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

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A list of authors and their affiliations appears at the end of the paper

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 in 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 yr 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.

Ecological successions—how communities change or replace one another over time—have been a cornerstone of ecology since its inception[1,2]. Primary successions, that is, the development of ecosystems where a severe disturbance opens up large areas lacking most 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 homogeneous 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 past century, some studies showed homogeneous 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 ref. 12). These 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 (that is, 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 in plant succession is difficult to quantify as it may vary over the succession period, but also as a function 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

e-mail: isa_cantera@hotmail.com

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, these emerging ecosystems typically show sparse vegetation[18] dominated by few species that exhibit a high degree of specialization to live in these 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 studies[20,21] could have overemphasized the importance of addition.

In principle, species accumulation could result from the joint effect of dispersal-related processes (that is, 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 in 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 the β-diversity (that is, 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 yr); 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, that is, assemblages of taxa inhabiting a deglaciated site with a specific age since glacier retreat. eDNA metabarcoding allows the production of 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 analyse plant communities, some uncertainty persists on the correspondence between patterns obtained with this approach and those obtained with traditional sampling[39,44,45]. 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, we first quantified the total β-diversity (β-total) between communities within each foreland and assessed whether 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 (that is, richness differences between communities due to species gain) and β-replacement components (that is, the substitution of one taxon by another, without affecting species richness), and the temporal patterns of these components were assessed. Finally, we assessed whether the detected patterns are progressive or show breakpoints over successions[22].

Diversity of plant communities in glacier forelands

After data filtering and removal of likely contaminants, 519 molecular operational taxonomic units (MOTUs) of vascular plants were detected with eDNA metabarcoding across the dated sites of the 46 glacier forelands (Supplementary Tables 1 and 2). eDNA metabarcoding detected 0–60 MOTUs per site (mean ± s.e. = 11 ± 0.6; Supplementary Fig. 1a) and 5–150 MOTUs per foreland (mean = 36 ± 4.4). No plant MOTUs were detected at 17 sites, all of which were <32 yr old (Supplementary Table 1). Using traditional floristic surveys, 365 vascular plant taxa were detected across 57 dated sites from 13 glacier forelands (Supplementary Tables 1 and 3), with 0–133 (mean = 24 ± 3) taxa per site (Supplementary Fig. 1a) and 15–162 taxa (mean = 48 ± 10) per foreland. Traditional sampling did not show any vascular plant species only in one site, which was deglaciated since just 2 yr (Carihuairazo glacier; Supplementary Table 1).

To assess the variation in community composition over time, as well as the relative contribution of taxa addition versus replacement to 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 significantly 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 in β-diversity components over succession

Bayesian generalized linear mixed models (GLMMs) were used to assess how the β-diversity components varied over succession, 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 (for example, early versus early or late versus late), while high values represent comparisons between communities at very different successional stages (for example, 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 and 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

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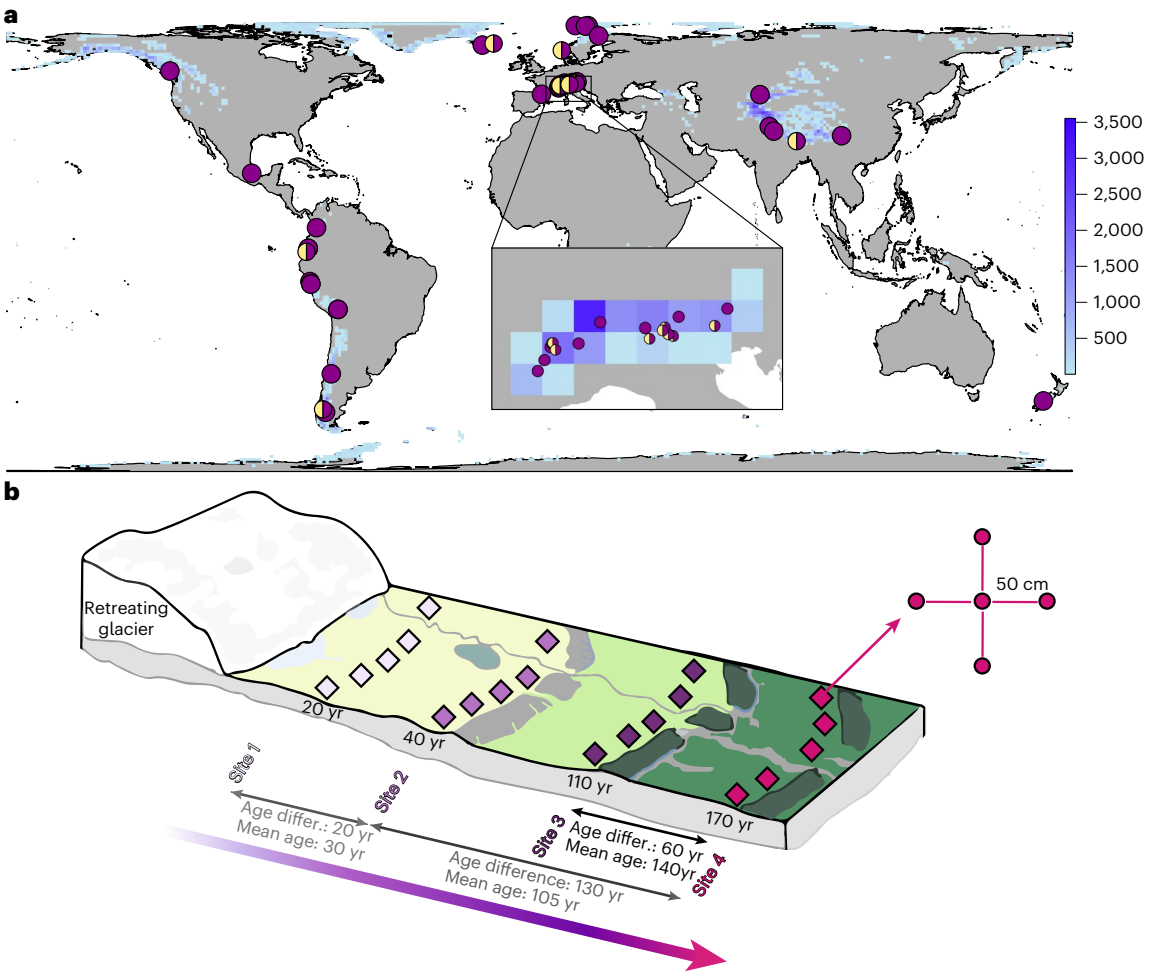

Fig. 1 | Sampling design. a, Global distribution of the 46 glacier forelands where plant communities were sampled in dated sites along chronosequences with eDNA from the soil (all circles). In 13 of these glacier forelands, we also gathered traditional plant inventories (purple and yellow circles). The background blue grid represents the number of glaciers for each 1 × 1° cell (www.glims.org) and ranges from 1 (pale blue) to 3,500 glaciers (darkest blue) (see colour bar). **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 colour indicates a site and its corresponding age class). For each site, we established ~5 plots (diamonds). Within each plot, we collected 5 soil subsamples within 1 m^2 (subsamples distribution 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.

negative for eDNA data and slightly overlapping zero for traditional data; Fig. 3b–d and 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 and Table 1), while it decreased with the mean age of compared communities (Fig. 3b–d and 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 so for traditional data (Fig. 3a–c and 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 and Table 1). Despite some differences across forelands, the relationships remained consistent when we excluded the forelands with a small number of sites (<8 sites per foreland; Supplementary Figs. 2 and 3). The pattern also remained consistent when we repeated the analyses using Sørensen's instead of Jaccard's index to calculate dissimilarities (Supplementary Fig. 4) and when we excluded rare MOTUs (Supplementary Fig. 5).

β-total, β-richness and β-replacement changed steadily over time, given that segmented regressions did not reveal significant breakpoints for the relationships between these variables and mean age (all $P > 0.05$). Models with breakpoints also showed higher Bayesian information criterion values compared with the linear ones (Supplementary Table 4).

Discussion

Predicting ecosystem responses to disturbance events and environmental changes requires understanding of 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 483 yr after deglaciation, both mechanisms provided an overall similar contribution to compositional

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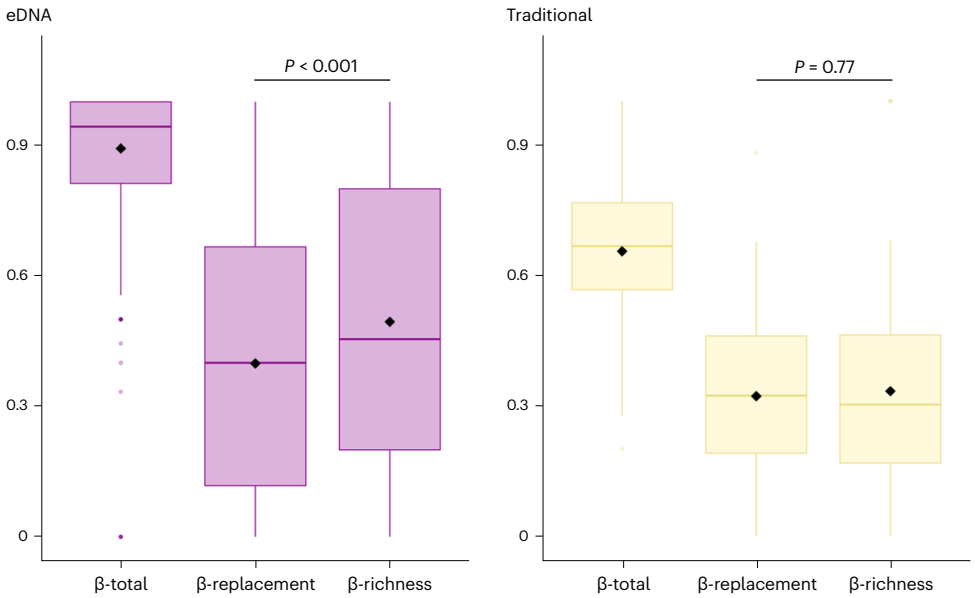

Fig. 2 | β-diversity components obtained with eDNA and traditional methods. Left: components obtained with eDNA (purple, 771 comparisons between communities within the same glacier foreland). Right: components obtained with traditional methods (yellow, 102 comparisons). Boxplots indicate median (middle 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 significant.

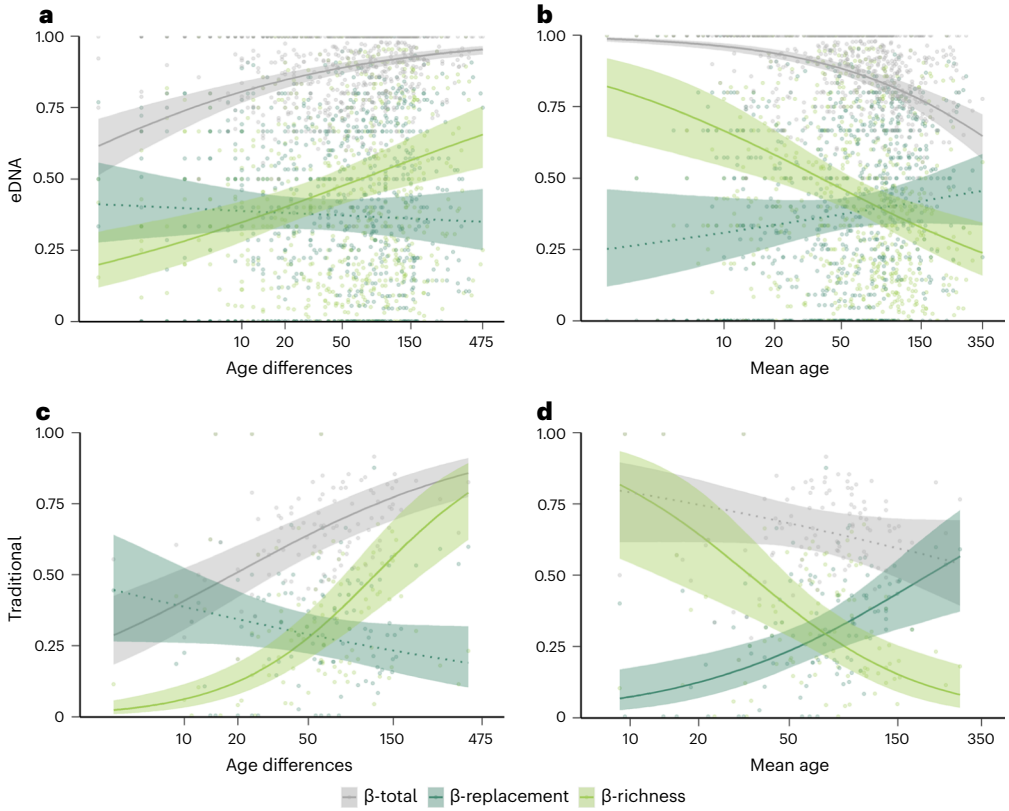

Fig. 3 | Patterns of β-diversity components over plant succession following glacier retreat. Changes in total dissimilarity (β-total, grey), taxa replacement (β-replacement, blue) and taxa addition (β-richness, green) with age differences (**a**,**c**) and mean age (**b**,**d**) between compared communities. **a**,**b**, Patterns obtained with eDNA data (771 comparisons). **c**,**d**, Patterns obtained with traditional data (102 comparisons). Points represent observed values and shaded areas are 95% CIs obtained from Bayesian GLMMs (see Methods). Parameters with 95% CIs overlapping or not overlapping zero are represented with dashed or solid 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.

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Table 1 | Results of the Bayesian GLMMs assessing the effects of age differences between compared sites and time (mean age of compared sites) on the different β-diversity measures

Dependent variable	Independent variables	eDNA (*N*=771 comparisons)					Traditional (*N*=102 comparisons)				
		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	0.03		
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**			**−0.7**	**−1.1**	**−0.4**		
β-replacement	Age differences	−0.05	−0.2	0.1	0.01	0.6	−0.2	−0.4	0.01	0.2	0.8
	Mean age	0.2	−0.08	0.4			**0.5**	**0.2**	**0.8**		

Glacier identity and cross-site identity of sites involved in the comparisons were included as random factors. Parameters with 95% CIs not overlapping zero are highlighted in bold. R^2_M, marginal *R*; R^2_C, conditional *R*.

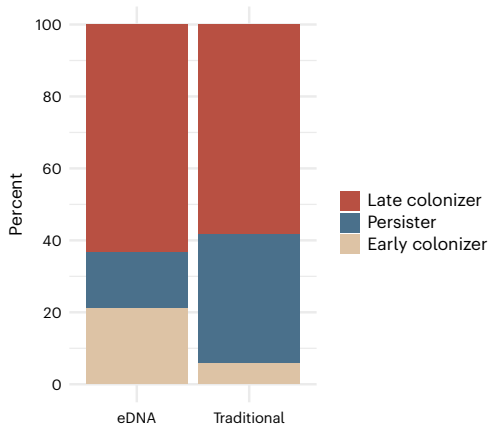

Fig. 4 | Proportion of early colonizers, persisters and late colonizers in glacier forelands. Percentages of taxa present exclusively in communities with ≤50 yr of succession ('Early colonizer'), taxa present exclusively in communities with >50 yr of succession ('Late colonizer') and taxa present in both groups of communities ('Persister'). Taxa categorization was performed within the chronosequence of each glacier foreland.

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 glacier 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, with replacement becoming dominant after >50 yr 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 and 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 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 yr of succession (Fig. 4). Such apparent incongruence could be explained by the ambiguity of the concept of 'severe' or harsh' environment. These terms apply to limiting conditions both linked to climate (conditions in high altitudes and/or latitudes) and to specific microhabitats 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-growing and opportunistic species adapted to alpine environments[36,52], with the gradual addition of more dispersal-limited species[32,53]. Later, these species are followed by mountain specialists that are more competitive because they are shade-resistant and/or exploit nutrients efficiently[24,34,36,51,54].

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

When we compared communities in early-successional stages (having on average <50 yr), richness differences contributed more than replacement in determining the dissimilarity between communities (Fig. 3a–b). Immediately after glacier retreat, soils are generally nutrient poor and affected by surface instability, but early colonizers do not inhibit the establishment of new colonizing taxa[1]. This may be explained either by neutral interactions (due to the predominant role of the environment[57] or of stochastic processes[9,10]) in these species-poor early stages, or by facilitative interactions[1] where the beneficiary species are not constraining the already established ones[58]. However, the importance of taxa addition quickly decreased over time and replacement became the dominant pattern for late-successional stages, suggesting 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 with 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 yr followed by a plateau and a decline in total

β-diversity[22,59,60]. However, in our study, the trends in β-diversity and its components during succession did not exhibit significant breakpoints (Supplementary Table 4), 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 (for example, 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 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 (Supplementary Fig. 1b) despite eDNA generally detecting fewer taxa per site. Similar to 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 DNA present in environmental samples[43], potentially favouring abundant taxa[62–64]. Amplifying rare taxa in soil samples can be particularly challenging, given the low diffusion rates of DNA in the soil and the presence of inhibitors[44,65]. Increasing the number of subplots per site, PCR replicates and sequencing depth can improve the detection of rare species. In most forelands, very recent sites without visible vegetation were not sampled by traditional methods but were sampled using eDNA. This might also contribute to the lower number of plants detected on average by eDNA. Another possible issue is that soil eDNA can detect past plant species[44]. However, the signal of these past species is weaker than that of current plant communities[40,41,44], and eDNA inventories generally better match traditional inventories of current plant communities than those of older ones[39]. Despite these potential limitations, eDNA yielded temporal patterns extremely similar to those obtained by traditional methods, confirming that the former provides reliable diversity estimates, particularly for β-diversity[66].

Some studies have questioned the robustness of the conclusions obtained from the analysis of chronosequences. Sites placed at similar distances from the glacier margin can differ from each other due to microhabitat conditions or the identity of first colonizers (priority effects), potentially following different trajectories[38,67]. Nevertheless, the analysis of temporal data from permanent plots yielded patterns of β-richness and β-replacement highly consistent with our results (see Methods and Extended data Fig. 1), confirming the robustness of conclusions drawn from the chronosequence approach. Moreover, the chronosequence approach assumes that there are no disturbances over succession and that terrain age is the community age, which is not always the case (see ref. 17). Even if we avoided sites clearly affected by geomorphological disturbances, glacier forelands are dynamic landscapes where disturbances might interact with temporal patterns according to their frequency and magnitude[17]. Indeed, despite retrieving general patterns of β-diversity changes, our data exhibited high variability, suggesting that other site-level environmental conditions, such as microclimate, soil properties and perturbations[13,17,47], can affect the dissimilarity between sites. 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 past 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, and competition becoming 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 in 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), eDNA was captured from the soil in 1,255 plots during 2014 to 2020 using a previously published protocol[70]. In the sampled forelands, information on the dates of glacier retreat is available from the literature, remote sensing images and field surveys[71]. 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 (Fig. 1b). 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[71]. We avoided sites clearly affected by geomorphological disturbance. Within each site, we sampled 2–10 plots (mean = 5, s.e. = 0.05; Supplementary Table 1). The plots within each site had similar distances to the glacier forefront and were, if possible, regularly spaced at distances of ~20 m (Fig. 1b). At each plot, we collected five soil subsamples within 1 m (Fig. 1b) at a depth of 0–20 cm and pooled them 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[72]. Before the collection of soil, all the sampling equipment underwent strict decontamination protocols (burned at >1,000 °C with a portable blow torch). In all countries, sampling was performed around the warmest season (for example, late July to early September in temperate areas of the Northern Hemisphere and February for temperate areas of the Southern Hemisphere).

Environmental DNA from the composite 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[73], 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[74] (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[75]. DNA extracts were randomized in 96-well plates together with extraction controls, bioinformatic blanks (that is, 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 for monitor contaminations, blanks allow identification of tag-jump issues and positive controls allow monitoring of potential cross-contamination of samples, as well as 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). Before 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. On the basis of 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 Ampli*Taq* 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[76]. 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 manufacturer protocol. Finally, we combined subsamples and sent them to Fasteris (Switzerland) where library preparation and sequencing were performed using the MetaFast protocol[75] and Illumina HiSeq platforms (paired-end approach, 2×150 bp), respectively.

The OBITools software suite[77] was used to perform the bioinformatic analyses of sequence data. First, forward and reverse reads were assembled with the 'illuminapairedend' programme, and the 'ngsfilter' programme 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' programme and filtered out those containing 'N' (representing that the nucleotide could not be determined) and/or with an unexpected sequence length (for example, <10 bp) and singletons. Subsequently, the 'obiclean' programme 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 removal of 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' programme (https://git.metabarcoding.org/obitools/sumaclust/wikis/home). This threshold was selected on the basis of preliminary bioinformatics analyses, as it represents the threshold minimizing the risk of merging different species in the same MOTU while avoiding splitting of different sequences of the same species in different MOTUs[78]. Finally, we performed a taxonomic assignment of cluster heads on the basis of the EMBL reference database (v.140). The reference database was built by carrying out an in silico PCR with the 'ecopcr' programme[79]. Next, we assigned detected sequences to MOTUs using the 'ecotag' programme, following a previously described procedure[77]. This programme 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 (for example, genus, family, order)[77]. To remove sequences detected at a low frequency that can be artefacts produced by PCR, contaminants and sequencing errors[43,66], we performed an additional filtering in R (v.4.3.1). 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 (that is, tag-jump errors). Then, we discarded MOTUs detected in only one sample, as they might represent singletons[80]; MOTUs detected in <2 PCR replicates of the same sample, as they can represent false positives[76]; and MOTUs detected in more than one extraction or PCR-negative control, as they might represent contaminants[43]. The complete codes and functions for bioinformatics and MOTU filtering are provided in Supplementary Codes 1–3. See Supplementary Table 5 for the number of sequences and MOTUs kept at each step of the procedure. Eleven of these 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 (Supplementary Table 2).

Traditional sampling

Even if the eDNA approach is emerging as a viable and reliable tool for sampling plant communities in soils[40,62,64,45], some features of the method remain poorly understood (for example, 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; Supplementary Table 1), 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. 20,54,81 for complete methodological aspects in these forelands. Literature data were complemented with inventories (Supplementary Table 3) collected along the chronosequences of 10 additional forelands (Supplementary Table 1). 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 (1–9, mean ± s.e.: 3.4 ± 0.4 plots per site, depending on site surface and geomorphological heterogeneity; Supplementary Table 1). Plots showed homogeneous altitudes, slopes and aspects throughout their surface. All plots within the same foreland had the same size. In most of the forelands, the plot surface was 25 m^2 (Supplementary Table 1), but in two forelands (Exploradores and Flaajokull), larger plots were used to better cover the geomorphological variability of the foreland (50–200 m^2; Supplementary Table 1). Nevertheless, all results remain identical if the two forelands with larger plots were removed from the dataset (Supplementary Table 6). Plots were located in dated sites in the central portion of the foreland, that is, in front of the terminal part of the glacier tongue, avoiding disturbed areas (for example, 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 in 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, that is, angiosperms, gymnosperms and pteridophytes (ferns, clubmosses and horsetails); however, to enable comparison with eDNA data, pteridophytes were not included in the 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 from 2 to 419 yr) 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[82]. 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 in ref. 46 to decompose β-total into β-replacement and β-richness, and to quantify the relative importance of these two processes. β-richness represents the richness

differences between compared communities associated with taxa losses and gains, irrespective of nestedness[46,83]. We additionally partitioned β-diversity into turnover (that is, replacement of some taxa by others between communities) and nestedness (that is, richness differences where a community is a strict subset of a broader community) following the approach in ref. 84. Despite retrieving patterns similar to the ones obtained with the approach in ref. 46 for traditional data (Supplementary Fig. 6), for eDNA data, the nestedness values were low and the total dissimilarity was mostly driven by the turnover component (see Supplementary Note 1 and Figs. 7 and 8). In fact, the method has been found to underestimate richness differences and overestimate the turnover component[46,83,85], which may be specially marked when assessing diversity with MOTUs (which is often the case for eDNA studies on a large geographic scale); the approach in ref. 46 appeared to be less sensitive to these issues. β-diversity partitioning was performed with the approach in ref. 46 through the 'BAT' R package[86], while the 'betapart' package[87] was used for partitioning as proposed in ref. 84.

Seven of the 13 chronosequences sampled with traditional sampling showed an important variability in the number of sampled plots per site (Supplementary Table 1). 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 ran the main Bayesian models with these measures. Full details and the R code used are provided in Supplementary Code 4.

Statistical analyses

First, we tested whether β-replacement and β-richness provide an overall different contribution to the β-diversity of pairs of communities. Two-sample randomization tests for paired samples were used to assess whether 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[88]. 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 (Supplementary Note 1).

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 (that is, 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 ($\beta \approx$ age differences + mean age + (1|glacier) + (1|site1) + (1|site2)). Age differences and mean ages were log-transformed and then scaled (mean = 0, s.d. = 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 ref. 89 ((value × (N − 1) + 0.5) / N; with N being the 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[90]. 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 with models with random intercept only (except for the β-richness and β-total models with traditional data; Supplementary Table 7). All the effects remain identical if random slope models were used instead of random intercept models (Supplementary Table 8). To illustrate the main results (Fig. 3), we kept all the available comparisons, but traditional data and eDNA data covered different temporal ranges. eDNA data ranged from 1–475 yr and 2–350 yr for age differences and mean ages, respectively, while traditional data ranged from 4–397 yr and 7.5–281.5 yr for age differences and mean ages, respectively. See Supplementary Fig. 9 (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[91,92]. 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'[91] 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 on the basis of the Bayesian information criterion (BIC). Simulations performed following the framework in ref. 92 confirmed that this approach can detect different threshold typologies in datasets with features similar to the ones analysed here (Supplementary Figs. 10 and 11). We used the packages ggplot2, cowplot, ggpubr and ggcorrplot to create figures, as well as stringr, reshape2, reshape, rlist, plyr, dplyr and 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 yr) trends of ecosystem development[67,93]. 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 ref. 67). For instance, the method might mis-estimate the relative contribution of replacement and taxa addition if the landscape context strongly differs between sites along the chronosequences. Therefore, we extracted data obtained in permanent plots from ref. 94 to validate the conclusions obtained through our analysis of chronosequences. Ref. 94 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 2 yr from 2005 to 2015. We used this compositional data from permanent plots to calculate and decompose β-diversity between communities using the approach in ref. 46. On average, taxa addition contributed to 85% of total β-diversity, whereas replacement contributed only to 15% (Extended Data Fig. 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 Fig. 1b). Overall, the analysis of permanent plots showed findings highly consistent with the analysis of chronosequences.

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
Raw sequence data (Sper01 marker) generated using the protocols described in the 'Methods' section are deposited in the 'Sper01_raw_sequences.zip' folder available in Zenodo (https://zenodo.org/record/6620359#.Y8E1OP6ZO5d)[95]. The data that support the findings of this study are provided as Supplementary Tables 1–3.

Code availability
Scripts for reproducing the results in this study are available as Supplementary codes.
Supplementary Code 1. Code reproducing bioinformatics steps.
Supplementary Code 2. Code reproducing taxonomic assignation.
Supplementary Code 3. R code for the MOTU filtering after bioinformatic 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 Code 4. R code to calculate beta-diversity and its components, run the main models and illustrate results.
Supplementary Code 5. R code to test the ability of our sampling design to detect breakpoints in segmented regressions.

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Author contributions

I.C., M.C. and G.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. Zerboni, 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. and 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. Zerboni, A. Zimmer, G.A.D., J.P. M.C. and G.F.F. participated in sampling and the initial development of the study. All authors reviewed and/or provided input on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Isabel Cantera.

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Isabel Cantera[1] ✉, Alexis Carteron[1,2], Alessia Guerrieri[1,3], Silvio Marta[1,4], Aurélie Bonin[1,3], Roberto Ambrosini[1], Fabien Anthelme[5], Roberto Sergio Azzoni[1,6], Peter Almond[7], Pablo Alviz Gazitúa[8], Sophie Cauvy-Fraunié[9], Jorge Luis Ceballos Lievano[10], Pritam Chand[11], Milap Chand Sharma[12], John Clague[13], Justiniano Alejo Cochachín Rapre[14,33], Chiara Compostella[6], Rolando Cruz Encarnación[14], Olivier Dangles[15], Andre Eger[16], Sergey Erokhin[17], Andrea Franzetti[18], Ludovic Gielly[19], Fabrizio Gili[1,20], Mauro Gobbi[21], Sigmund Hågvar[22,23], Norine Khedim[24], Rosa Isela Meneses[25,26], Gwendolyn Peyre[27], Francesca Pittino[18], Antoine Rabatel[28], Nurai Urseitova[17], Yan Yang[29], Vitalii Zaginaev[30], Andrea Zerboni[6], Anaïs Zimmer[31], Pierre Taberlet[19,23], Guglielmina Adele Diolaiuti[1], Jerome Poulenard[15], Wilfried Thuiller[19], Marco Caccianiga[32] & Gentile Francesco Ficetola[1,19]

[1]Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, Milano, Italy. [2]Université de Toulouse, Ecole d'Ingénieurs de Purpan, UMR INRAE-INPT DYNAFOR, Toulouse, France. [3]Argaly, Bâtiment CleanSpace, Sainte-Hélène-du-Lac, France. [4]Institute of Geosciences and Earth Resources, CNR, Pisa, Italy. [5]Laboratory AMAP, IRD, University of Montpellier, CIRAD, CNRS, INRA, Montpellier, France. [6]Dipartimento di Scienze della Terra 'Ardito Desio', Milano, Italy. [7]Department of Soil and Physical Sciences, Lincoln University, Lincoln, New Zealand. [8]Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Osorno, Chile. [9]INRAE, UR RIVERLY, Centre de Lyon-Villeurbanne, Villeurbanne, France. [10]Instituto de Hidrología, Meteorología y Estudios Ambientales IDEAM, Bogotá, Colombia. [11]Department of Geography, School of Environment and Earth Sciences, Central University of Punjab, VPO- Ghudda, Bathinda, Punjab, India. [12]Centre for the Study of Regional Development - School of Social Sciences, Jawaharlal Nehru University, New Delhi, India. [13]Department of Earth Sciences, Simon Fraser University, Burnaby, British Columbia, Canada. [14]Área de Evaluación de Glaciares y Lfagunas, Autoridad Nacional del Agua, Huaraz, Peru. [15]CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France. [16]Mannaki Whenua - Landcare Research, Soils and Landscapes, Lincoln, New Zealand. [17]Institute of Water Problems and Hydro-Energy, Kyrgyz National Academy of Sciences, Bishkek, Kyrgyzstan. [18]Department of Earth and Environmental Sciences (DISAT), - University of Milano-Bicocca, Milano, Italy. [19]Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Grenoble, France. [20]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy. [21]Research and Museum Collections Office, Climate and Ecology Unit, MUSE-Science Museum, Trento, Italy. [22]Faculty of Environmental Sciences and Natural Resource Management (INA), Norwegian University of Life Sciences, Ås, Norway. [23]UiT – The Arctic University of Norway, Tromsø Museum, Tromsø, Norway. [24]Univ. Savoie Mont Blanc, Univ. Grenoble Alpes, EDYTEM, Chambéry, France. [25]Herbario Nacional de Bolivia: La Paz, La Paz, Bolivia. [26]Universidad Católica del Norte, Antofagasta, Chile. [27]Department of Civil and Environmental Engineering, University of the Andes, Bogotá, Colombia. [28]Univ. Grenoble Alpes, CNRS, IRD, Grenoble-INP, Institut des Géosciences de l'Environnement (IGE, UMR 5001), Grenoble, France. [29]Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu, China. [30]Mountain Societies Research Institute, University of Central Asia, Bishkek, Kyrgyzstan. [31]Department of Geography and the Environment, University of Texas at Austin, Austin, TX, USA. [32]Dipartimento di Bioscienze, Università degli Studi di Milano, Milano, Italy. [33]Deceased: Justiniano Alejo Cochachín Rapre. ✉e-mail: isa_cantera@hotmail.com

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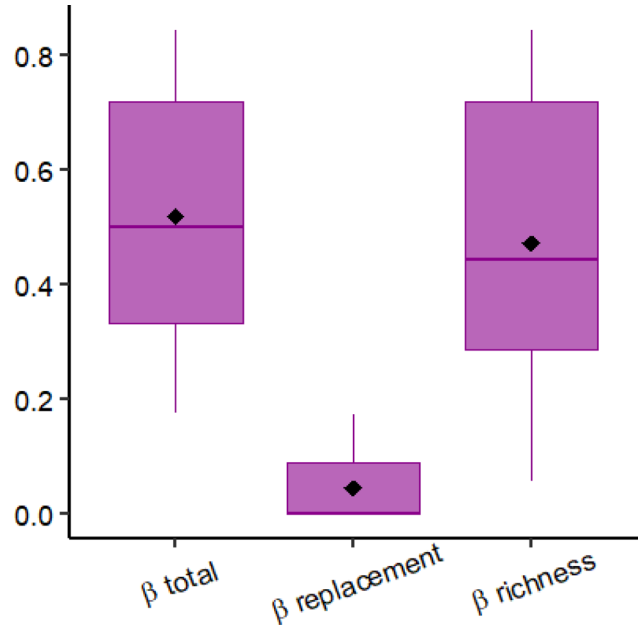
a



b

Dependent variable	Independent variables	*B*	95C.I.		85C.I.		$R^2_{Conditional}$	$R^2_{Marginal}$
			lower	upper	lower	upper		
β-total	Age differences	0.8	**0.4**	**1**	**0.5**	**0.8**	0.9	0.8
	Mean age	-0.4	-0.8	0.04	**-0.6**	**-0.1**		
β-richness	Age differences	0.8	**0.5**	**1.1**	**0.6**	**1**	0.9	0.8
	Mean age	-0.6	**-1.1**	**-0.05**	**-0.9**	**-0.3**		
β-replacement	Age differences	-0.1	-0.5	0.4	-0.4	0.2	0.6	0.4
	Mean age	0.5	-0.2	1.2	**0.05**	**1**		

Extended Data Fig. 1 | Patterns of β-diversity and its components over time measured with temporal data to support the conclusions based on the chronosequence approach. Data was obtained from Fickert & Grüninger[94], which sampled vascular plants with traditional methods in permanent plots during the first decade after the deglaciation of two glaciers in the Alps ($N = 30$ comparisons). **a**. β-diversity components. Boxplots indicate median (middle line), 25th, 75th percentiles (box), as well as 1.5 * interquartile range (whiskers) and outliers (dots). Diamonds indicate the mean values. **b**. Results of the Bayesian generalized mixed models assessing the effects of mean age and age differences between compared sites on the different β-diversity measures. Glacier identity and identity of sites involved in the comparisons were included as random factors. Parameters with 95% CI non-overlapping zero are highlighted in bold.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	Sequence data were analyzed using the OBITools software suite (Boyer et al. ,2016. doi:DOI: 10.1111/1755-0998.12428) and the SUMACLUST program (https://git.metabarcoding.org/obitools/sumaclust/wikis/home). As reference database for genetic assignement, we used EMBL release 140 (https://www.ebi.ac.uk/about/news/service-news/release-140-enas-assembledannotated-sequences-now-available-0) Statistical analyses were performed using R version 4.0.5 (2021-03-31)z. R packages: -BAT (2.8.1) and betapart (1.5.4) to measure and decompose beta-diveriisity. -brms (2.15.0) for Bayesian generalized linear mixed models and performance (0.8.0) to obtain R^2 of the models. -segmented (1.4.1) to to build linear mixed models with breakpoints. -ggplot2 (3.4.0); cowplot (1.1.1); ggpubr (0.4.0); and ggcorrplot (0.1.3) for figures. -stringr (1.4.0); reshape2 (1.4.4); reshape (0.8.8) ; rlist (0.4.6.2); plyr(1.8.8) ; dplyr (1.1.3) to format data. -EnvStats (2.8.1) test the differences on the contribution of β-richness and β-replacement, as well as between beturnover and nestedness Detailed description of methods is available in the methods sections: "eDNA sampling", "β-diversity measures" and "Statistical analyses".

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

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).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	not applicable
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
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Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We used both environmental DNA and traditional surveys to quantify the relative importance of species addition versus replacement in total beta-diversity of vascular plant communities colonizing glaciers forelands.
Research sample	We obtained data from 323 dated sites (eDNA campling: 266 sites; Traditional sampling : 57) covering 1-483 years since glacier retreat across 46 glaciers around the world. 2-9 sites per glacier forelands (average: 5) were selected along the chronosequences of glacier retreat (i.e., the chronological sequence of specific geomorphological features along deglaciated terrains for which the date of glacier retreat is known). A site represented one plant community and correspond to an age class indicating the past position of the glacier at known dates.
Sampling strategy	At each site, we established 1-20 plots regularly spaced for a more repeatable biodiversity estimate of the plant community. To characterize communities, 1255 plots and 258 plots were used for the eDNA and traditional analyses, respectively.
Data collection	eDNA data: At each plot, we collected five soil subsamples within one meter and at a depth of 0–20 cm and pooled them together to form a composite sample of ~200 g per plot. 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 and stored until molecular analyses. Environmental DNA from the soil samples was extracted in a dedicated room 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 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). We used the Sper01 primer pair, which targets the P6 loop of the trnL intron in chloroplast DNA of Spermatophyta (seed plants). Traditional data: We gathered observational inventories of plant communities from 13 of the 46 glacier forelands that were sampled with eDNA. In three cases (Carihuairazo, Pasquale, Rutor) 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. Literature data were complemented with unpublished data collected along the chronosequences of nine additional forelands. Within each site, vascular plants were recorded in multiple homogeneous plots within each site (1-9; mean ± SE: 3.2 ± 0.4 plots per site), depending on site surface and geomorphological heterogeneity. In most of the forelands, the plot surface was 25 m2 (Table S1). In two forelands (Exploradores and Flaajokull), larger plots were used to better cover the geomorphological variability of sites (50-200 m2; Table S1). 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. Plots showed homogeneous altitudes, slopes, and aspects throughout their surface. 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. 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.
Timing and spatial scale	From 2000 to 2020, we sampled 1255 soil samples in 46 glacier forelands epresentative of some of the main mountain chains of Europe, Asia, the Americas and Oceania and 258 plant vascular plant inventories located across 13 glacier forelands in the Andes (n=2), the Alps (n=8), Iceland (n=1), Nepal (n=1), and Norway (n=1).
Data exclusions	We excluded sites impacted by recent landslides / fluvial erosion. The eDNA from each plot was analysed in four replicated PCRs. We then removed MOTUs with best identity < 90%, detected in only one sample (as they represent singletons), MOTUs that were detected in <2 PCR replicates of the same sample, as they can represent false positives and MOTUs detected in less than 20 reads 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). The complete code and functions are provided in supplementary information (Supplementary Script 1). Finally, 11 MOTUs were removed from eDNA inventories because they were probably food contamination, as the families do not exist in the studied ecosystems and include species used as food. See Table S7 for the number of sequences and MOTUs kept at each step of the procedure. To enable comparison with eDNA data, Pteridophytes were not included in the traditional data analyses.
Reproducibility	The coordinates of sampled sites are available with the dataset, thus sampling is reproducible. The results presented in the manuscript were obtained by analysing the output of statistical models; all data and script are available and the results are thus fully reproducible. Data availability Raw sequence data generated using the protocols described in the "Material and Methods" section is deposited on Zenodo https://zenodo.org/record/6620359#.Y8E1OP6ZO5d. The data that support the findings of this study are provided as supplementary materials (Tables S1-S3). Scripts for reproducing the results in this study are available as Supplementary codes. Supplementary code 1: Code reproducing bioinformatics steps. Supplementary code 2: Code reproducing taxonomic assignation. Supplementary code 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 code 4: R code to calculate beta-diversity and its components, run the main models, and illustrate results. Supplementary code 5: R code to test the ability of our sampling design to detect breakpoints in segmented regressions.
Randomization	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).
Blinding	Site location was replaced by a code for molecular analyses. Therefore, plant inventories derived from environmental DNA data were obtained without prior knowledge of site location, dating and environmental variables.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	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).
Location	Samples were collected in 46 glacier forelands, covering five continents from the equator to polar regions. The contry were they were sampled and the precise location (latitude, longitude) are available in TableS1.
Access & import/export	The access to all sites, eDNA collection and samples export was legally permitted. When applicable, the study complies with access and benefit permits authorizing collection, transport, analysis and divulgation of DNA data used in this study.
Disturbance	No disturbance was caused by the field work.

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Materials & experimental systems

n/a	Involved in the study
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☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging