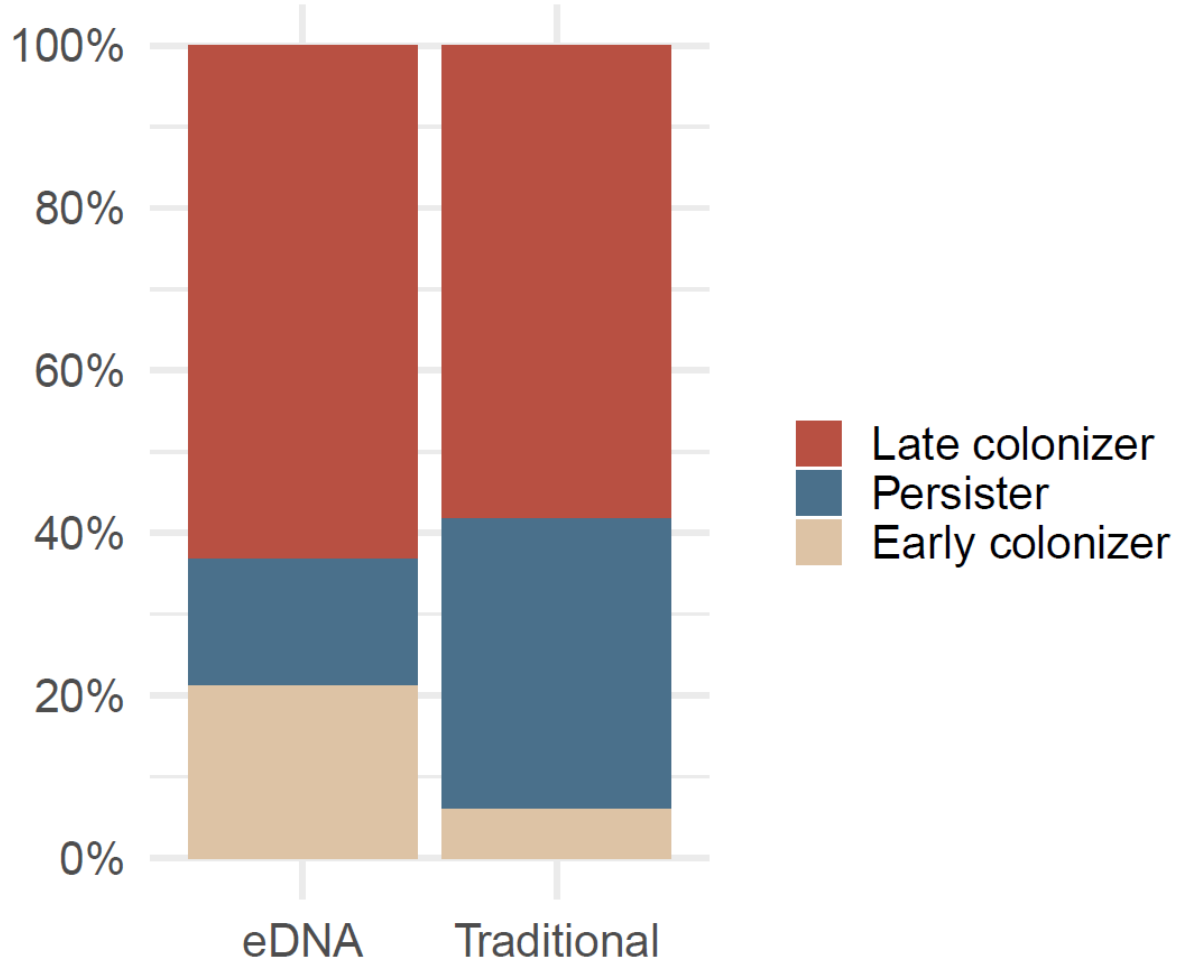

Fig. 2

Tables

Table 1: Results of the Bayesian generalized mixed models assessing the effects of time (mean age of compared sites) and age differences between compared sites on the different β-diversity measures. Glacier identity and cross-site identity of sites involved in the comparisons were included as random factors. Parameters with 95% CIs) non-overlapping zero are highlighted in bold. R^2_M: marginal R^2; R^2_C: conditional R^2.

		eDNA (*N*=771 comparisons)					Traditional (*N*=102 comparisons)				
Dependent variable	Independent variables	*B*	95%CI		R^2_M	R^2_C	*B*	95%CI		R^2_M	R^2_C
			Lower	Upper				Lower	Upper		
β-Total	Age differences	**0.5**	**0.4**	**0.6**	0.1	0.3	**0.5**	**0.3**	**0.6**	0.3	0.9
	Mean age	**-0.7**	**-0.8**	**-0.5**			-0.2	-0.5	0.02		
β-Richness	Age differences	**0.4**	**0.2**	**0.6**	0.05	0.6	**0.9**	**0.6**	**1.2**	0.3	0.8
	Mean age	**-0.5**	**-0.7**	**-0.2**			**-0.8**	**-1.1**	**-0.4**		
β-Replacement	Age differences	-0.05	-0.2	0.1	0.01	0.6	-0.2	-0.4	0.02	0.2	0.8
	Mean age	0.16	-0.08	0.4			**0.5**	**0.2**	**0.8**		