Science of the Total Environment 967 (2025) 178740

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Incipient soils: New habitats in proglacial areas of the Maladeta massif (Central Pyrenees)

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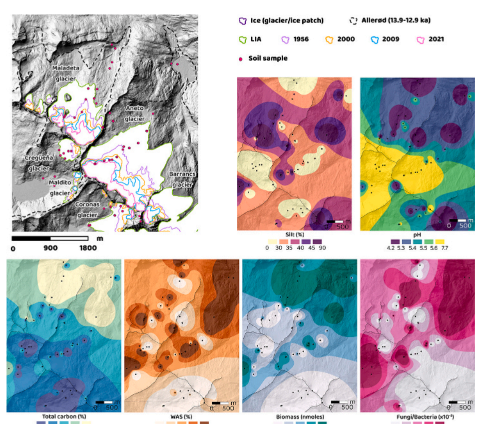
HIGHLIGHTS

- Since LIA, only remains the 12% of the glacierised surface of the Maladeta massif.
- The characteristics of the soils were highly variable across the massif.
- There was no relationship between deglaciation age and soil development degree.
- Silt content was key to soil property development and microbial community growth.
- The duration of the snow cover also played an important role in the development of incipient soils.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniel Alessi

Keywords:
Incipient soils
Pyrenees
Deglaciation
Microbiology
Little Ice Age

ABSTRACT

Since the Little Ice Age, the deglaciation of the Maladeta massif (Central Pyrenees) has been almost continuous; since then, the glaciated surface has been reduced by 87.9 % until 2021. This deglaciation has led to an increase in proglacial areas, allowing the development of new habitats. In this study, 86 samples of incipient soils (>5 cm depth) were selected with a large range of different characteristics (elevation, orientation, density of plant cover or soil colour). Geochemical, microbiological and statistical analyses were carried out under the hypothesis that time of deglaciation control the evolution of these incipient soils. The results showed soils with highly variable characteristics, although in general neutral-acid pH (median 5.5), water aggregate stability was relatively high (median 64 %), but they exhibited very low carbon content (median 1 %). In these very incipient soils, the time of deglaciation seems not to be a key factor that determined the development of the soils. In spite of this, the duration of the snow cover (strongly influenced by the topography) is a factor that explains the degree of

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https://doi.org/10.1016/j.scitotenv.2025.178740
Received 21 September 2024; Received in revised form 2 February 2025; Accepted 3 February 2025
Available online 16 February 2025

development of the soils. Moreover, the percentage of silt content has shown to give the soil a higher reactivity, water and nutrient retention capacity and greater stability. A pattern of biological succession was found, from bacteria as colonisers, to fungi in subsequent stages, and finally to plants colonising the habitats. Finally, the impact of mountain tourism in the most transited sector of the Maladeta massif (the Aneto cirque) showed a high concentration of nitrate (>50 mg kg^{-1}), which favours primary productivity and increases the abundance of microorganisms. This study shows the importance of the microtopography and the presence of silt in the development of very incipient soils in recently deglaciated areas.

1. Introduction

The Little Ice Age (LIA), represents the last global glacial event in the majority of mountainous regions (Solomina et al., 2016; García-Ruiz et al., 2020). Since then, and associated with climate change, the glaciers have retreated almost continuously (Dyurgerov, 2001; Oerlemans, 2005). Glacier retreat has facilitated the formation of new environments and often permitted the development of incipient soils and the colonization of vegetation. These soils are well represented in the proglacial zones (or alpine glacial foreland), which are defined as the area between the actual front of the glaciers and the terminal moraines deposited during the LIA expansion (Eckmeier et al., 2013).

As an initial hypothesis, it was postulated that the characteristics and degree of development of these soils could be primarily influenced by the date of deglaciation of the region (Huggett, 1998), following a chronosequence, as observed in other several proglacial areas worldwide, including the Werenskiold glacier (Svalbard; Kabala and Zapart, 2012) and the Pindari proglacial area (Indian Central Himalaya; Prokop et al., 2021). Thus, areas that were deglaciated earlier than others would have had enough time for soil development. However, several studies have indicated that additional variables may also play a role in the development of soil in high mountain environments (Baumler and Zech, 1994; Simon et al., 2000; Burga et al., 2010; Leonelli et al., 2024; Temme, 2019), where extreme climate conditions hinder the well-known soil development (Kabala et al., 2021). Other studies demonstrated that even if the soil evolved according to the normal soil chronosequence, other factors, such as the morphology of the surrounding reliefs, could mask the relation among time and soil development (Masseroli et al., 2023). In soils that have recently undergone deglaciation, their composition and properties change as plant communities develop. For example, certain components such as organic matter or total nitrogen (TN) often increase. In addition, the composition and structure of the microbial communities and plant development experience substantial changes (Ohtonen et al., 1999; Nemergut et al., 2007; Bajerski and Wagner, 2013; Rime et al., 2015; Ficetola et al., 2024; D'Amico et al., 2014).

The Pyrenees host the southernmost remaining glaciers in Europe (Grunewald and Scheithauer, 2010). However, in the recent years, the rapid glacial retreat resulting from increased temperatures has facilitated the development of incipient soils and vegetation, in isolated patches within a few years of the ice melt. In particular, the Maladeta massif, the most glaciated massif in the Pyrenees, with glaciers and ice patches preserved on both north- and south-facing slopes (Vidaller et al., 2021), only conserves the 7 % of the LIA glaciated surface. This quickly shrinkage has led the development of incipient soils in very extreme weather conditions. One of the most intriguing aspects of this massif is that during the LIA, it hosted glaciers in all orientations (Vidaller et al., 2024), allowing for the investigation of how these new soils have emerged over time and which factors mostly determine their characteristics. Besides, the monitoring of the last remaining glaciers of this massif carried out in the last years has allowed to determine an age of deglaciation for each sample with a high precision based on its location respect to LIA moraines or respect to glacial surface determined by satellite imagery (Vidaller et al., 2024) or by drones surveys (Izagirre et al., 2024; Vidaller et al., 2021).

Furthermore, human recreational activities have a discernible impact on the mountainous environment. Anthropogenic activities have significantly altered the global biogeochemical cycles of nitrogen and sulphur (Vitousek et al., 1997), with these activities reaching the highest mountain elevations in recent decades, including mountaineering and climbing. Acidification has been identified as a potential threat to ecosystems (Rodhe and Herrera, 1988), with the process being caused by acid rain and the replacement of base cations from the cation exchange complex (CEC) of the soils by hydrogen ions (Bouwman et al., 2002). Additionally, nitrogen inputs can affect biodiversity, as nitrogen is the primary nutrient limiting plant and microbial production in terrestrial ecosystems. Increases in nitrogen inputs, in whatever form, can lead to shifts in plant species composition towards nitrophilous species (Bobbink et al., 1996; Richardson et al., 2023).

The objective of this study is to determine if the time of deglaciation was the principal factor that condition the development of the very incipient soils in recently deglaciated areas. To achieve this, several geochemical, microbiological and statistical analyses were carried out in 86 soil samples from the proglacial area of the Maladeta massif (Central Pyrenees, Spain). To investigate if there were other factors that control the development of incipient soils a wide range of characteristics, including orientation, elevation, slope, duration of snow cover, and deglaciation period were considered. To stablish the age of deglaciation, a comprehensive reconstruction of the deglaciation of the Maladeta massif, based on precise remote sensing products and the OSL dating of selected moraines (Vidaller et al., under review), was carried out. This study represents a novel approach to the study of very incipient soils, combining geochemical and microbiological analyses.

2. Study area

The Maladeta massif is situated in the Central Pyrenees (Fig. 1a), delimited by the Ésera, Vallibierna and Salenques valleys (Fig. 1b). It is the highest massif in this mountain range, with >40 peaks exceeding 3000 m above sea level (a.s.l.). These include the Aneto peak (3404 m a.s.l.), the highest point in the Pyrenees, and the Maladeta peak (3312 m a.s.l.). In terms of geology, this massif is situated within the Axial Zone of the Pyrenees, where the highest elevations are composed of granites that were formed during the Variscan orogeny (García-Sansegundo et al., 2013; Ríos-Aragüés et al., 2002). The current landscape has been modelled by glacial and periglacial processes during the last deglaciation (Copons et al., 1997; García-Ruiz et al., 1992; Martínez de Pisón, 1989, 1990; Vidaller et al., 2024). The glacial geomorphology of the area is characterised by the presence of sharp ridges, glacial cirques and polished bedrock. In contrast, the accumulation of debris on the slopes represents the primary periglacial landform.

Nowadays, the 0 °C isotherm for this massif is located around 3200 m a.s.l. (based on data from the Spanish State Meteorological Agency-AEMET, database of the Besurta and Renclusa weather stations, the Clima y Nieve database from Llanos del Hospital, and Posets-Maladeta Natural Park database from the Aneto station, and a lapse rate of 0.525 °C every 100 m). The mean annual temperature for the period 2008–2021 was 4.5 °C, and the mean annual precipitation was over 1300 mm (as measured at the Renclusa hut at 2140 m a.s.l.; AEMET database). Winter and spring are the wettest seasons. The snow precipitation during these months are heavily underestimated by under catch (Buisan et al., 2015). Due to its elevated position and orientation, the Maladeta massif is the most glaciated massif in the Pyrenees, hosting two of the four largest glaciers in the range: the Aneto (39.6 ha in 2023) and Maladeta (15.6 ha in 2023) glaciers, both situated on the northern slope. There is also another glacier in this slope, Tempestades glacier (5.5 ha in 2023). Four additional ice patches remain in this massif: Maladeta West, Aneto (secondary body) and Barrancs in the north slope, and Coronas in the south slope (Fig. 1b; Vidaller et al., 2021; Izagirre et al., 2024). The prevailing cold conditions permit the maintenance of permafrost at elevations exceeding 2800 m a.s.l. (Serrano-Cañadas et al., 2019).

3. Material and methods

The main objective of this research was to determine if the time of deglaciation was the principal factor that condition the development of the very incipient soils in recent deglaciation areas, or if, on the other hand, there are additional factors that control their development. To accomplish this objective, a transect of soil sampling was carried out in four cirques in the Maladeta massif with a wide range of orientation, elevation, slope, duration of snow cover, plant cover and deglaciation period. To determine the characteristics of each soil sample topographical, geochemical, sedimentological, mineralogical and microbiological analyses were carried out.

3.1. Glacier shrinkage cartography and soils classification per deglaciation period

In this study, several techniques have been employed in order to define the age of the obtained soil samples:

- The extension of the glacier during the LIA has been delineated from the LIA moraines, which are well developed in all the cirques, and dated in the Aneto cirque (Vidaller et al., under review). The glaciers were delineated manually, tracing the frontolateral moraine ridges until they reached the walls of the cirques. Additionally, photographic evidence has been employed to delineate the boundaries of these glaciers.
- The images from 1956 were sourced from the National Aerial Orthophotography Plan (PNOA). The boundaries of the glaciers were digitised and delineated manually over the orthophotos, following the methodology proposed by Vidaller et al. (2024).
- The 1981 glacier extent was delineated manually in accordance with the methodology proposed by Vidaller et al. (2023) and based on the orthophoto provided by the Spanish National Geographic Institute (IGN).
- The outlines of the glaciers in 2000 and 2009 were determined by applying the Normalised Differential Snow Index (NDSI) to Landsat satellite images as Debnath et al. (2018).

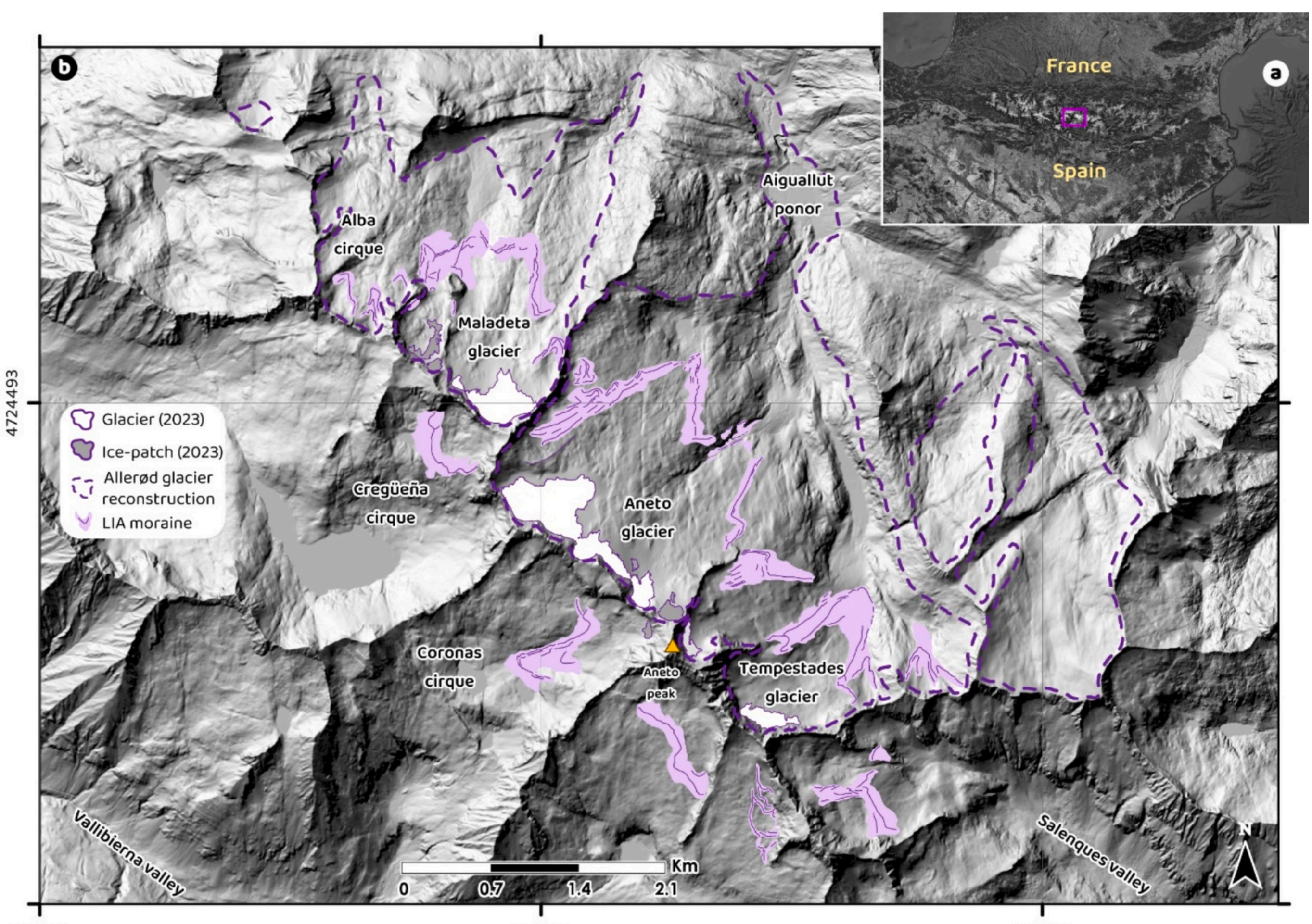

Fig. 1. (a) Location map of the Maladeta massif in the Pyrenees. Map obtained from Google Earth and modified. (b) Location of the main cirques, valleys, the LIA moraines (obtained from Vidaller et al., 2024), glaciers and ice-patch (2023 surface delimited in purple, obtained from Izagirre et al., 2024) of the Maladeta massif, and the reconstruction of the end of the Allerød period (12.9 ka BP, this reconstruction has been obtained from cosmogenic dates of the northern slope of the massif and the information was extrapolated to the rest of the massif, based on the geomorphological landforms; Vidaller et al., under review; Vidaller et al., 2024).

- Finally, the glaciated areas in 2021 were determined through the utilisation of drone surveys. The glacier outline was also delineated manually following Vidaller et al. (2023).

In order to classify the soil samples according to their age, the soils were grouped in eight clusters:

1- Lowland soils: 5 developed soil samples with horizons and covered by grass.
2- PreLIA: 17 soil samples in areas that were deglaciated between the Allerød (13.9–12.9 ka) and the LIA moraines. The difference between Lowland soils and PreLIA soils is the percentage of plant cover, Lowland soils present a 100 % of plant cover.
3- LIA-1956: 29 soil samples originated between the LIA maximum and 1956.
4- 1956-2000: 7 soil samples generated during the period 1956–2000.
5- 2000-2009: 5 soil samples generated during the period 2000–2009.
6- 2009-2021: 5 soil samples generated during the period 2009–2021.
7- Passes and peaks: 14 soil samples whose age is unknown, but they present similar topographic conditions since they are all found in passes and peaks of the massif.
8- Soils 0: 4 very incipient soil samples formed just after ice melted without vegetation.

3.2. Soil samples collection and processing

A total of 86 samples were collected during summer 2020 and 2021 across the massif, with the objective of capturing a high variability in topographic conditions and glacial cirques along the deglaciation chronosequence. Consequently, soil samples were collected from locations exhibiting a wide range of orientation, elevation, slope, duration of snow cover, and deglaciation period. To check consistency in the distribution of soils characteristics, two samples were often collected in close proximity to each other (with similarities in soil characteristics, including vegetation cover, wind protection, and soil colour). The uppermost centimetre of soil was discarded to avoid analysing soils with oxidation processes (due to the interaction atmosphere-soil), to prevent contamination with any other agent and to avoid any other alteration of the samples. The depth of soil samples collected ranged from two to five centimetres, depends on the availability of soil in each place (but all of the soil patched presented >5 cm depth). In instances where the soil was partially covered by gravels, these were also removed.

In all cases, a minimum of 0.5 kg of soil was collected. Half of this quantity was stored in a fridge at the Pyrenean Institute of Ecology (IPE-CSIC, Zaragoza, Spain), while the remaining portion was dried in an oven set at 50 °C for a period of 24 to 48 h, the duration of the drying process dependent on the moisture content of each soil sample. Following the completion of the drying process, the samples were subjected to sieving, with only the smallest fraction (<2 mm) retained for subsequent analysis.

3.3. Topographic and duration of snow cover analysis

The basic topographic information (coordinates and elevation) of each sample was obtained during the field campaign with a GPS device to geolocate each sampling point. A digital elevation model (DEM) of 2 m, obtained from the IGN and the ArcGIS 10.5.1 software (ESRI), was used to calculate the orientation using the *Aspect* tool and the slope using the *Slope* tool. The potential radiation under clear sky conditions was calculated for the whole year and for the ablation season (June–September) using the *Solar Radiation* tool.

For the duration of snow cover for the years 2018–2021, a series of maps were created utilising the Google Earth Engine, R and Sentinel 2 images with cloud cover below 60 % between 15th February and 31st August. The presence of snow in each pixel was determined using the algorithm proposed by Gascoin et al. (2019). The duration of the snow cover of each sample was calculated as the mean of the four-year studied period.

3.4. Geochemical, sedimentological and mineralogical analysis

Most of the geochemical analyses were carried out at the IPE-CSIC laboratories. The moisture content (%) was determined by weighing the samples before and after drying them in an oven, being the water content the difference between the two weights. Samples for grain size analysis were heated for 24 h at 80 °C in 3 % H_2O_2 to eliminate organic matter and measured with a laser grain-size analyser MasterSizer 2000 according to Vicente de Vera-García et al. (2023). Shepard's texture diagram (Shepard, 1954) was used to classify the samples into sand (>63 μm) and silt (<63 μm; note than in this study silt and clay have been considered together).

pH was measured using a calibrated combination electrode in a solution of 10 g soil in 25 ml distilled water. Electrical conductivity ($EC_{1:5}$) was measured using a conductivity meter with temperature probe and conductivity cell in a dissolution of 10 g of sample suspended in 50 ml of distilled water.

Total carbon (TC), total inorganic carbon (TIC), total sulphur (TS) and total nitrogen (TN) were analysed using a LECO SC144 DR analyser. For TIC analysis, organic matter was previously removed at 460 °C for 300 min in a muffle furnace. Total organic carbon (TOC) was calculated as the difference between TC and TIC (Vicente de Vera-García et al., 2023). Inorganic carbon content was also measured using the Bernard calcimeter according to Lamas et al. (2005). As the amount of inorganic carbon was too low, close to the detection boundary for both methods, the TC was considered equivalent to TOC.

The water stability of soil aggregates (WAS) was determined according to the single sieve method of Kemper and Rosenau (1986) using a modified Yoder apparatus (Yoder, 1936). Briefly, 5 g of air-dry aggregates of 1–2 mm diameter were placed on the top of a 0.25 mm mesh sieve and sieved in distilled water for 3 min (34 strokes min^{-1}; stroke length 1.3 cm). After this time, the soil retained on the sieve was transferred to an aluminium dish, oven-dried and weighed. Sand correction was performed by dispersing the stable aggregates with sodium polyphosphate (5 g l^{-1}) and sieving through the 0.25 mm sieve. WAS is expressed as the percentage of the weight of the aggregates retained on the sieve (sand-free basis) to the initial weight of the aggregates.

An elemental composition was carried out by determining the CEC at the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC, Spain). Exchangeable cations were extracted with 1 M ammonium acetate, washed with ethanol and extracted with 10 % NaCl. CEC was determined by UV–visible spectrophotometry using a segmented flow autoanalyzer. The exchangeable ammonium and nitrate contents of the soils were also determined after extraction with 1 M KCl and subsequent analysis by UV–Vis spectrophotometry using a segmented flow autoanalyzer.

Total concentration of the main elements associated to mineral components (Al, Fe, Ti, Si, Ca, Mg, Na, K) were carried out using an ICP-OES 720-ES (Varian) at the El Zaidin-CSIC experimental station (Granada, Spain). Samples were digested with HCl and HNO_3 (1:1:3 de H_2O: HCl:HNO_3) in an UltraWAVE (Milestone) at 220 °C for 15 min. The total concentration of iron (Fe_T) and aluminium (Al_T) was extracted in an Ethos Plus Microwave Lab Station by adding 8 ml of a mixture of HNO_3/HF (3:5, *v*/v) (Otero et al., 2005). The efficiency of the extraction process (>90 %) was determined by analysis of certified reference materials (MESS-3 and Soil SO_3). The mineralogy of 42 of the samples was determined by means of X-ray diffraction (DRX) using a Bruker-AXS D5005 powder diffractometer at the GEO3BCN-CSIC facility in Barcelona, Spain. The results are presented in terms of the percentage composition of each mineral in each soil sample.

3.5. Biological analysis

3.5.1. Plant cover

The percentage of plant cover of each soil parcel was measured following the Daubenmire method (Daubenmire, 1956) by visual determination during the field work, described and applied later by Seefeldt and Booth (2006). With a parcel of 20 × 50 cm, the plants were grouped in a fictitious way, and the plant cover was quantified as the percentage of the parcel covered by all plants. For each soil patch the dominant species has been identified and grouped by their main presence in each soil age group defined.

3.5.2. Microbiological analysis: phospholipid-derived fatty acids

Soil samples were lyophilised, and aliquots of 2 g were used for lipid extraction. Lipids were extracted with a one-phase chloroform-methanol-phosphate buffer solvent. Phospholipids were separated from non-polar lipids and converted to fatty acid methyl esters prior to analysis, following the methodology described by Buyer and Sasser (2012). The resulting fatty acid methyl esters (FAMEs) were separated by gas chromatography using an Agilent 7890 A GC system (Agilent Technologies, Wilmington, DE, USA) equipped with a 25-m Ultra-2 (5 %-phenyl)-methylpolysiloxane column (J&W Scientifc, Folsom, CA, USA) and a FID detector. Identification and quantification of FAMES was performed using the PLFAD1 method of Sherlock software version 6.3 from MIDI, Inc. (Newark, DE, USA). The internal standard 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA) was used for quantification of FAMEs. Total microbial biomass was estimated by summing the concentration of all individual PLFAs and expressed as nanomoles of PLFA per gram of soil.

Specific PLFAs were used as biomarkers to quantify the biomass of broad taxonomic microbial groups according to their characteristic fatty acids: eukaryotic, Gram-negative and Gram-positive bacteria (hereafter G- and G+, respectively), actinobacteria, fungi (considering saprophytic and arbuscular mycorrhizal together) (Frostegård and Bååth, 1996). The ratios between G+ and G- bacteria, fungi/bacteria and G- stress were also used to describe the composition of the microbial community.

3.6. Statistical analysis

A range of statistical analysis was considered to analyse and group the characteristics of the soils, and the main properties that condition the development of these new ecosystems after the ice melt. The normality and homogeneity of variance has been tested with the Kolmogorov-Smirnov test to check data distribution. A correlation matrix, using Kendall's rank correlation coefficient, has allowed to simplify the variables studied in the statistical analysis and to discard redundant variables, such as annual and ablation (June–September) radiation or sand and silt percentage.

As the first hypothesis was if the age of the soil determines the evolution of very incipient soils, Mann-Whitney test (none of the considered variables had normal distribution) were made between each pair of deglaciation period soil groups to determine if there were significative differences between groups. Also, two Principal Component Analysis (PCA; one for the geochemical variables and another one for the microbiological ones) were done to group the samples attending of similar characteristics and to relate the variables analysed to the soil development process. Furthermore, PCA were used to prove if the development process was conditioned by the period of deglaciation. To check if there were other characteristics that control the development of incipient soils three differential analysis (DA) were done. A first one considering once again the period of deglaciation (PreLIA, LIA-1956, 1956–2000, 2000–2009, 2009–2021, Passes and peaks, Soils 0 and Lowland soils), another one considering the cirques where the samples were collected (Aneto, Maladeta, Coronas and Cregüeña cirques), and finally a last one considering three snow duration periods (>201, 180–201 and <180 days with snow cover).

In addition, we compared the characteristics of the most contrasted soils in their degree of development (the most developed soils - MDS and the least developed soils - LDS) considering the biomass, WAS and TC variables. Ten samples were selected from each group. To select a sample for the MDS group, it was necessary that the sample was in the top 10 of the higher values of at least two variables between biomass, WAS and TC. Conversely, for the LDS group, the sample had to be in the lower top 10 of at least two of the three possible variables.

Finally, a redundancy analysis (RDA) was done in order to determine which topographic and geochemical variables are more influent for the development of the microbiology.

4. Results

4.1. Glacier evolution since the LIA

The year 1850 was regarded as the maximum extension of the glaciers during the LIA (e.g. Grove, 2004), although in the Maladeta massif the Aneto LIA moraine was dated at 400 ± 50 years (Vidaller et al., under review). At this time, 707.06 ha of the Maladeta massif was covered by glaciers, distributed in 16 glaciers. Additionally, there were 6.6 ha of ice patches. By 1956, almost a century later, the glacial surface had diminished by 60.3 %. The remaining nine glaciers, which had undergone some division since the end of the LIA, covered 280.6 ha. From 1956 to 1981, the rate of shrinkage was slower, from 4.0 ha yr^{-1} for the 1850–1956 period to 1.1 ha yr^{-1} for the 1956–1981 period. Consequently, the glacierised area reduced by 64.2 % since the LIA. In the year 2000, only 175 ha remained (a reduction of 74.8 % since the LIA), and the rate of shrinkage (3.9 ha yr^{-1}) was similar to that observed for the period LIA-1956. The area covered by ice patches remained relatively stable at 10.1 ha. During the period 2000–2009, the rate of shrinkage accelerated to 5.7 ha yr^{-1}, with losses amounting to 82.14 %. The Maladeta glacier fragmented into the eastern and western ones. The smallest glaciers underwent degradation, and ice patches covered 3.6 ha (Table 1). During the period between 2009 and 2021, the Coronas glacier became an ice patch. The Aneto glacier, meanwhile, was divided into two bodies between 2015 and 2016. By the end of this period, the glacierised area had shrunk to 85.21 ha, representing an 87.9 % reduction compared to the end of the LIA.

4.2. Topographic characteristics of the soil samples of different deglaciation periods

For each of the eight age groups into which the soil samples were classified, the slope (Fig. 2a, b) and radiation during the ablation period (Fig. 2c) were very similar. Logically, the elevation (Fig. 2d) of the soils was one of the main characteristics to differentiate each deglaciation period. Lowland soils group was at the lowest elevation, followed by PreLIA soils, while Passes and peaks soils were the highest group, followed by the most recent soils (2009–2021). Similarly, the duration of snow cover (Fig. 2e) was very different in the Lowland soils with a median of 151 days compared to the median of 187 days in the 2009–2021 soils. Finally, looking at the distribution of soils in the glacial cirques (Fig. 2f), the Aneto cirque hosted the greatest variation of deglaciation periods, while in the Cregüeña cirque the LIA-1956 soils dominated.

4.3. Geochemical, microbiological and vegetation characterisation

Granitic rocks were the common substrate in all the study area, with a mineralogical composition dominated by quartz (34 %), Ca-rich albite (30 %), muscovite (13.4 %), intermediate microcline (11.2 %) and clinochlore (8.7 %). There were also other minerals whose content was much lower (around 1 %) and they were only present in some samples. Some of them were pyrophyllite, montmorillonite, potassic pargasite, beidellite and Fe-rich magnesian hornblende. Mineralogical

Table 1
Glacierized area (ha) for each year that compound this study. Data of 1850 corresponds with the LIA maximum. The data of 1956 and 1981 were obtained from orthophotos, the data of 2000 and 2009 from LANDSAT imagery, and the data of 2021 from drone surveys. (*) Modified from Vidaller et al. (2023).

Glacier		LIA maximum	1956	1981	2000	2009	2021
Alba	Western	5.65	3	2.44	–	–	–
	Central	1.98	–	–	–	–	–
	Eastern	4.12	–	–	–	–	–
Maladeta	Western	126.36	65.01	59.41	47.84	7.22	4.82
	Eastern					29.16	20.24
Aneto	Principal	251.32	121.28	112.53$^{(*)}$	89.88	68.15	46.10
	Secondary						3.93
Barrancs		132.83	17.57	14.74	12.75	8.43	3.77
Tempestades	Principal		31.65	30.33	16.73	10.72	6.35
	Pass	4.57	–	–	–	–	–
Ixalenques	Western	6.84	–	–	–	–	–
	Eastern	2.03	–	–	–	–	–
	Secondary	6.84	–	–	–	–	–
Salenques	Western	51.42	4.81	3.77	–	–	–
	Eastern		17.67	15.64	5.88	–	–
Russell		9.31	–	–	–	–	–
Llosas		31.98	–	–	–	–	–
Coronas		42.55	15.47	11.31	4.88	2.6	–
Maldito		9.29	–	–	–	–	–
Cregüeña		19.97	4.16	3.08	–	–	–
Total glaciers (ha)		707.06	280.62	253.25	177.96	126.29	85.21
Ice patches (ha)		6.58	11.3	7.67	10.08	3.57	0.90
Glacier losses (%)			60.31	64.18	74.83	82.14	87.95

composition appears not to be useful to distinguish soil samples since they all have very similar mineral content. On the other hand, other soil characteristics were highly variable across the massif (Fig. 3).

The Maladeta massif was primarily distinguished by a neutral-acid pH (median 5.5; Fig. 3a) and a low electric conductivity (median 382 μS cm^{-1}), which exhibited remarkable homogeneity. The moisture content was relatively low, with a median value of 9.5 %. It is noteworthy that, despite their incipient nature, the WAS percentage was relatively high (median 64.1 %; Fig. 3k). This is in contrast to the TC, which is very low (median 0.9 %; Fig. 3b). The lower values of TC were found in the southern slope of the massif, meanwhile, the higher values were in the lower elevations of the northern slope. In line with the low content of organic matter, the content of TN (median of 0.1 %) and the TS were very low (median of 0.02 %). With regard to inorganic nitrogen fractions (Fig. 3d), nitrate exhibited considerable spatial variability, with very low values in most of the Maladeta massif (32.8 ± 65.7 mg kg^{-1}), with the exception of Aneto cirque, where values were markedly elevated (mean 72.4 ± 104 mg kg^{-1}; Fig. 3d). Within these, ammonium concentrations (Fig. 3c) were higher in the lower elevations (median 10.3 mg kg^{-1}; 30 to 200 mg kg^{-1}) than in the upper elevations (0 to 20 mg kg^{-1}). The lower values of silt (between 0 and 30 %) were found in the south slopes and at intermediate elevations. Regarding to the chemical elements, the distribution of Ca (Fig. 3f) was very variable (median: 5.1×10^3 mg kg^{-1}), but the higher values were found near the front of the glaciers. A similar distribution was found in the case of the P (Fig. 3g). Mg (median: 7.4×10^3 mg kg^{-1}; Fig. 3h) and Fe (median: 20.7 $\times 10^3$ mg kg^{-1}; Fig. 3i) which presented their maximum values at the higher elevations, excluding Passes and peaks. Finally, the S content (median: 0.1×10^3 mg kg^{-1}; Fig. 3j), related with the organic characteristics of the soils, presented their lower values at the south face of the massif and at the higher elevations of the north face, meanwhile the lower values were at the lower elevations of the northern slope.

The total microbial biomass was mainly higher in the lower areas of the Maladeta massif (Fig. 3l). Given this distribution, these areas also had a higher fungi/bacteria ratio (Fig. 3m). Meanwhile, the eukaryotes were more abundant at the south face of the massif (Fig. 3n), where the ice melted before. On the other hand, the highest plant cover surface was found at the north face and samples at lower elevation, where the percentage reached the 100 % (Fig. 3o).

In the case of the vegetation, the dominant species of each soil patch was determined. Lowland soils and PreLIA soils were characterised by the dominance of poaceae, Carex, *Vaccinium uliginosum*, *Pedicularis pyrenaica*, *Calluna vulgaris*, *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *Pinus uncinate* and *Juniperus alpine*. The Passes and peaks soils presented spermatophytes such as *Leucanthemopsis alpine*, *Saxifraga pubescens* or *Androsace ciliata*. The soils deglaciated after LIA were characterised by the dominance of *Leontodon pyrenaicus*, *Poa alpine* and *Saxifraga moschata*.

It was notable a paucity of correlation observed among the considered variables. In general terms, elevation and snow cover duration (and any of the other topographical variables) did not correlate with any other variable. There were significative correlations between TC, TN, TS and nitrates, also between Al, Mg and Fe elements. As anticipated, the organic geochemical variables, such as high TC, were correlated with high microbial biomass.

4.4. *Soil characteristics related to deglaciation periods, glacial cirques and snow cover duration*

Table 3 shows the pairs of soil groups (based on deglaciation age periods) that exhibited the largest statistically significant differences for the different considered soil characteristics, while Table S1 in Supplementary material shows all existing statistical differences among soil groups. Mann-Whitney analysis did not show many significant differences between soil characteristics and deglaciation periods. Thus, significant differences were not observed between the groups LIA-1956, 1956–2000 and 2000–2009. The most different groups were lowland soils from PreLIA compared to both Passes and peaks and from LIA-1956 groups. The moisture content allowed to differentiate the Passes and peaks group from most of the others. The electrical conductivity did not exhibit statistically significant differences among groups. However, pH demonstrated notable differences between older and younger groups, with the largest discrepancies observed between the Passes and peaks and the 2009–2021 groups. The percentage of WAS was the only variable with significant differences between Lowland soils and LIA-1956 groups. The TC content was a key differentiating factor between the lowland soils and Passes and peaks groups from 2000 to 2009, as well as the PreLIA group and LIA-1956. In terms of chemical elements, regarding the amount of Ca, the largest differences were between Passes and peaks and 2000–2009; in the case of P between Lowland soil group and PreLIA group. Mg was different between Lowland soil group and 2009–2021; meanwhile in the case of Fe the greatest discrepancy was

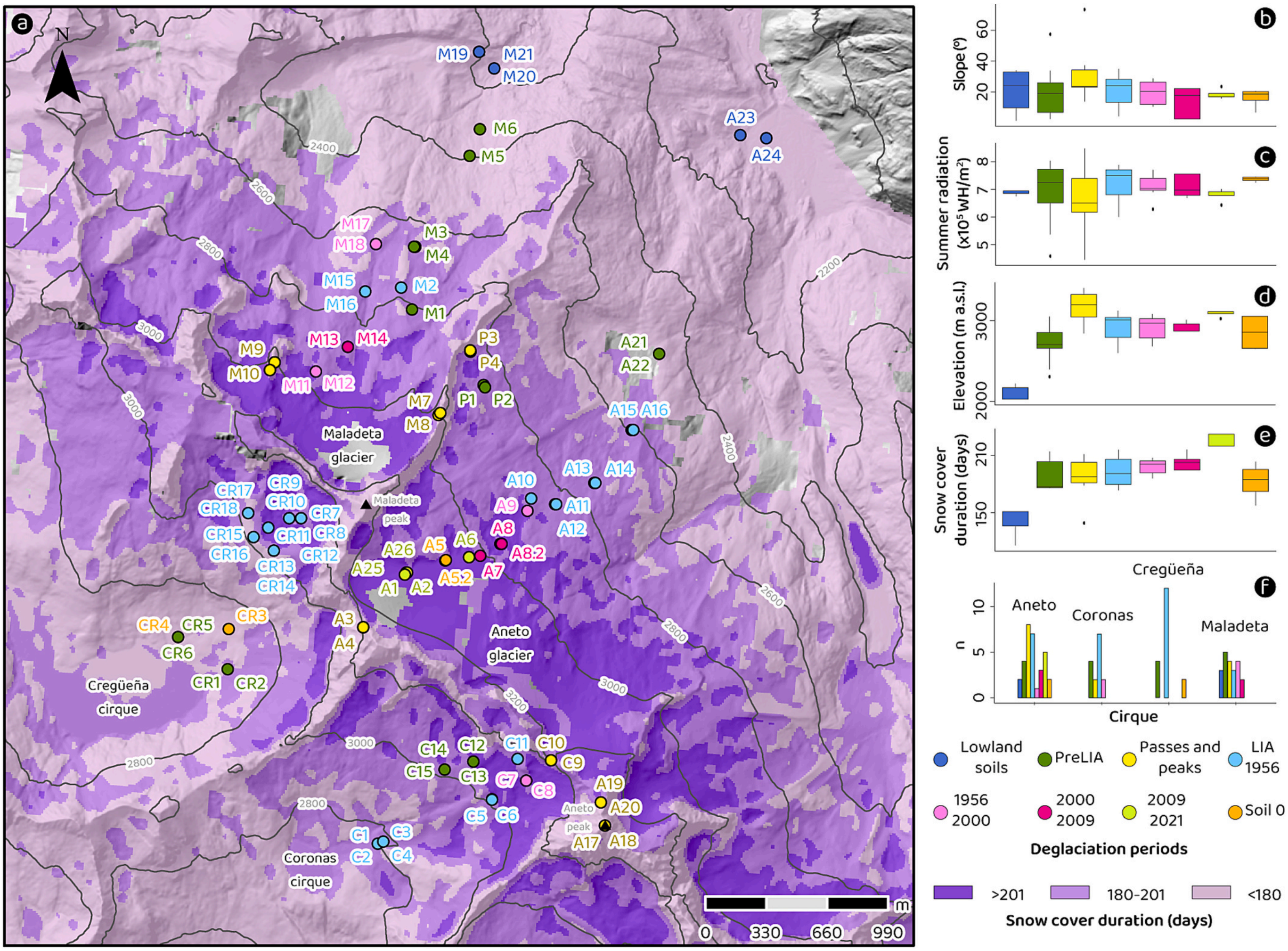

Fig. 2. (a) Location map of the soil samples collected in the Maladeta massif, in the Aneto (A), Maladeta (M), Cregüeña (CR) and Coronas (C) cirques. The colour of the dots referred to the period of deglaciation that has been assigned. The colour of the base map refers to the duration of snow cover. (b) Boxplot that represents the distribution of slope for each deglaciation period. (c) Boxplot that represents the distribution of summer (June–September) solar radiation for each deglaciation period. (d) Boxplot that represents the distribution of elevation for each deglaciation period. (e) Boxplot that represents the distribution of snow cover duration for each deglaciation period. (f) Bar diagram that represents the number (n) of soil samples of each deglaciation period at each cirque.

between Lowland soils and PreLIA; and in the case of S the main difference was between PreLIA and Soil 0 groups. The ammonium concentration exhibited the greatest discrepancy between the PreLIA and the other groups, with the greatest divergence observed between the LIA-1956 and the 2009–2021 groups. Nitrate concentration discrepancies were observed between Passes and peaks and Lowland soils. The percentage of silt distinguished the groups LIA-1956 from PreLIA, Passes and peaks, 1956–2000 and 2009–2021 (Table 2).

In relation to the microbiological variables, they exhibited notable similarity across most of the groups compared (Table S1 in Supplementary material). Biomass was different between PreLIA and Passes and peaks, LIA-1956 and 2000–2009 groups. Eukaryote in Lowland soils differed from 2009 to 2021, and PreLIA from Pass and peaks. G- bacteria only differed in Lowland soil and PreLIA groups. In the case of G+ bacteria, in addition to this difference, LIA-1956 was different from Lowland soil, Passes and peaks and 2009–2021, and 1956–2000 from Lowland soils and 2009–2021. Actinobacteria was different between Passes and peaks groups from others. Fungi were different in the pairs Lowland soil of Passes and peaks and 1956–2000, and 2009–2021 of Passes and peaks, 1956–2000 and Soil 0. The presence of anaerobe was different in the pairs 2000–2009 with Lowland soils, PreLIA, and 2009–2021. Finally, the G- stress only differentiated the pair formed by the Passes and peaks and the 2009–2021 groups. Regarding plant cover, most groups exhibited notable distinctions from one another, with the highest median discrepancy between the Lowland soils and Soil 0 groups (Table 2).

The two PCA analyses identified 6 components to classify the samples. The characteristics of each component can be seen in Figs. S2 and S3 in the Supplementary material. Fig. 4 shows that soils associated to most of the discriminated components can be found in the majority of the deglaciation periods. Thus, in most periods of deglaciation was possible to find soil samples with the highest correlation with components 1 and 2 (the ones that accumulate most of the variance), and very often in the same period were found positive and negative correlation values.

However, the PCA analyses revealed some interesting patterns. When considering soils from the same deglaciation period, elevation proved to be a highly influential variable. However, when considering soils at the same elevation, the percentage of silt emerged as the most significant variable, with the deglaciation period also exerting a notable influence (Fig. 5 and Fig. S2 in Supplementary material). Soils at higher elevations exhibited longer periods of snow cover, higher pH levels, and were associated with a lower percentage of silt, moisture content, electric conductivity, TS and plant cover percentage (Fig. 5 and Fig. S2 in Supplementary material). Low percentage of silt was indicative of a low organic content. Regarding soil variables, a strong association

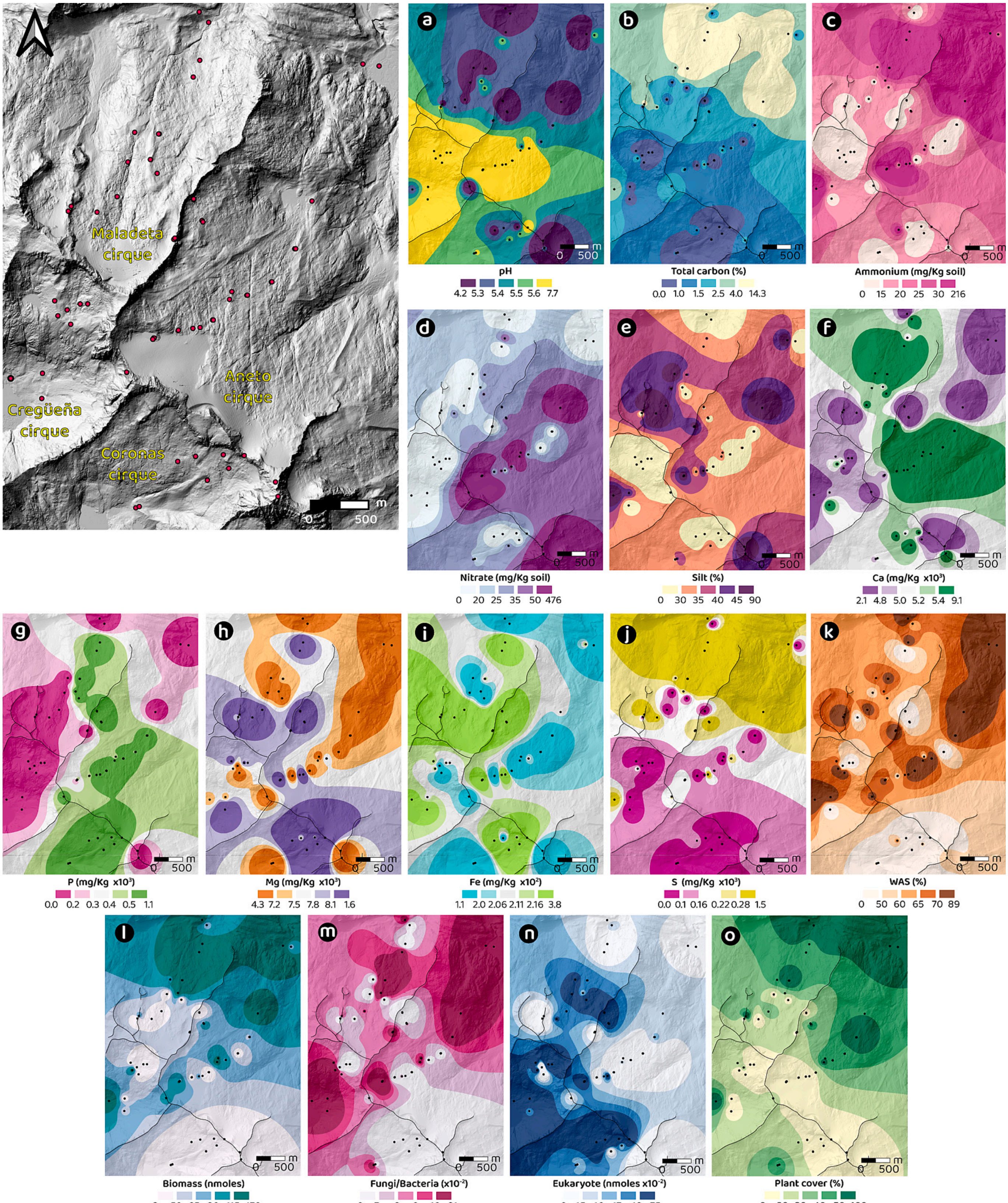

Fig. 3. Main map shows the location of the soil samples (red dots) in the different cirques (Aneto Maladeta, Coronas and Cregüeña cirques). Spatially interpolated values of (a) pH, (b) total carbon (TC), (c) ammonium, (d) nitrate, (e) silt, (f) total calcium (Ca), (g) total phosphorus (P), (h) total magnesium (Mg), (i) total iron (Fe), (j) total sulphur (S), (k) water stability of soil aggregates (WAS), (l) biomass, (m) ratio fungi/bacteria, (n) eukaryote, and (o) plant cover. Maps was derived by interpolating (inverse distance weighting method) the results of each variable in the study area using QGIS software.

Table 2
Pairs of age period groups that most differentiated each other for each variable according to Mann Whitney test. The upper deglaciation period group of each cell corresponds with the group with highest values.

Humidity (%)	1956–2000 Passes and peaks	Na (g kg^{-1} soil)	Soil 0 LIA-1956	Biomass (nmoles)	PreLIA 2000–2009
pH	2009–2021 Passes and peaks	P (g Kg^{-1} soil)	PreLIA Lowland soil	Eukaryote (nmoles)	PreLIA Passes and peaks
WAS (%)	Lowland soil 1956–2000	S (g Kg^{-1} soil)	PreLIA Soil 0	G- (nmoles)	Lowland soil PreLIA
TC (%)	PreLIA LIA-1956	Si (g Kg^{-1} soil)	Passes and peaks 2000–2009	G+ (nmoles)	1956–2000 Lowland soil
Al (g kg^{-1} soil)	PreLIA Lowland soil	Ti (g Kg^{-1} soil)	Lowland soil 1956–2000	Actinobaterias (nmoles)	2009–2021 1956–2000
Ca (g kg^{-1} soil)	2000–2009 Passes and peaks	Exchangeable NH_4^+	PreLIA 2000–2009	Fungi (nmoles)	2009–2021 1956–2000
Fe (g kg^{-1} soil)	PreLIA Lowland soil	Ammonium (g kg^{-1} soil)	2009–2021 LIA-1956	Anaerobe (nmoles)	PreLIA 2000–2009
K (g kg^{-1} soil)	Passes and peaks Soil 0	Nitrate (g kg^{-1} soil)	Passes and peaks Lowland soil	Fungi/bacteria	2009–2021 1956–2000
Mg (g kg^{-1} soil)	2009–2021 Lowland soil	Plant cover (%)	Lowland soil Soil 0	G+/G-	LIA-1956 Lowland soil
Mn (g kg^{-1} soil)	2009–2021 Lowland soil	Silt (%)	2009–2021 LIA-1956	G- Stress	2009–2021 Passes and peaks

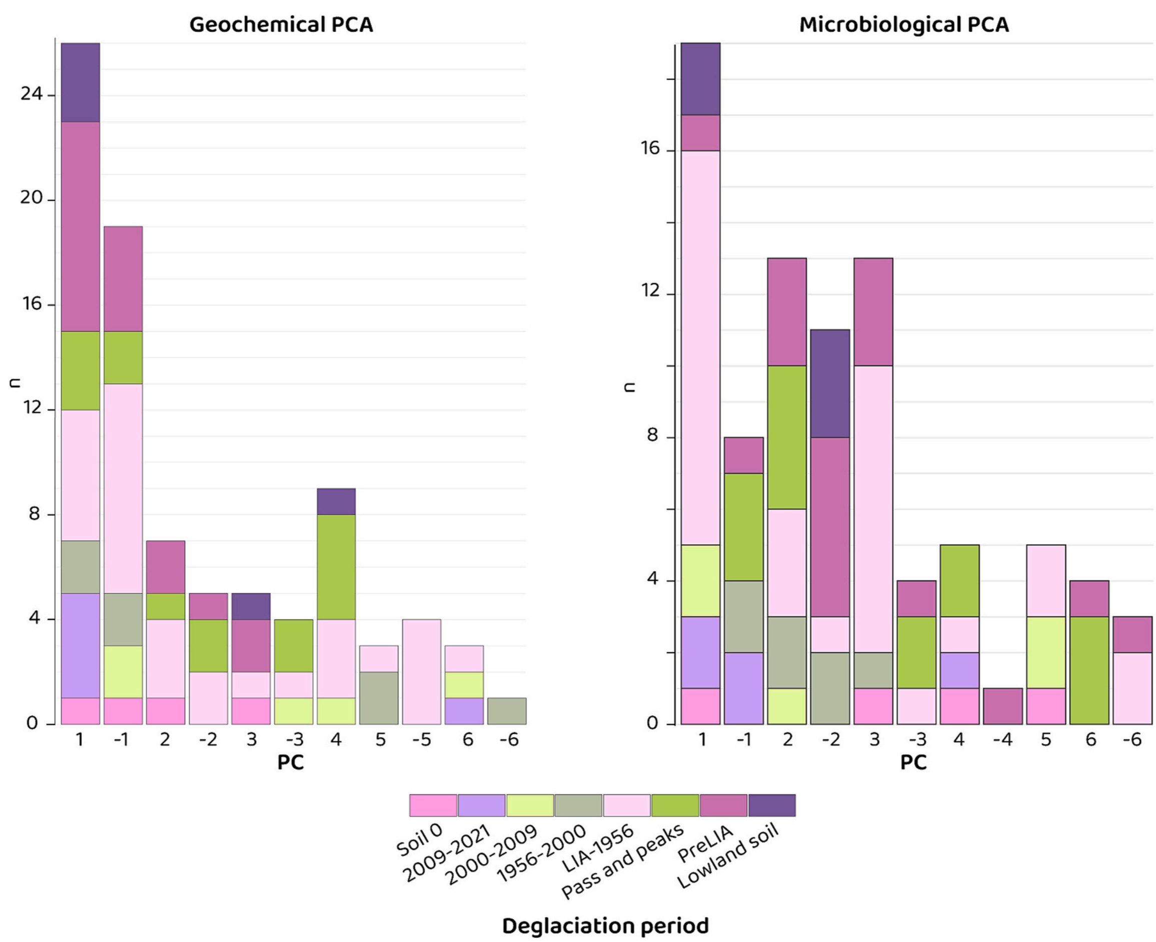

Fig. 4. Geochemical (left) and microbiological (right) PCA. The bars of the diagrams show the number (n or frequency) of samples that are more related to each component (positive and negative). The colours are associated to the different periods of deglaciation.

between TC, TN, silt and ammonium contents was observed (Fig. 5). These variables were also strongly associated with a higher plant coverage and TS content (Fig. 5 and Fig. S2 in the Supplementary material).

Higher microbial biomass frequently implied higher relative abundance of fungi (Fig. 5). Similarly, bacteria, were also more prevalent in areas with higher snow cover and elevation (Fig. S3 in the Supplementary material). With regard to the microbiological variable, the deglaciation period assigned to each soil group played a more significant role than in the case of the geochemical variables. In the younger soils, the ratio of fungi to bacteria was lower (Fig. 5 and Fig. S3 in the Supplementary material). A high pH is associated with a high relative

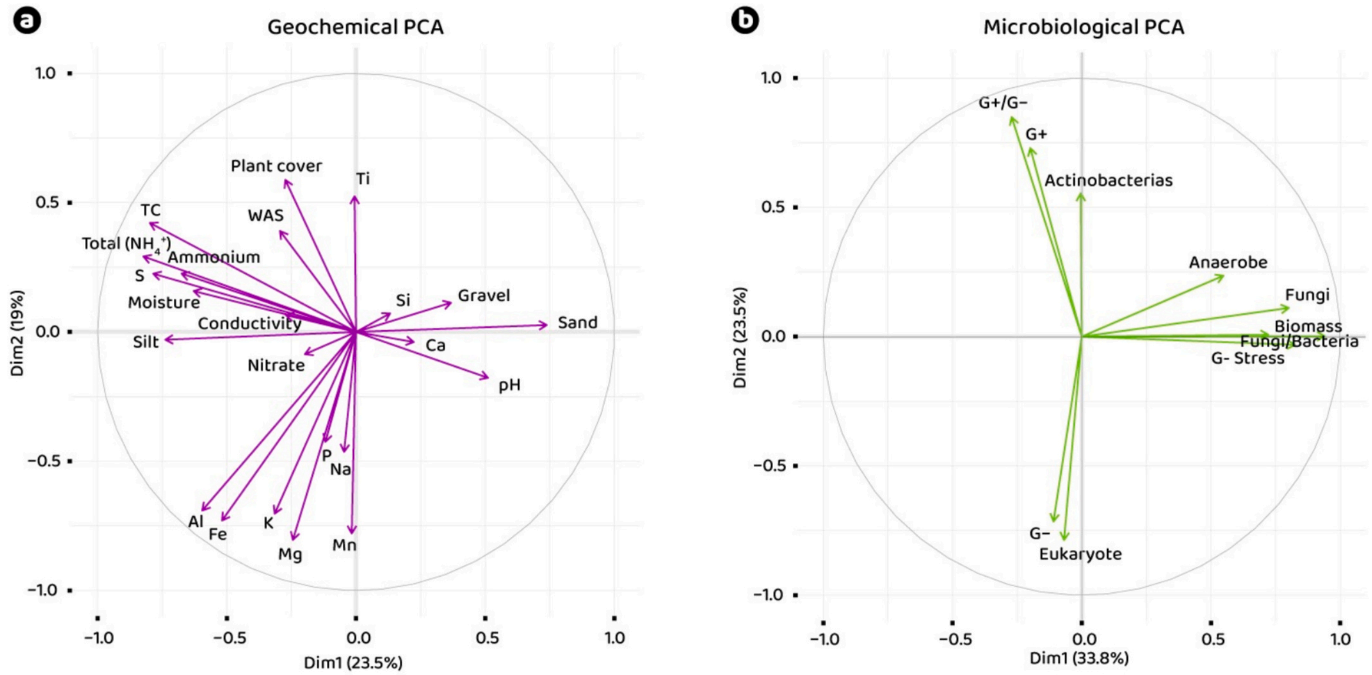

Fig. 5. PCA plots for the geochemical (a) and microbiological analysis (b). The geochemical variables include the chemical elements, grain-size, pH, conductivity, moisture, nitrate and ammonium content, WAS and plant cover. For this plot, the period of deglaciation was not considered.

abundance of eukaryote, low levels of stress (higher G-stress index) and a higher relative abundance of fungi (Fig. S3 in the Supplementary material).

Differential analyses (DA) did not show a clear discrimination between soil properties and groups based on deglaciation periods (age-chronosequence), while characteristics of groups based on cirques and snow duration exhibited more differences (Fig. 6). Based on deglaciation groups (Fig. 6a), the most different group was the Lowland soils, followed by the Soil 0 group, 2009–2021 group and the Passes and peaks group. PreLIA soils presented the highest percentages of plant cover, meanwhile, the higher content of P was in the Passes and peaks group. On the other hand, the most recent soils presented high percentage of silt, pH, Na and Mg. Fungi were more abundant in the older soils, meanwhile G- bacteria in the younger ones. Additionally, the Al content was higher in the most recent soils. Considering the data provided by the different cirques (Fig. 6b), the soils of Cregüeña cirque exhibited the most notable differentiation, displaying elevated levels of pH, Ti, and Al. The Aneto cirque samples exhibited elevated concentrations of nitrate, ammonium, TS, Fe and silt. The samples from Coronas cirque showed elevated levels of P and Mg, yet displayed reduced pH, TC, Al_T, and TN. Conversely, samples of the Cregüeña and Maladeta cirques exhibited elevated TC, Al_T, pH, and TN. TS presented lower values in Cregüeña cirque and higher values in Aneto cirque. The lowest values of P were observed in Cregüeña cirque and the highest in Coronas cirque. Regarding the snow cover duration (Fig. 6c), the intermediate period (180–201 days) revealed the highest percentage of silt, biomass and plant cover. Conversely, areas with shorter snow cover duration exhibited higher concentrations of chemical elements, such as Mn, P, Al, Fe. Samples from sites with longer snow cover duration (exceeding 201 days) displayed elevated moisture levels, elevated nitrate and ammonium concentrations, and were situated at higher elevations. Additionally, the *P* values were higher at sites with longer snow duration. A significant difference was observed in the total Fe content depending on snow duration, with higher values recorded in areas with longer snow cover.

4.5. *A comparison of the characteristics of soils that are most and least developed*

The most developed soils (MDS, based on biomass, TC and WAS) was found at PreLIA, Lowland soils, and one from LIA-1956 (Fig. 7); and were abundant in the Maladeta and Aneto cirques. This eight samples were characterised by lower elevation, with a median of 2474 m a.s.l. and 176 days of snow cover duration. These soils presented higher humidity (26.5 %) and higher silt fraction (median: 49.7 %). The conductivity was 424 μS cm^{-1} and the pH in median was 5. Considering the organic variables, the plant cover reached the 65 % in median and the median of microbial biomass was 256 nmoles. The biofilic elements (TS and TN) were higher in this group, as well as the nitrate and ammonium. Referring to the microbiology, the relative abundance of bacteria in this group was lower, in contrast with that of fungi, which was higher. Also, this group presented higher values of G- stress (i.e. low stress) related with the oxidative stress (Fig. 7).

On the other hand, most of the least developed soils (LDS) were found at LIA-1956 group, and a few samples of PreLIA group. These samples were located in the Coronas and Cregüeña cirques and at high elevations (3044 m a.s.l. in median) with more days (214 days) of snow cover. The humidity of these soils was very low (7 %), and the grain-size was larger (dominated the sand fraction). The conductivity was lower (285 μS cm^{-1}) meanwhile the pH was higher (6.2 as median). For the LDS, the organic variables were near to cero. The ammonium and nitrate were very low (5 and 4 mg kg^{-1}, respectively), but exhibiting very high dispersion. The content of bacteria was higher in this group, but there were no fungi (Fig. 7).

5. Discussion

Soil formation is the result of the interaction of five formation factors (climate, relief, parent material, living organisms and time; Jenny, 1941); in our case we considered time (duration of the deglaciation period), relief (topography), living organisms (plant cover, microbial biomass) and climate (snow cover duration); while the parent material for all samples was the same (granite) but with potential local small

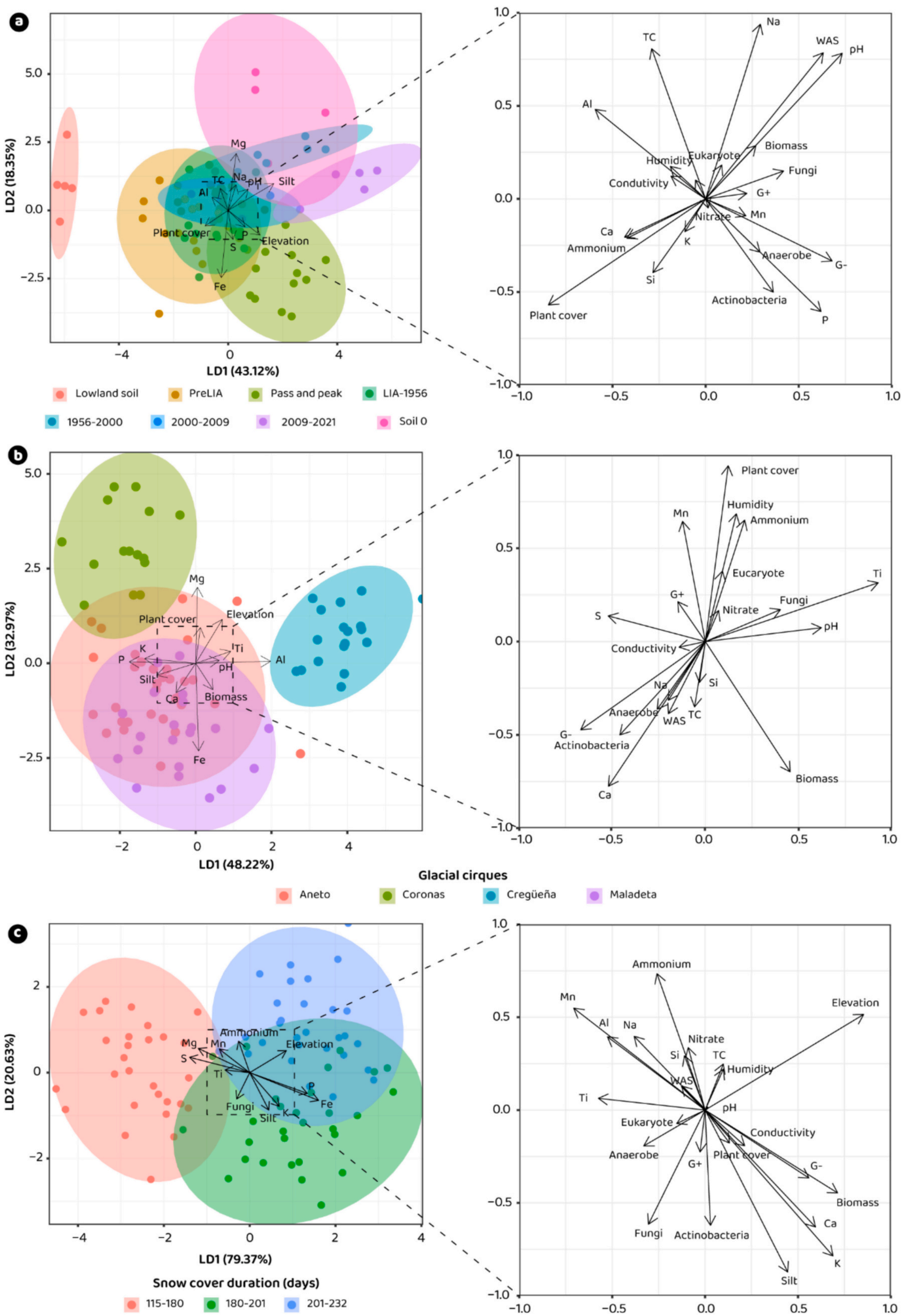

Fig. 6. Plots of the DA analysis. (a) DA considering the eight periods of deglaciation. (b) DA considering the four studied cirques (Aneto, Maladeta, Coronas and Cregüeña cirques). (c) DA considering the snow cover duration periods (<180, 180–201 and >201 days of snow cover duration). For these analyses geochemical and microbiological variables have been considered together. The variables with the greatest influence are not displayed; only those that are less prominent are magnified.

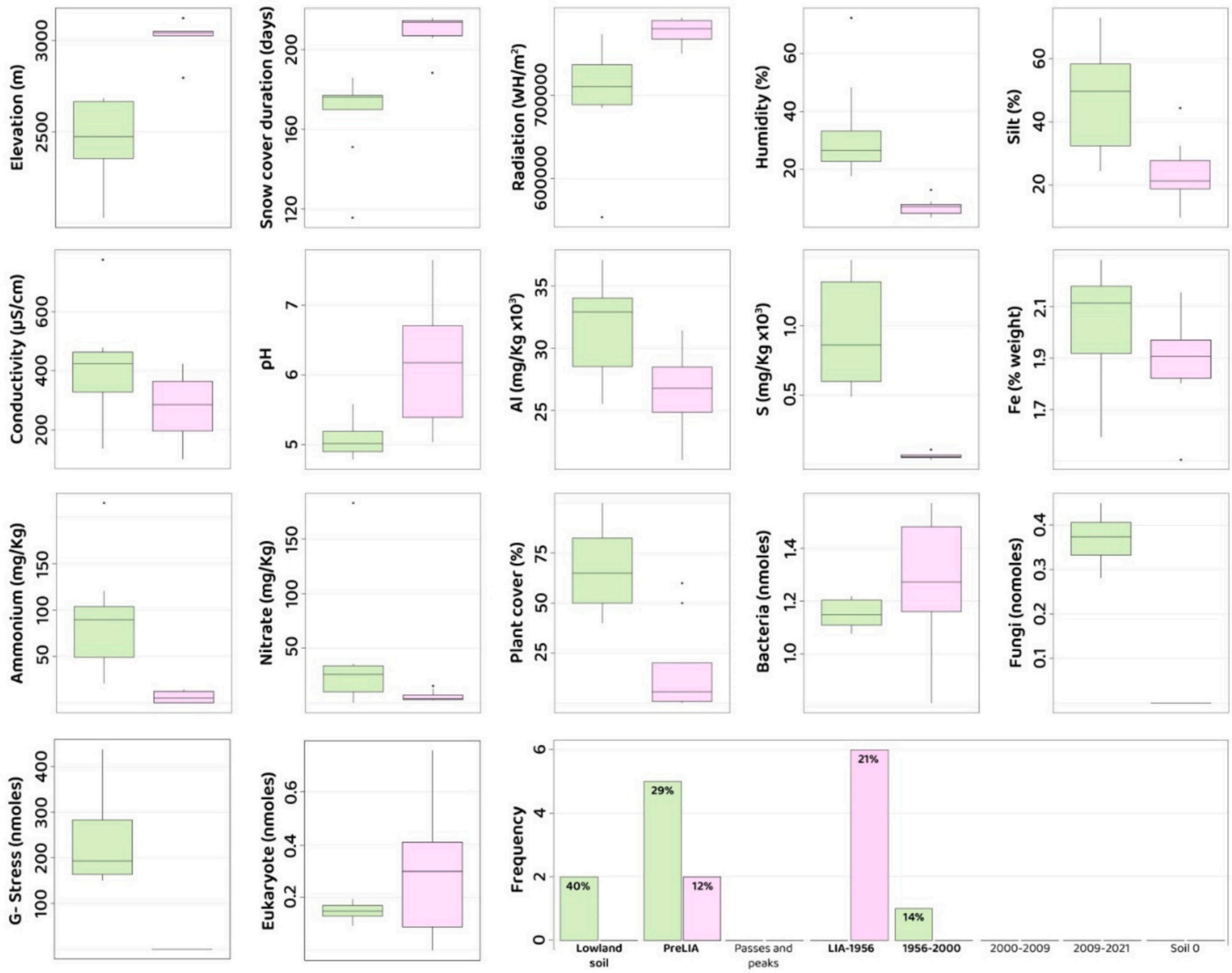

Fig. 7. Boxplots of the variables analysed that best represents the difference between the most developed soils (MDS) and the least developed soils (LDS) groups. Green plots correspond with the most developed soils and pink plot to the least developed soils. Each boxplot represents the median and quartiles of each group (MDS and LDS). In the case of the period of deglaciation analysis, the bars inform about the number (frequency) of soil samples that corresponded to each deglaciation period and development state (MDS and LDS).

variations in chemical, mineralogical composition and grain-size.

5.1. Erratic pattern in the development of the incipient soils

In general, the results did not show clear differences between different groups of soils (separated by the period of deglaciation) nor between cirques or snow cover duration. Soils from the LIA to 2009 were very similar and without variables that statistically differentiated them, indicating that time was not an important factor on controlling soil development (Targulian and Krasilnikov, 2007). The age groups statistically different to the others were Lowland soils, Passes and Peaks, Soil 0 and 2009–2021. This was also evident when comparing the most and least developed soils (MDS and LDS, respectively). The MDS were formed by soils from the Lowland and PreLIA groups. However, the LDS were expected to belong to Soil 0 or 2009–2021 groups, but instead they corresponded to PreLIA and LIA-1956 groups.

Despite the limited development of the soils, it has been possible to identify some soil components that are key factors in the evolution of the incipient soils towards a new mountain habitat. The percentage of fine material (silt and clay) was an essential component that gave the soil a higher reactivity, moisture, water and nutrient retention capacity and greater stability (higher WAS). Consequently, all elements related to the biogenic component (TC, TN, TS, ammonium, nitrate) showed the highest contents. Also a higher content of organic matter favored a better soil aggregation, acidification and soil stability, as was demonstrated in previous studies (Zumsteg et al., 2012). This fine material could be provided by the glacier meltwater or from the weathering of the bedrock. Even though the soils were formed decades ago, the organic content was very limited when the content of silt was low. Likewise considering samples of the same deglaciation period, the duration of the snow cover (related with the elevation) influenced the development of the soils. Shorter snow cover duration increased the colonization of the microbiological components and plants. In contraposition to other investigations, these results showed that the chronosequence is not a really important variable, and other parameters influence more on the development of incipient soils. Paradoxically, this result may be explained by the very high resolution of the used chronosequence series. Several studies considered samples of almost completely developed soils with ages of hundreds of years, but this research was focused in a high number of periods for a relatively short time interval.

The correlation analysis did not show correlation between the topographic variables with geochemical and microbiological characteristics. We hypothesise that the microtopography created by glacial erosion and distribution of boulders played a more important role in the development of incipient soils (i.e., accumulation of silty sediment in small depressions or concavities) and the subsequent revegetation than the topography at higher scale (i.e. altitude, aspect, etc) or the time that the terrain was deglaciated. Also, glacial, periglacial, fluvio-glacial, and slope dynamics promote transportation and sediment mixing adding uncertainty to the real age estimation of the soil patches (Masseroli et al., 2023).

In alpine areas, soil development is often non-linear with the chronosequence. There are other factors that affect the development of incipient soils. For example, geomorphological features controls the granulometry and the distribution of geochemical elements due to erosion by runoff, snow and ice melt (Navas et al., 2024). The input of nonweathered material causes continuous rejuvenation of the profiles (Leonelli et al., 2024). Fine regolith and sediments are often deposited and stabilised on concave surfaces or close to large rocks and later evolved into incipient soils that were laterally discontinuous (Leonelli et al., 2024). Sometimes, there is an alternation of phases of soil erosion and phases of thickening of the soil by continuous sediment input deposited by geomorphological processes (Egli and Poulenard, 2016; Johnson and Watson-Stegner, 1987). This way, during the initial phase of soil development and considering the same parent material (granite) the geomorphic process, including the microtopography of the proglacial area, notably influence soil physical and chemical properties (Temme, 2019).

As in other studies (Ficetola et al., 2024), very incipient soils (2009–2021 group in our study) presented higher values of nutrients, such as carbon, sulphur or nitrogen. These nutrients may came from glacier and snow microorganisms (Khedim et al., 2021; Rime et al., 2016; Zimmer et al., 2024) and are probably essential for triggering the formation of early-stage communities of microorganisms and other pioneer organisms (Bardgett et al., 2007).

5.2. Biological succession pattern

Most of the microbiological variables were correlated to each other. Redundancy analysis indicated that the pH, snow cover duration, WAS and TC percentages were the main variables that control the microbiological colonization of the soils, as it was also supported by other studies (Zumsteg et al., 2012; Jiang et al., 2021). Other variables, as the moisture, were also influential. In the case of carbon content, other studies showed that the content of organic carbon increases with soil age showing an increment of acidity with soil age (Masseroli et al., 2023). Also, the carbon content influences on the pH values, because the organic matter accumulation acidifies the soils (Bernasconi et al., 2011; D'Amico et al., 2015). More importantly, the evolution of the biological variables, depends on the interaction with the physical variables, but especially depend on the environmental stress (Matthews, 1992).

High amount of fungi was correlated with higher G- stress, so less stress in the habitats. The microbiological characteristics were very similar in all the deglaciation period groups, only the percentage of plant cover differentiated most of the groups, which was in agreement with the importance of the rhizosphere in creating favourable microenvironments for the development and biodiversity of microorganism populations (Leyval and Berthelin, 1991; Otero et al., 2013; Dlamini et al., 2022). The snow cover duration (highly controlled by the elevation) influences the evolution of the microorganism. At higher elevation and snow duration the ratio G+/G- is higher. Also, the geochemical variables influence in the evolution of the microbiology. High pH implies higher relative abundance of eukaryotes and lower of G+ bacteria, as pH was a significant factor influencing soil microbiology (Lauber et al., 2009). On the other hand, high percentage of silt favored a higher moisture content, which in turn promotes anoxic conditions and the development of anaerobic microorganism. The variables biomass and plant cover were associated to a high content of carbon, meanwhile the high percentage of WAS was mostly related with a high value of G- stress (that implies low oxidative stress), and the presence of fungi.

The fine material of the soil favours greater stability and increased water retention, so that bacteria can colonise the habitat, then WAS were generated through the action of the microorganism, and finally, when there was a stable substrate, the vegetation took hold the soils. Attending to the succession of the microorganism, younger soils showed lower ratio fungi/bacteria, confirming that the first to colonise these new habitats are the G+ bacteria, followed by G- bacteria, and were subsequently replaced by fungi. This succession probably was influenced by the water availability (Stawska, 2017). Initial microbial colonisers fix C and N into bioavailable forms, which in turn promote the development of more complex and efficient microbial communities as well as later plant establishment (Donhauser and Frey, 2018). Many bacteria were also better adapted (than were fungi) to life in barren, early-successional sediments, so they were more abundant than fungi in areas with higher snow cover and elevation. They can be photoautotrophs, heterotrophs or chemoautotrophs and many can fix atmospheric nitrogen (Nemergut et al., 2007; Schmidt et al., 2014), whereas fungi are all heterotrophs, and none can fix nitrogen. So, once the bacteria had extracted nutrients from the minerals in the bedrock and from the atmosphere, the fungi started to grow up. They were more dependent on fixed sources of carbon and nitrogen than bacteria and may not have as many available niches before there was significant accumulation of organic matter during succession (Zumsteg et al., 2012).

After the microorganism colonization, vegetation can inhabit these ecosystems, and fungi were closely associated with plants (Paul et al., 2004; Ficetola et al., 2024). Plant distribution and cover are related to geomorphic processes, whereas sediment transport and biogeomorphic stability thresholds have been identified as driving factors of colonization patterns in glacier forefields (Haselberger et al., 2021, 2023). The plant colonization contributes to the development of organic matter of the incipient soils. Also, the colonization of nitrogen-fixed plant species favour the increase of nitrogen in the soils (D'Amico et al., 2015). This feedback mechanism between the arrival of specific species to incipient soils, and their effects on the geochemistry and microbiological properties of the soil at patch scale should be object of further research. In this study most of the species identified were spermatophytes and bryophytes, showing that the incipient stage of the plant colonization is logically related with the adverse weather conditions found in the high mountains and the short time soils have been uncovered by ice and snow.

5.3. The impact of the human footprint

The soils of the Aneto cirque, and also of the Maladeta cirque, presented high levels of ammonium and, above all, nitrate, in some samples with values similar to those of ornithic soils (typical of those observed in seabird colonies; see for example De la Peña-Lastra et al., 2020; Otero and Fernández-Sanjurjo, 1999). Normal values in non-cultivate soils are <10 mg kg^{-1} (Weil and Brady, 2017), meanwhile in the case of Aneto samples, this values are higher than 50 mg kg^{-1}. This is a very transited area by the mountain tourism. The Aneto peak (3404 m a.s.l.) is the highest peak of the Pyrenees and the objective of many mountain climbers. It is calculated that during the summer months, between June and September, around 13,000 people transit this cirque (Posets Maladeta Natural Park database). There is only one hut, Renclusa hut, located at 2140 m a.s.l., so it is common to find people every day bivouacking above 2500 m a.s.l.

The presence of a higher amount of ammonium and nitrate in this cirque is associated with a high amount of N, which favours primary productivity, i.e. other biophilic nutrients such as P, S or C. Higher microbial biomass, higher fungi abundance and anaerobic organisms were positively associated with higher TC, TN, NH_4^+, NO_3^- silt, whereas they were negatively associated with snow duration. Besides that, fungi were the most important microorganism groups for microbial biomass. The high amount of N, could promote and accelerate the development of biomass and plant cover (Robertson et al., 1997; Chen et al., 2004).

These findings highlight the complex interactions of cold conditions, including snow and ice, in these regions, with the presence of mountain tourism, evidencing the fragility of these areas with regard to tourism activities.

6. Conclusions

Since the LIA, the Maladeta massif glacier has been receding steadily, leaving only 12 % of its glaciarized surface in 2021. This has allowed the rapid development of new habitats. This study shows the main characteristics of 86 samples of incipient soil located at the proglacial area of the Maladeta massif. The results of this study did not show a clear relationship between the time in which the area was deglaciated and the degree of development of a soil. In this way, there were no statistical differences between soils from the LIA to 2009, showing that a longer period of time is required for the correct generation of incipient soils.

The content of clay and silt was found to be a key factor in the development of soils properties and microbial communities. It gives the soil a higher reactivity, water and nutrient retention capacity and greater stability. So, silt amount favours the colonization of bacteria, that at the same time favours the development of WAS, the colonization of fungi, and finally the establishment of plants and higher diversity of microorganisms. Low pH and high S values were also characteristic of the most developed soils. In the case of the microorganism, there is a succession pattern in which the first colonisers are the G+ bacteria, followed by the G- bacteria, fungi and other eukaryotes. The duration of the snow cover (very related to the elevation) also played an important role in the development of incipient soils. Thus, the better conditions for the development of soil microbiology are low altitudes with a high percentage of silt.

Mountain tourism is an important factor to be considered in the development of these new habitats. The incipient soils are very vulnerable areas, in which the contamination due to the anthropic activities may alter the presence of nutrients and therefore, the colonization of microorganisms. Environment eutrophication (e.g. high concentration of inorganic N) decrease the biodiversity, although the biomass increases due to the colonization by pioneers and nitrophilous species, but this acid soils do not allow the succession pattern of the microorganism.

CRediT authorship contribution statement

Ixeia Vidaller: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xosé Luis Otero:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **José Mariano Igual:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Gabriel Nuto Nobrega:** Writing – review & editing, Formal analysis. **Tiago Osorio Ferreira:** Writing – review & editing, Formal analysis. **Ana Moreno:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Juan Ignacio López-Moreno:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the LIFE-Climate4Pyrenees co-funded by the European Union and SNOWDUST (TED2021-130114B-I00) funded by the Agencia Española de Investigación, and funds from the research group of Aragón: Geomorfología y Cambio Global. Ixeia Vidaller is supported by the grant FPU18/04978 and is enrolled in the PhD programme at the University of Zaragoza. José M. Igual was supported by project "CLU-2019-05-RNASA/CSIC Unit of Excellence", funded by the Junta de Castilla y León and co-financed by the European Union (ERDF "Europe drives our growth"). Other support was provided by the Consellería de Educacion, Universidade e Formacion Profesional-Xunta de Galicia (Axudas á consolidacion e estruturacion de unidades de investigacion competitivas do SUG del Plan Galego IDT, Ambiosol Group ref. ED431C 2022/40).

We acknowledge the help of Esteban Alonso, Chemary Carrera and Marcel Galofré during the field work, the help of Maria Victoria López-Sánchez with the WAS analysis, the help of Juan José Jiménez with the interpretation of the results and the help of Rafael Vidaller, José Antonio Sesé and Victor Ezquerra with the plant identification. Thanks, are also owed to AEMET, Clima y Nieve Pirineos and Posets-Maladeta Natural Park for providing the climatic data of Renclusa hut, Llanos del Hospital and Aneto weather stations; to Posets-Maladeta Natural Park for the field work permits necessary to this investigation, and to IPE laboratories for facilitate the laboratory works.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178740.

Data availability

The results of the analyses employed in the present study can be located in the repository: https://doi.org/10.5281/zenodo.14864208.

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