Llamas (*Llama glama*) enhance novel proglacial ecosystem development: an experimental approach in the Cordillera Blanca, Peru

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Article

Keywords:

Posted Date: April 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2693855/v1

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Version of Record: A version of this preprint was published at Scientific Reports on September 24th, 2023. See the published version at https://doi.org/10.1038/s41598-023-41458-x.
Abstract

Worldwide, mountain glaciers are shrinking rapidly and projected to disappear largely by 2100. Consequently, large areas are becoming available for novel alpine ecosystems. These harsh environments, however, slow down primary succession. In this study with a local community, we conducted an inclusion experiment to investigate if and how *Llama glama* influences soils and vegetation primary succession following glacial retreat. At the foot of the Uruashraju glacier in the Cordillera Blanca, Peru (~ 4680 m.a.s.l.), we established four llama inclusion plots and four control plots that we studied from 2019 to 2022, 24–40 years after glacial retreat. After three years, the llama plots had significantly increased soil organic carbon and soil nitrogen. In the llama plots, we found a large, significant increase in vascular plant cover (+ 57%) between the second and third years of experimentation, and we identified four new species that were not present in 2019. Our results suggest that *Llama glama*, through their latrine behavior and role as a seed disperser, enhances the primary succession and novel ecosystem formation in recently deglaciated landscapes. Our study provides scientific support that rewilding of native Andean camelid may favor adaptation to glacier retreat and strengthen the conservation and management of novel proglacial ecosystems.

1. Introduction

Glaciers are retreating rapidly, and 49 to 83% of worldwide glaciers (excluding ice sheets) will disappear by 2100. The opening glacier forelands provide an opportunity for novel alpine ecosystems to develop. Here, we investigate to what extent llamas may influence soils and vegetation primary succession following glacial retreat and, thus, influence novel proglacial ecosystem formation on a timescale relevant to the rapid pace of anthropogenic climate change.

Since the beginning of the 20th century, much work has been done on primary succession in proglacial environments, including recent key advances on biotic colonization and pedogenesis. The main ecological constraints to plant succession after glacier retreat include dispersal limitation, deficit in facilitators, and abiotic stress from low temperature, low soil moisture, and low soil fertility. In general, the first colonizers are wind-dispersed, while animal- or water-dispersed individuals are absent. Nevertheless, high dispersal capacity alone does not ensure successful succession. Indeed, the very first colonizers after glacial retreat may be opportunistic species that disappear when water and temperature changes as the glacier recedes. Thus, a dispersal lag and an establishment lag constrain primary succession. Nurse species are mostly absent during early succession, which limits facilitation when other species need this most to overcome extreme environmental stress (stress-gradient hypothesis). Establishing and organizing novel alpine plant communities in proglacial landscapes is therefore complex and slow, and studying this necessitates long-term observation and experimentation.

Manipulative experiments offer great potential for understanding the role of biotic and abiotic factors in driving seed dispersal and plant establishment during primary succession. Notably, *in situ* experiments...
might inform us about how external drivers (e.g., livestock grazing, wildlife re-introduction, and tourism) can transform primary succession trajectories.

Crucial to understanding primary vegetation succession is identifying the drivers of proglacial pedogenesis. First, proglacial pedogenesis is slow: skeletic or lithic Leptosols require decades to form and more distinct soil horizons may take hundreds of years. Proglacial soil formation rates vary depending on topography and morphodynamics, climate factors, parent materials, time, and organisms (i.e., plant-soil interactions, microbial interactions, biological soil crusts). Proglacial soils are usually phosphorus and nitrogen limited. Although microorganism colonization is key during soil development, harsh proglacial microclimates and nutrient limitations impede microbial activity. Plant colonization is tightly linked to many soil characteristics (e.g., texture, structure, depth, organic fraction, and nitrogen).

Herbivory in glacier forelands might also affect primary succession. Experimental studies have reported a positive short-term effect (i.e., 7–10 years) of herbivory on plant community assembly and ecosystem functioning on bare landslide surfaces (with Trichosurus vulpecula) and on Taiga river floodplains (with Alces alces). First, herbivory affected plant species composition that, in turn, accelerated the successional trajectory. In addition, browsing decreased the biomass of the dominant species and therefore provided greater opportunities for colonization and access to nutrients by other species (e.g., nitrogen-fixing species). The effects of herbivory on plants, however, has a complex periodicity, in which biomass increases in seven to 10 years but there is a negative impact on the biomass of the colonizing pioneer species from one to three years.

Second, mammal herbivores enrich soil N and C and soil microbial communities because their wastes increase plant biomass, especially of nitrogen-fixing species. Mammal herbivores also feed upon and transport mycorrhizal fungi. For example, interactions between fungi and large ungulates facilitated primary succession at Mount St Helens. Long term (>30 years) ungulate herbivory can lead to the invasion of unpalatable species, but it can also increase the presence of highly palatable species. In addition, mammals might play a role in exo- and endo- seed dispersion. Mammals, including muskox, lemming, fox, and ermine are potentially significant dispersal vectors in glacier forelands. Finally, trampling can also affect vegetation positively or negatively by improving seedling recruitment by pressing seeds into the uppermost soil layer or by increasing lichen clonal growth through fragmentation and spreading processes.

In alpine proglacial environments, Andean camelid latrines might create more favorable conditions such as resources-rich substrates, sites for seedling establishment, and seed sources, for vegetation establishment (Vicugna vicugna, Lama guanicoe). Reider and Schmidt suggested that vicuña latrines shortcut a 100 + year lag between glacier retreat and primary succession in the Cordillera Vilcanota, Peru. Other studies in different ecosystems have highlighted soil nutrient accumulation by alpaca or vicuña latrines.
Besides soil nutrient enrichment, trampling by large ungulate might also influence soil formation positively or negatively by creating biogeomorphologic changes. First, trampling intensity affects mycorrhizae diversity, which affects soil fertility. Second, trampling can favor pedogenesis by incorporating litter into the soil, enhancing microbial processes and likely influencing the fate of litter carbon, soil organic carbon, and nitrogen mineralization. Third, by causing bioturbation, trampling can expose more mineral soil to erosion and generate finer particles. Moreover, herbivore trampling can compact soils, which might affect soil infiltration, erosion, and nutrient mineralization. Nevertheless, camelid—versus cattle—trampling creates less compaction and can improve ecosystem functioning through water redistribution.

Globally, experimental approaches in proglacial landscapes are crucially missing. A few studies implemented experimental research designs in glacier forelands, which mainly included seed burial and experimental warming. Moreover, all studies took place in the European Alps, while pressing ecological challenges related to deglaciation exist globally. Thus far, we have no study that includes experiments with large herbivores, including camelids, in deglacierized landscapes. However, this type of approach is necessary to understand more precisely the modalities of post-glacial plant colonization.

The globally most discussed socio-environmental challenges connected to glacier retreat include natural hazards, water use competition, local agriculture, hydroelectricity, mining, and tourism. Thus, questions about adaptation and risk reduction are paramount. While glaciers melt worldwide, emerging proglacial ecosystems are becoming important components of the freshwater and carbon cycles. They might also act as sediment sinks that buffer upstream hazards and be refugia for cold-adapted species. The establishment and organization of novel proglacial systems is a key concern for adaptation to glacier retreat in future-and existing-glacier-free valleys. We urgently need to uncover mechanisms that could leapfrog the main limitations of proglacial ecosystem development and overcome these existing and growing socio-environmental challenges.

In this study, we used an inclusion experiment to understand if the presence of llamas (Llama glama) in glacier forelands can enhance primary succession after glacier retreat. We evaluated the effects of llamas on (1) soil pedogenesis and (2) primary vegetation succession in a proglacial ecosystem of the Tropical Peruvian Andes, where glaciers are exceptionally vulnerable to global warming. First, we hypothesized that the llama latrines would drive soil enrichment, catalyzing soil formation processes; soils would have higher nitrogen and carbon contents in the presence of llamas. Second, we hypothesized that the presence of llamas enhances the primary succession of the vegetation. With llamas, we expected an increase in plant biomass, as well as higher nitrogen and phosphorus foliar contents because of soil enrichment. Finally, we hypothesized that the llamas would function as seed transporters between lowlands, highlands, and valleys, by carrying seeds in their hooves, wool (epizoochory), or stomach, and stimulate seed germination via endozoochory. Our study aims to replicate a natural phenomenon to enhance ecosystem formation and inform conservation and/or land management strategies in proglacial landscapes.
2. Results

2.1. Environmental characteristics of the plots

In 2019 there was no significant difference between the mean slope of the control and llama plots. Similarly, there was no significant difference in sand, gravel and rock and block contents between the llama and control plots (Table S3). Between June 2019 and May 2021, we observed lower daily minimum (-0.45°C; p-value = 3.8e-10) and higher daily maximum temperatures (+3.88°C; p-value = 0.001) within the llama plots than within the controls (Table S3).

Table 1: Data collected during the field evaluations of the experiment.

<table>
<thead>
<tr>
<th>Data collected</th>
<th>May 2019</th>
<th>Dec. 2020</th>
<th>May 2021</th>
<th>June 2022</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floristic &amp; geomorphic surveys</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Plant functional traits</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Soil sampling</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Llama latrine sampling</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

2.2. Effect of llama presence of soil development

After 3 years of treatment, we observed an effect of the llamas on the silt and sand contents (Figure S1). Both the full 3-years model (2019 and 2022 data) and the 2022 model showed a significant increase in silt within the llama plots (respectively p-value = 0.017; Table S5 and p-value = 0.055; Table 2). Only the full 3-years model showed a significant decrease in sand within the llama plots (p-value = 0.019; Table S5). Between 2019 and 2022, SOC and IC obtained by the LOI method and N were affected positively by the llamas (respectively +0.2% SOC, p-value = 0.04; +0.1% IC, p-value = 0.047 and +0.05% N, p-value = 0.04; Fig. 3, Table 2, and Table S4). The SOC data from the EA method also showed a significant effect of the llamas on the SOC in 2022 (+0.16% SOC, p-value = 0.04). The δ\(^{13}\)C signature of SOC in 2022 did not differ significantly between the treatments as well as pH (Table 2). The organic C of the latrine (15.4%) was approximately 9 times higher than the SOC of the control plots in 2022 (1.74%). Similarly, latrine total N (5.07%) was 36 times higher than the N in the 2022 control plots (0.14%; Table S7).
### Table 2
Significance of the effects of the llamas on soil properties in 2019 and 2022: texture (clay, silt and sand), pH, organic carbon (SOC) by LOI and EA methods, inorganic carbon (IC), nitrogen (N), and $\delta^{13}C$.

<table>
<thead>
<tr>
<th></th>
<th>2019</th>
<th></th>
<th></th>
<th></th>
<th>2022</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est.</td>
<td>Std.error</td>
<td>Df</td>
<td>t value</td>
<td>Pr($&gt;t$)</td>
<td>Est.</td>
<td>Std.error</td>
<td>Df</td>
</tr>
<tr>
<td>Clay</td>
<td>1.197</td>
<td>2.064</td>
<td>22</td>
<td>2.179</td>
<td><strong>0.040</strong>*</td>
<td>22.122</td>
<td>1.741</td>
<td>22</td>
</tr>
<tr>
<td>Silt</td>
<td>1.945</td>
<td>1.357</td>
<td>22</td>
<td>1.434</td>
<td>0.166</td>
<td>5.406</td>
<td>2.663</td>
<td>22</td>
</tr>
<tr>
<td>Sand</td>
<td>-6.441</td>
<td>2.939</td>
<td>22</td>
<td>-2.191</td>
<td><strong>0.041</strong>*</td>
<td>-5.995</td>
<td>4.447</td>
<td>22</td>
</tr>
<tr>
<td>pH</td>
<td>0.093</td>
<td>0.069</td>
<td>19</td>
<td>1.354</td>
<td>0.192</td>
<td>0.25</td>
<td>0.163</td>
<td>19</td>
</tr>
<tr>
<td>SOC by LOI</td>
<td>-0.015</td>
<td>0.094</td>
<td>19</td>
<td>-0.155</td>
<td>0.878</td>
<td>0.205</td>
<td>0.096</td>
<td>19</td>
</tr>
<tr>
<td>SOC by EA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.158</td>
<td>0.075</td>
<td>19</td>
</tr>
<tr>
<td>N by EA</td>
<td>-0.009</td>
<td>0.008</td>
<td>19</td>
<td>-1.158</td>
<td>0.261</td>
<td>0.048</td>
<td>0.011</td>
<td>19</td>
</tr>
<tr>
<td>$\delta^{13}C$</td>
<td>-0.233</td>
<td>0.136</td>
<td>19</td>
<td>-1.716</td>
<td>0.102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>-0.007</td>
<td>0.044</td>
<td>19</td>
<td>-0.155</td>
<td>0.878</td>
<td>0.097</td>
<td>0.045</td>
<td>19</td>
</tr>
</tbody>
</table>

2.3. Primary succession of vegetation

- **Vegetation biomass**

In 2019, the mean subplot cover of the llama (7.96%) and control (11.38%) plots were not significantly different ($p$-value = 0.12; **Table S8**). Between 2019 and 2022, the presence of llamas had a significant ($p$-value < 0.001) positive effect on plant cover (**Table S9**). Between 2020 and 2021, the llama and control plots did not show significant increases in vegetation cover (for each the $p$-value = 1; Fig. 4; **Table S10**). In contrast, from 2021 to 2022, the mean cover in the llama plots increased from 8.91–13.97% (+57%, $p$-value < 0.001; Fig. 4; **Table S8** and **S10**). Linear regressions on the percentage change in cover between 2019 and 2022 in function of the 2019 subplot cover showed that the control subplots with higher cover in 2019 have gained less cover during the three years than the subplots with lower cover in June 2019 ($p$-value = 0.004, R-squared = 0.24). The trend is lowered within the llama subplots ($p$-value = 0.018, R-squared = 0.18; **Figure S2**). We did not detect a significant effect of the llamas on the BSC.

Across the four surveys of the experiment, we observed a total of 14 plant species. Of these, 13 were identified at the species level (**Table S2**). Results from the generalized mixed effect model showed no effect of the llama treatment on species richness between 2019 and 2022 ($p$-value = 0.534; **Table S9**).
Nevertheless, the *Belloa piptolepsis*, *Oritrophium limnophilum*, *Senecio rufescens*, and an unidentified juvenile individual (*sp.*) appeared only in the llama plots in 2022. Also, *Melpomene peruviana* appeared only within the control plots in 2022 but was already present in 2019 (Table S2). The four species with the highest total cover were *Cinnagrostis rigida*, *Senecio sublutescens*, *Minoides kunthiana*, and *Agrostis tolulescensis* (Table S2). Mixed effects models ran separately for the four species showed that the llama presence had a higher effect on the plant covers of *S. sublutescens* and *M. kunthiana* (respectively, *p*-value < 0.001 and *p*-value = 0.003; Table S12; Figure S3).

- **Plant traits**

Plant traits analysis revealed an effect of the llamas on the LDMC of *Pernettya prostrata* (ANOVA on randomized block *p*-value = 0.085). We observed higher LDMC within the samples collected within the llama plots but far from latrines (i.e., Llama: no latrine) than the samples collected in the control plots (+ 28.7 mg.g⁻¹) and samples with latrine influence (+ 32 mg.g⁻¹; Figure S4; Table S13). There is a positive effect of the llamas on the LDMC when there is no latrine influence (*p*-value = 0.036; Table S14). However, contrast tests did not show significant differences between groups. We did not observe significant changes in LDMC, N, and P for the other species sampled. Subplot greenness was positively affected by the llamas (Table S9). There were significant decreases in plant greenness between 2020 and 2021 in the control plots (*p*-value ≤ 0.001) and the llama plots (*p*-value ≤ 0.001; Fig. 5; Table S11). Although there was no significant increase in greenness between 2021 and 2022 for both treatments, we observed higher greeness index within the llama plots than within the control plots in 2021 (*p*-value = 0.002) and in 2022 (*p*-value = 0.002; Table S11). Finally, we detected a negative effect of the llamas on plant fertility (*p*-value = 0.034; Table S15 and S16; Figure S5). During the four floristic surveys we have reported several observations of pasture eaten within the subplots in both the control and llama plots (Table S17). We did not detect an effect of the llamas on plant necromass and height.

### 2.4. Seed and germination potential

In total, 266 seeds were found within the llama fecal pellets’ samples (Fig. 6). Seeds of 12 plant species belonging to seven families were identified within the pellets (Table S18 and S19). The viability tests showed that five of the seed species identified still maintain their germinative power (Fig. 6; Table S18).

### 3. Discussion

Over three years, llamas had a positive impact on proglacial pedogenesis through a modification of the soil texture and increases in SOC and N. First, the very high SOC, N, and P measured in the latrines samples demonstrate that Andean camelid latrines act as nutrient hotspots within the proglacial landscape. The increase in SOC and N observed in the llama plots soil samples are almost certainly due to a translocation of nutrients via surface runoff from the latrines to the adjacent areas. Since, Andean camelids graze in latrine areas because of their nutritional benefits, there is also an intra-
systems redistribution of the nutrients within the proglacial pasture from old to new latrine sites, in addition to a transfer from downstream to proglacial systems. Besides latrine nutrient addition, camelid trampling might also facilitate litter and BSC incorporation to the subsurface soil, accelerating its decomposition and favoring C and N enrichment 36.

The $\delta^{13}C$ signatures of the llama and control plots are consistent with previous observations in glacier forelands and demonstrate the presence of ancient carbon 46. The slight depletion (but not significant, $p$-value $= 0.1$) in $\delta^{13}C$ between the control and llama plots could support an enrichment in modern carbon in line with the observed increase in SOC. Indeed, $\delta^{13}C$ is higher in low-carbon soils and decreases as SOC increases due to recent organic carbon inputs 14.

Soil texture can serve as proxy for proglacial pedogenesis. Proglacial chronosequence studies have reported a reduction of the fraction of sand and an increase in silt with increasing age 12,47. We suggest that here the observed increase in silt is due to llama bioturbation processes 36 that favor silt incorporation. Second, the increase in plant cover would have reduced erosion processes and increased the capture of recent, carbonate-rich sediment within the denser vegetation, creating feedback effects of the vegetation on soil development (i.e., biogeomorphic interactions 48). These texture changes reveal an accelerated soil development with the llamas and would lead to soil's behavior and microtopography changes, which in turn will affect the spatial redistribution of water and soil resources, and proglacial ecosystem functioning and productivity 39,47. Future analysis will investigate nitrogen isotope signature and microbial activity (e.g., mycorrhizae), to improve our understanding of how the llamas affect belowground communities and therefore the overall nutrient cycles in glacier foreland.

After three years, the presence of llamas had a substantial impact on the primary vegetation succession at the Uruashraju glacier foreland, in line with hypothesis 2. The optimization of aboveground net primary production by mammals is reported in grass and shrubland systems 25,49, and few studies also reported positive pathways in arctic tundra 50,51. In the glacier foreland of the Uruashraju, the llamas have improved the development of the aboveground vegetation seen in the increased plant cover and overall greenness of the fenced plots. These changes are likely to have significant effects on the landscape evolution, enhancing vegetation-soil feedback cycles 47,52. For example, S. sublutescens had the highest cover increase in the llama plots and is very likely to produce significant quantities of litter that will be redistributed to the soil surface through llama trampling and litter decomposition 35. Thus, the effects of the llama latrine behavior on soil pedogenesis cascades through increased plant cover 30. The effects of the llamas on plant cover do remain low after three years, but this is a short period to observe significant change in proglacial ecosystems where ecological dynamics are slow 2. Indeed, most of experimental studies are carried out for longer periods (i.e., 10 to 35 + years) 21,22.

Llamas have modified the functional traits of the proglacial plant community. Studies show that plant, leaf thickness, and LDMC greenness is tightly linked to plant growth, chlorophyll production, and correlated with leaf N 53,54. The increase in subplot greenness within the llama plots suggests higher
photosynthetic activity and plant growth, which is very likely connected to the latrine soil nutrient enrichment. To complement this, the higher LDMC values of *P. prostrata* observed within the “llama - no latrine influence” group in comparison to the “controls” suggests a defense mechanism of *P. prostrata* responding to a grazing effect \(^5\). The individuals of *P. prostrata* suffering from grazing and not benefiting from the enriched soil may have lowered their growth rate and invested more resources in structural protection of the photosynthetic tissues, as an adaptation to avoid the effect of defoliation (i.e., avoidance strategy \(^5\)). While the lower values found within the llama plots with “latrine influence” suggest a nutrient enrichment effect from the latrines and therefore a reverse response of *P. prostrata* LDMC, lowering the negative consequences of the llama grazing (i.e., tolerance strategy). The llama latrine behavior has locally transformed a low-nutrient system into a more productive system, and individuals benefiting from the enriched substrate have faster regrowth traits \(^5\). We hypothesize that variation in climatic conditions (precipitation during the wet versus dry season) explain the higher greenness index observed in January 2020 and the strong decrease in greenness between January 2020 and May 2021.

In contrast with the plant cover and greenness gains, there is a decline of plant fertility due to the llamas. Because of the high P of the latrines we expected an increase in plant fertility \(^5\). This unexpected outcome may be linked to the presence of eaten pasture within the llama plots, which might also have biased our plant cover and plant height observations for 2020 and 2021. To overcome this concern, in 2022 we waited 3 weeks between the last grazing treatment and our field evaluation, giving time for the plants to grow again after the grazing event. We also reported eaten pasture within the control subplots in 2020 and 2021, indicating either that the llamas escaped at least once from the fences, despite attentive care, or the llamas grazed within the control area during their arrival on site while the farmers were distributing the animals between plots.

Our results also suggest a decrease in competition between species within the llama plots. The mixed model positive coefficient of the 2019 plant cover means that plots with higher cover in 2019 displayed stronger positive effects from the llamas. In the control plots the 2019 initial cover explained a fourth of the variation of the percentage change in cover between 2019 and 2022, suggesting that in the absence of llamas, the subplots with low cover are more likely to experience higher rates of plant cover increase because of reduced plant-plant competition. Whereas in the llama plots the strength of the relationship is lower, indicating that other factors (i.e., the llamas) might have reduced competition through the physical effect of grazing \(^5\). Further, over longer periods, substrate enrichment might favor ruderal species that have been previously replaced by more competitive and stress tolerant species during primary succession \(^5\). The presence of llamas in proglacial landscapes may modify primary succession trajectories and allow plant communities to overcome the harsh environmental conditions.

We did not observe a notable change in plant richness within the experiment. Although, we registered four new species that appeared only within the grazed areas. Similarly, we noted the disappearance of one species within the llama plots in 2022 that was present in 2019. This might be due to the temporal
variability in moisture inputs within the proglacial systems, since *C. ovata* is mainly found in wet terrains \(^\text{58}\). Even if we consider the environmental variations as random between llama and control plots, the higher temperature range observed in the llama plots could slow down plant growth (*Table S3*). Long-term monitoring is necessary to fully test our hypothesis. Finally, future research will need to investigate the feedbacks between plant defoliation from herbivory and its effect on soil biota and all factors on the interactions of mycorrhizae \(^\text{59}\).

Ungulates are known to act as dispersal agents through endozoochory, carrying seeds on their coats, or between their hooves (epizoochory), or simply spitting out seeds after mastication or rumination \(^\text{60,61}\). Here, we show that llamas have the potential to disperse seeds of different plant families and types from lower elevation or neighboring valleys to the proglacial habitat (*Table S9*). We found viable seeds from five different species: *Alchemilla sp.*, *Luzula sp.*, *Pennisetum sp.*, *P. prostrata*, and *Sporobolus sp.*, of which only the *Pernettya* and *Luzula* were identified during the floristic surveys of the experiment, indicating an external seed input. The seeds of *Pernettya*, *Sporobolus*, and *Luzula*, being viable and endozoochore, evidence the role of Andean camelids as seed transporters by endozoochory \(^\text{62}\). Notably, the *Luzula racemosa*, which was not present in 2019 within the experiment but found in 2022 in the llama plots, is present at lower elevation in the valley (57 years after deglaciarization; *Table S1*) and therefore might have been brought to the experiment by the llamas. In view of the fact that llamas move regularly between vegetation communities for foraging and visiting, both endo- and exozoochorous dispersal provides an important potential mechanism for colonization. The seed viability test was done using latrines old for at least one month. Therefore, we recognize that different physical and chemical treatments of the seed (e.g. digestive treatment, feces humidity and deterioration) might have produced different viability results or also impacted seed dormancy and therefore germination. We hypothesize that no viable seeds could also have been transported via llamas exozoochory from the lower ecosystems. For example, species such as *Plantago tubulosa* and *Werneria pygmaea*, peat-forming cushion plants \(^\text{63}\) and *Alchemilla pinnata* \(^\text{62}\) have been found to be part of the Andean camelid diet, and might be transported via llama endo or exozoochory to the more recently deglaciarized terrains. Finally, we do not exclude the role of the llamas as facilitator of the spread of invasive exotic plants by epizoochoory or endozoochory such as *Rumex acetosella* \(^\text{64}\). Further research is needed to confirm the role of the llamas as exo and endo-zoochore dispersers \(^\text{31}\).

In the Andes, the Puna ecosystem was a primary center of ungulate domestication \(^\text{65}\). However, since the Spanish conquest in the 1500s, European livestock (e.g., cattle, horse, donkey, and sheep) have almost completely replaced domestic camelids and ancestral herding practices. Introduced livestock—often associated with overgrazing—have several negative ecological impacts in Andean ecosystems \(^\text{65}\). First, exotic animals can alter the structure and composition of the native vegetation \(^\text{64}\), the hydrologic soil functions \(^\text{38}\), and can increase erosion by soil compaction and by uprooting plants \(^\text{65}\). Here we showed that the presence of Andean camelid has positive effects on novel proglacial ecosystems that may result in positive effect for high alpine biodiversity and ecosystem functioning. The reintroduction or rewilding of native Andean camelid may favor adaptation to glacier retreat while benefiting the local economy.
Andean camelids are known for their fiber and meat production, and are also involved in tourism activities and Andean folklore. Rewilding interventions as strategies for biodiversity conservation and ecosystem functioning are novel management alternatives that could be applied to the benefit of proglacial landscapes.

Conclusion

Our study shows that Andean camelids (*Llama glama*) can enhance soil pedogenesis and primary vegetation succession in glacier forelands. In three years, the latrine behavior of the llamas led to soil organic carbon and nitrogen enrichment and changes in soil texture, suggesting enhanced proglacial pedogenesis pathways. Similarly, we reported a significant increase of the plant cover and the appearance of four new species. We found that llamas can act as seed transporters bringing seeds from lower elevations or neighboring valleys to the proglacial habitats and possibly facilitating colonization processes, albeit more years might be necessary to also observe an increase in plant richness. These results have several implications. First, llamas might help overcome some of the constraints of primary succession after glacier retreat like dispersal limitation, low soil fertility, and plant competition, lowering dispersal and establishment lags and enhancing ecological connectivity between deglacierized landscapes. Second, our experimental simulation of a natural process, suggests that other Andean camelids displaying similar behavior might also enhance proglacial ecosystem establishment (i.e., Vicuña, Alpaca, and Guanaco). Lastly, our findings may have implications for the conservation and managements of novel proglacial ecosystem around the world. Locally, our study supports the goal of national and local efforts in re-introducing Andean camelids from local communities while decreasing the cattle density. The experimental protocol designed in this study will allow long-term monitoring to continue to test the changes observed in our three-year study and discover what changes may happen at longer timescales.

4. Materials And Methods

4.1. Study Area

We conducted our experiment in the Uruashraju glacier foreland located in the Quebrada Pumahuacanca within the National Park of Huascaran (NPH), Cordillera Blanca, northwestern Peru (Fig. 1). The Cordillera Blanca is the world's most extensively glacier-covered tropical mountain range. In the 1960s, the imminent extinction of the overhunted and emblematic Vicuña, a native Andean camelid, prompted the creation of the NPH. This area is semi-arid and has highly seasonal precipitation with 80% of the 700mm (~3500m.a.s.l.) to 1000mm (~4550m.a.s.l.) per year falling between October and May (Kaser et al., 2003). The mean annual air temperature at 3450m.a.s.l. is approximately 12–14°C with strong diurnal variability but little seasonal variability due to its tropical location (9°S). The geology of the Quebrada Pumahuacanca is dominated by granodiorite and tonalite, with outcrops of the meta-sedimentary Jurassic Chicama formation, which consists of shale, pyritic siltstone, and quartzite with volcanics in...
some areas. Sulfdie-rich lithologies occur within the glacier foreland and their oxidation after the retreat of the ice is linked to low pH values.

The Pumahuacanca Quebrada (from 4050 to 5605 m.a.s.l.) has patches of high Andean woodlands (e.g., *Polylepis* spp. and *Gynoxis* spp.), wetland 'bofedales' dominated by cushion-forming plant communities (e.g., *Distichia muscoides*), and grasslands dominated by tussock grass such as *Cinnagrostis rigida*. Certain species like *Wemeria nubigena* indicates the presence of non-native livestock and overgrazing. Within the NPH, Pasture User Committees (CUP) use these high pasture ecosystems to graze their livestock. This is the case of the CUP of Arwaycancha in the Quebrada Pumahuanca. In recent years, the uncontrolled introduction of exotic livestock for agropastoralism and tourism activity has caused degradation of pastures and the disappearance of some native species within the Park. Due to their soft footsteps and prehensile split upper lips Andean camelid minimize impact on the groundcover compared with introduced livestock. The NPH has thus supported camelid breeding initiatives as an ecosystem and biodiversity conservation strategy.

The study site (77° 19' 20" W, 9° 35' 47" S) lies between 4680 and 4700 m.a.s.l. in a terrain deglacierized between 1979 and 1995 (Fig. 1). This research was in collaboration with the NPH and the *Llama 2000 Asociación*, a local community of farmers, who own the llamas. The farmers' own village of Canrey Chico, Olleros has contaminated water due to natural acid rock drainage (ARD) that threatens their agriculture. Previous floristic plot surveys carried on in the Uruashraju glacier foreland identified 47 vascular plant species (Zimmer et al., unpublished data; Table S1) We selected the Uruashraju foreland based on the *Llama 2000 Asociación's* shared interest in our research questions, the presence of llamas in the adjacent Quebrada, data availability for deglacierization chronology and floristic comparisons within the overall glacier foreland (Table S1), and the ecological representativeness of the site. Considering the socio-geoecological aspects of the region, we expect our results to be generalizable to the overall Cordillera Blanca and, possibly, to other mountain ranges.

**4.2. Context and experimental design**

The *Llama 2000 Asociación* launched the *Llama 2000* Project to increase the value of local llama breeding and conserve an essential part of their Inca biocultural heritage. In 2019, the community aimed to strengthen its activity promoting the sustainable management of llamas and vicuñas to develop community-based tourism along the Qhapaq Ñan trail, enhance local economy, and develop climate change adaptation strategies. We collaborated with the *Llama 2000* community and the NPH to test a novel adaptation strategy to glacier retreat by introducing llamas in the Uruashraju glacier foreland. Manipulative experiments better predict the causal impacts of short-term ecological change and allow for a better control of confounding factors, which here included livestock disturbance, llama stocking rate and visit frequency to the site, and camelid movement between Quebradas.

In June 2019 we set up a randomized complete block design with four monitoring blocks — i.e., four llama fences and four controls (Fig. 2A and B) — across a chronosequence since deglacierization: the
south corner of the experiment was ice-free in ~1979 and the north corner in 1995 (Fig. 1C). We built eight plots (925m$^2$ each) over 12,500 m$^2$ of low to moderate slopes. Within each plot, we randomly established eight (1m$^2$) permanent subplots (Fig. 2C). From June 2019 and to 2022, in each fenced plot, three llamas grazed for three days each month (cf. Appendix 1 – Experimental design).

4.3. Field Data Collection

To test the effects of llamas — grazing, trampling, and latrine behavior — on proglacial ecosystem development following our three hypotheses (i.e., soil enrichment, vegetation succession, and seed dispersal), we collected: (1) pedological, (2) plant diversity and productivity data, and (3) llama latrine samples. The data collection took place at different times (Table 1).

First, in May 2019, before introducing the llamas, we conducted the first floristic and geomorphic evaluations of the 64 subplots (i.e., Hypothesis 2). At each subplot, we identified all the vascular species (Table S2). We estimated visually their relative surface cover, density, necromass, presence of fertility attributes (i.e., reproductive organs), and their average height. As well, we recorded the presence of biological soil crust (BSC) and measured its height and relative cover. In each subplot, we also estimated soil granulometry — percentage cover of sand (<2mm), gravel (<2cm), and rock (<10cm), and block (>10cm). Every year from 2019 to 2022, we replicated the same biotic and abiotic data collection for each of the 64 subplots to study the effects of the presence of llamas over time. In addition, in 2020, 2021, and 2022 we visually assigned a greenness index (0, 1, or 2) to each subplot based on the vegetated proportion of the plot been green.

In addition, we measured three plant functional traits: leaf dry matter content (LDMC), total nitrogen (N), and phosphorus (P) to study plant productivity (i.e., Hypothesis 2). Plant leaf N is directly related to photosynthesis and respiration and reflects the trade-off between greater photosynthetic capacity and potentially suffering more herbivory. Leaf P is also involved in respiration and energy transfer and is linked to reproduction. LDMC correlates negatively with potential relative growth rate and positively with leaf lifespan. Leaves with high LDMC tend to be relatively tough and are thus assumed to be more resistant to physical hazards (e.g., herbivory) than are leaves with low LDMC. Therefore, LDMC is an indicator of a plant’s resource use strategy: usually, stress tolerant and slow growing species found in stressed environments display high LDMC and low N, whereas low LDMC and N-rich leaf are characteristic of competitors and ruderals with fast resource acquisition strategies. LDMC is also a predictor of soil fertility: LDMC decreases when soil fertility increases. For the trait measurements, we selected four species based on (1) their presence in the 8 plots (Table S2), (2) their potential as ecosystem engineers (i.e., P. prostata: slope stabilization; pers. obs., S. sublutescens: high litter production and micro-habitat creation; pers. obs.), and (3) their palatability (A. tolucensis; P. prostrata; pers. obs.).

In June 2022, we collected 10 leaf samples from different individuals of the same species within each plot. Each sample is a composite of a minimum of three individuals, summing to approximately 15 to 40
leaves per sample depending on the species. We used stratified sampling within the four llama plots, collecting half of the 10 samples from individuals located inside or below a latrine and the other five samples from individuals located three meters outside latrines (cf. Appendix 1 – Field data collection). The collection of plant material was carried out in accordance with relevant recommendations and authorizations of the SERNANP (licenses N°008-2019-SERNANP-JEF and N°005-2022-SERNANP-JEF). All methods were carried out in accordance with relevant guidelines. As importantly, because we measure and count the same plant specimens present within the permanent subplots in the control and the llama plots every year, we did not collect the full plant specimens; this would have been destructive for the experiment and biased the floristic evaluations results for this study but also for the long-term monitoring of the experiment. Therefore, the plant species identified and studied have not been deposited in a public herbarium, since this process requires the full specimen (including vegetative material and root). Accordingly, the plant identification was done in the field by botanists from the Museum of Natural History of the National University of San Marcos (UNMSM), Lima, Peru (Sebastián Riva and Jean Salcedo). Regarding the measurement of the plant traits (nitrogen, LDMC, phosphorus), we only collected the minimum amount necessary for the analysis, collecting only the leaves directly from the plants in the field, and trying to be as minimally destructive as possible for the experiment.

Then, to infer the effect of the llamas on soil development (i.e., Hypothesis 1) in May 2019 (before introducing the llamas) and June 2022 (three years after initiation), we collected three soil samples distributed in the upper, middle, and lower slopes of each llama plot. Each sample is itself a composite of three subsamples collected within 1m², between 3 to 15cm deep. We excluded from the sampling any pure organic surface layer, litter, and vegetation (cf. Appendix 1 – Field data collection).

Additionally, we collected covariate variables that might potentially affect our response variables: subplot slope degree and soil temperature. To measure soil temperature, we buried two HOBO 8K Pendant data loggers (five to 10cm depth) per plot within the upper and lower parts of the plot. Since June 2019, the 24 data loggers have recorded temperature every four hours. The slope and soil temperature measurements allowed us to account for confounding factors such as a slope effect on soil composition or vegetation cover or an effect of the proximity of the glacier on soil temperature.

Finally, to estimate the fertilization effects of the llamas on the soil composition and their role as seed dispersers (i.e., Hypothesis 3; Fig. 2D), we collected three fecal samples from three different llama latrines in each llama plot. The Environmental Quality Laboratory (EQL) in Huaraz, Peru analyzed samples for nutrients, and the Museum of Natural History of the UNMSM in Lima, Peru analyzed seeds in the samples (cf. Appendix 1 – Field data collection).

4.4. Laboratory and herbarium method

Laboratory analysis

To infer the role of llamas on pedogenesis, we analyzed soil pH, particle size distribution, soil organic (SOC), inorganic (IC) carbon, soil nitrogen (N), and carbon isotopes (δ¹³C). We analyzed the 48 soil
samples at the University of Texas at Austin, USA, mostly at the Soils and Geoarchaeology Laboratory. Soil pH was determined using a soil:solution ratio of 1:2. Because estimating SOC in proglacial soil is complex (due to low SOC and carbonate or clay contents), we used two different methods: sequential loss on ignition (LOI) and elemental analyzer (EA). First, we analyzed the 48 samples from 2019 and 2022 using the LOI method for SOC and IC (cf. Appendix 1 – Laboratory analysis). Second, for the 24 samples collected in 2022, we measured SOC and $^{13}$C by isotope ratio mass spectrometry. Values of $\delta^{13}$C provide information about the origin of the SOC. Bardgett et al. $^{46}$ linked high $\delta^{13}$C values to the presence of ancient carbon, and Kielland & Bryant $^{22}$ used $\delta^{13}$C values to examine the effects of herbivory on soil carbon composition. We measured N using an EA CHNS-O. Finally, at the Laboratory for Environmental Archaeology of UT Austin, we performed Particle Size Analysis (PSA) after deflocculation (cf. Appendix 1 – Laboratory analysis).

The EQL in Huaraz, Peru measured leaf LDMC, N, and P, as well as the total organic C, total N, and total P of the fecal samples. We measured LDMC only for *P. prostrata* and *S. sublutescens* since we did not expect major changes within the tussock grasses $^{77}$. N and P were measured for all species. At UNMSM in Lima, we evaluated the viability of the seed using the Tetrazolium test (*Table S11*; cf. Appendix 1 – Laboratory analysis).

### 4.5. Data Analysis

Data analyses were performed using the statistical software R version 4.2.2. To test our first hypothesis (i.e., llamas catalyze soil formation), we first computed summary statistic for all the soil response variables (texture, pH, SOC, IC, N, and $\delta^{13}$C) for the control and llama plots. Afterward, we use linear mixed models to test the significance of the variation assignable to the llama treatment on a randomized block design. We first ran a model based on the experiment's full 3-years (2019 to 2022). Second, we ran two separate models, one for the 2019 data and one for the 2022, to remove the variable “Years” from the model to gain degrees of freedom and reduce standard errors. We report the results of all models.

To test our second hypothesis, we assessed plant community changes based on species richness — count of species per subplot to estimate the taxonomic diversity of the subplots, plant cover, and plant functional traits (i.e., LDMC, N and P, plant height, necromass, greenness, and fertility) between the control and llama plots. Plant height and necromass were community weighted for each subplot. As well, we computed the percentage of species with the presence of fertility attributes for each subplot (i.e., number of species with fertility attributes/subplot richness), and we calculated a percentage change in plant cover between 2019 and 2022. First, we calculated summary statistics. Then, we used mixed models to analyze the effect of llamas on plant cover, height, necromass, and fertility. We computed the total percentage cover of each species for the overall experiment to select the four species most representative of the plant community. Then we ran distinct mixed models for the four species to test for the effect of the llamas on the species selected. We used Generalized Linear Mixed Models with a Poisson distribution to test the effects of the llamas on plant richness and greenness (*lm4* library). The initial plant cover value reported in 2019, were used in the plant cover analysis as a co-variate to increase the power of the
test. Multiple post-hoc comparisons of means across llama and control treatments were assessed with Bonferroni tests. Afterward, we used linear regressions to assess the effect of the initial subplot cover on the percentage change in cover between 2019 and 2022 and the 2022 plant cover. Normality and homoscedasticity conditions were assessed before running statistical test and log transformation were applied to the plant cover dataset to improve normality and reduce skewness.

To analyze variation in soil temperature we use the period with complete data from 23.06.2019 to 22.05.2021. We calculated summary statistics and used Wilcoxon tests to assess the difference between control and llama treatments. We tested for significant differences in slope gradient and granulometry of the subplots between the llama and control plots with T-tests and Wilcoxon tests (ggpubr library).

Declarations

Acknowledgements

This work was funded by the United States National Science Foundation under the Human-Environment and Geographical Sciences Program (Award no: 2105826) and National Geographic Society (Grant no. EC-58123R-19). This study was carried out under the scientific research authorization from the SERNANP (licenses N°008-2019-SERNANP-JEF and N°005-2022-SERNANP-JEF) authorizing the collection of plant material. We would like to express our deep gratitude to the Community Llama 2000 (in particular to Calixto Huerta Trujillo, Alejandro Cruz Salvador, and Jorge Martel Alvarado) for their continuous involvement and contribution to the experiment. We would like to acknowledge the human and material facilities provided by the National Park of Huascarán. We thank MSc. Giovana Patricia Vadillo Galvez from the Plant Physiology Laboratory (UNMSM) for the support in the identification of the seeds, and Pier Cisnero Jurado, Joshua Lopez Robles, Edu Jara Tarazona, Fernando Valverde Damián, and Inés Sachahuaman Balbin for their unconditional help in the field. Thanks to the Laboratory for Environmental Archaeology of UT Austin for the use of their equipment and to the multiple undergrad students of funding from the Soils and Geoarchaeology Laboratory of UT Austin for helping with the soil analysis. Finally, we thank Dr. Eugenio Arima for his statistical advice. No experiments on live animals were carried out.

Authors’ contributions

AZ, TB and FA conceived the ideas and designed the experiment; AZ, SR and JS collected the data; AZ, FA, and TB analyzed the data; AZ led the writing of the manuscript. FA and TB reviewed and edited the manuscript, and RC analyzed and provided the deglaciarization data. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Conflict of interest

The authors have no conflict of interest to declare.

References


Figures
Figure 1

Study site: Santa River watershed (A), Rio Negro sub-watershed (B), and the Uruashraju glacier foreland (C). Base layers originated from © Esri, © NASA, © NGA, © USGS, and © FAO; all other layers were created and edited by the authors.
Figure 2

Design of the experiment (A), *Llama glama* within a llama plot (B), 1m² subplot (c), seedling germinated from llama feces found within the experiment in June 2022 (D).
**Figure 3**

Effects of llamas on soil organic carbon (SOC by LOI) and nitrogen (N) (95% confidence intervals) in 2019 and 2022. Means not sharing any letter are significantly different by Bonferroni tests ($p \leq 0.05$).
Figure 4

Effects of llamas on plant cover (95% confidence intervals). Means not sharing any letter are significantly different by Bonferroni tests ($p \leq 0.05$).
Figure 5

Effects of llamas on greenery (95% confidence intervals). Means not sharing any letter are significantly different by Bonferroni tests ($p \leq 0.05$). Note: January is the Wet Season.
Figure 6

Seed viability per species found within the llama pellets (n=266).

Supplementary Files

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