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Research Article

Assessing Microbial Diversity in *Páramo* Soils (Multistrategy Analysis): Effects of Potato Farming and Livestock Grazing in Nevados National Natural Park, Colombia

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High Andean *Páramos* are very fragile neotropical ecosystems. Moreover, biodiversity in these areas is threatened by the anthropic activities of agriculture, cattle raising, and mining and has been little studied. Changes associated with potato farming and livestock grazing on microorganisms of *Páramo* soil of the Nevados National Natural Park (Nevados NNP) were assessed by (1) determination of physical and chemical properties (physicochemical matrix) and enzymatic activities associated (enzymatic activity matrix) with different biogeochemical cycles (C, N, and P); (2) microbial community functional diversity via evaluation of functional groups associated with carbon, nitrogen, and phosphorus cycles using cultivation-dependent techniques (arable functional group matrix and most probable number matrix); and (3) microbial diversity using cultivation-independent techniques that employ the hypervariable V5–V6 region of the *16S rRNA* gene and pyrosequencing (*16S*-454 genus matrix and *16S*-454 OTU matrix). Four of the six evaluated matrices (physicochemical, enzymatic activities, most probable number, and arable functional groups) revealed significant differences according to land use. The strategy adopted by the arable functional group matrix, in which the diversity of nitrogen fixation, phosphate solubilization, and cellulolytic compounds was evaluated, showed the most significant impacts of the different factors (land use, season, and elevation), especially those caused by potato cultivation and livestock. These results indicate that the initial impacts of potato farming and livestock grazing on the microbial community in El Bosque Village are better detected by functional diversity analysis than by molecular analysis of the *16S rRNA* gene V5–V6 variable region. The results may have been caused by the type of molecular marker used in the analyses and the type of agricultural practices used by peasant farmers, which affect the functional diversity of the soil community. Among these practices are the maintenance of fallow periods greater than 7 years between each potato crop and the small proportion of cattle in relation to the total land area of the village. As the findings can be interpreted as an indicator of the early impacts of potato cultivation and livestock on microbial diversity, the effects of implementing community management plans, applying agroecological models, retaining biocultural memory, and changing agrarian structure are relevant for mitigating future changes.

1. Introduction

Neotropical ecosystems known as *Páramo*s extend across vast regions situated above the high Andean forest line (3000–3800 m.a.s.l.) and the perpetual snow limit (4400–4800 m.a.s.l.) within the northern reaches of the Andes Mountains [1–4]. These areas are found in Ecuador, Venezuela, Costa Rica, and Colombia, with Colombia having the greatest extension of this ecosystem [5]. Bioclimatically, the *Páramo* ecosystem is characterized by extreme environmental conditions of great biological influence: low atmospheric pressure, air density, and average temperature [6, 7]. Furthermore, due to its capacity to retain substantial amounts of water in its hydromorphic soils and to control flow through watersheds, *Páramo* land is considered an ecological unit of great importance for the regulation of water flow [8] (Figure 1).

The *Páramos* are strategic biomes of critical importance that have been called "biodiversity hotspots within hotspot areas" due to their location in the Tropical Andes and the simultaneous condition of containing high biodiversity and highly threatened species [3, 9, 10]. *Páramo* regions are renowned for their essential role in generating, regulating, and preserving water resources and for their scenic value, a considerable number of plant and animal species that are exclusive to the region, and soil-associated microbiota, which is the foundation supporting the formation and growth of *Páramo* plant communities [7] (Figure 2).

The Nevados NNP is located in the Colombian central mountain range and has about 66% of the total park area composed of *Páramo* ecosystems [11]. Current potato cultivation and livestock activities within *Páramos* of the Nevados NNP combine intensive application of fertilisers and chemical pesticides, monoculture, and abundant irrigation with traditional practices by peasant farmers in the zone. Despite the relevance of the *Páramo* and the need to adopt measures for the protection and restoration of soil in this park [9], only a limited amount of research has investigated the impacts of these agricultural practices on the diversity of soil microbes.

Microorganisms are essential elements of soil because they transform organic matter and are important in the different biogeochemical cycles [12, 13]. Microbes are also considered bioindicators because they are very sensitive to different changes in edaphic systems, and they have been used to evaluate soil quality and predict its degradation [3, 14, 15]. The edaphic microorganisms may be affected by agricultural practices and human intervention in natural ecosystems. Thus, it has been suggested that proper analysis of the effects of land use and these practices on soil microbial should include evaluations at three different levels [16]: (a) the soil process level, at which aspects such as biomass, respiration rates, and enzymatic activities and their interrelations are important, even though they do not provide specific information at the taxonomic level or microbial community level; (b) the community or specific microorganism level (performed by cultivation-dependent methods), in which qualitative and quantitative changes can serve as important indicators; and (c) the microbial community level (performed by cultivation-independent methods due to limitations of cultivation-dependent methods), whereby the bacterial genetic diversity observed in soils by cultivation-independent methods can be 200-fold greater than that obtained by cultivation-dependent methods.

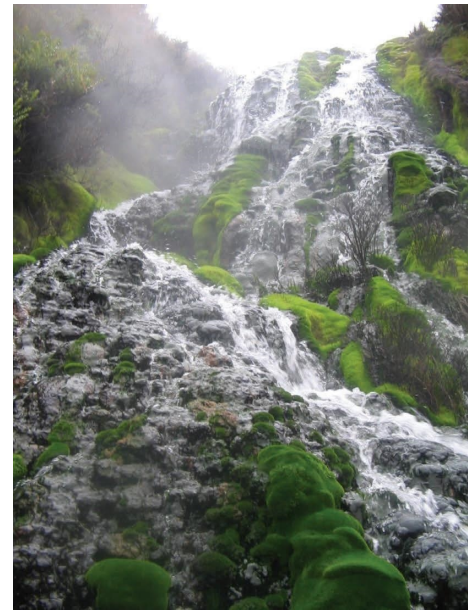

Figure 1: Páramo's photography. Nevados National Natural Park: surroundings of Coquito stream, taken by Lizeth Manuela Avellaneda-Torres.

Figure 2: Páramo's photography. Nevados National Natural Park 2, taken by Lizeth Manuela Avellaneda-Torres.

To date, studies have been conducted on plant biodiversity in *Páramo*s [17], carbon stored in *Páramo*s soils [18, 19], bioprospecting on cellulolytic microorganisms [20] and phosphate solubilizers in *Páramo* soils [21], and changes in the properties of *Páramo* soils by fires [22] and by afforestation [23]. However, there is scant information on the impacts of potato farming and livestock grazing on the soil microbial biodiversity in *Páramo* regions. We hypothesize that the potato crop and cattle farming would alter soils and the microbial communities present. Thus, the aim of this investigation was to assess potential alterations with potato crop and livestock grazing on microbial communities of *Páramo* soils of the Nevados NNP by using different strategies related to (a) soil processes, physicochemical parameters, and

enzymatic activities associated with nitrogen phosphorus and carbon cycles; (b) functional diversity of microorganism community by determining abundance and diversity of functional groups (C, N, and P) using cultivation-dependent techniques; and (c) microbial diversity by employing the *16S rRNA* gene V5–V6 hypervariable region and pyrosequencing. In addition, this research sought to establish the physicochemical, enzymatic, and microbial parameters that best explain changes in *Páramo* soils under potato crop and livestock grazing.

2. Materials and Methods

2.1. Description of Research Area. The research was carried out in Hamlet El Bosque (Pereira, Risaralda) in the Nevados NNP, Colombia. El Bosque is located at 3650 m.a.s.l., is surrounded by ice-capped mountains (Ruiz, Santa Isabel, and Tolima), is the only human settlement inside the protected area [24], and is the only area where agricultural and livestock activities occur in the park.

Agricultural activities, which involve the combined use of agrochemicals and local know-how, entail the removal of the native *Páramo* vegetation, a system of rotating potato (*Solanum tuberosum*) crops with pasture (livestock grazing) in biannual cycles, with fallow periods that can exceed seven years. For potato cultivation, agrochemicals such as carbofuran, parathion, methamidophos, chlorpyrifos, profenofos, mancozeb, propineb, mefenoxam, phenothrin, and synthetic N : P : K chemical fertilisers are applied, based on seller recommendations. However, farmers do not keep records of quantities, doses, and frequencies of chemical applications [3, 25]. Potato crop management is performed via manual ploughing processes without the use of specialised machinery. Livestock is reared with the main purpose of obtaining milk and cheese. The main cattle breed is Normande, and the density of livestock per hectare is 0.24–0.36. The grasses that are planted on the site are orchard grass (*Dactylis glomerata*), ryegrass (*Lolium* sp.), and plegadera (*Lachemilla* sp.) [3], for which no fertilization or maintenance activities are performed [3].

The soil samples obtained are those that have accumulated at the foot of nearby steep slopes, which are covered by thick layers of volcanic ash. Within the study area, one can observe ancient scree deposits buried beneath layers of pyroclastic materials. Soil samples were collected from three farms in the village (Buenos Aires, El Edén, and La Secreta). According to the general study of soils in Colombia, the sampled farms are located within two climatic units. The agroecosystem in Buenos Aires is situated close to Lake Otun, at elevations ranging from 3,600 to 4,000 m.a.s.l. It experiences daily average temperatures ranging from 6 to 9°C, with an average annual precipitation between 2,000 and 4,000 mm [3]. In this region, the microclimate is caused by the vigorous movement of nearby winds caused by the proximity to the Volcano Santa Isabel [3, 26]. The agroecosystems El Edén and La Secreta are situated at elevations ranging from 3,000 to 3,600 m.a.s.l., with average daily temperatures ranging from 9 to 12°C and average annual precipitation between 1,000 and 2,000 mm [3].

2.2. Sampling Design. Soils were sampled from three agroecosystems within the village of El Bosque: Buenos Aires (3,769 m.a.s.l.), El Edén (3,590 m.a.s.l.), and La Secreta (3,432 m.a.s.l.). In all cases, the slope was between 50 and 75%. In each farm, three categories of land use were assessed: *Páramo* (greatest possible conservation) (Figures 3 and 4), potato cultivation (*S. tuberosum*) (Figure 5), and livestock (Figure 6). Soil samples were collected on each type of land by taking 10 subsamples along a zigzag in a 10 m × 10 m quadrant. Sampling took place during both the dry and rainy seasons. A study design incorporating three factors was used: soil use, elevation, and sampling time. Each factor included a nested component known as the observation window or quadrant, which was executed at three distinct sites within every combination. Every factor combination underwent assessment at the three different sites, resulting in a total of 54 samples: 3 types of land use ×3 elevations ×2 seasons ×3 observation windows or quadrants. Furthermore, each sample underwent analysis in three replicates [3, 27].

Soils were sampled from the first 20 cm of soil after the elimination of the layer of plant material covering the surface. Furthermore, plant root balls were gently lifted out of the soil, and the plants were gently shaken to collect soil that remained on the roots. The typical vegetation of the sampling areas was for the potato crop (*S. tuberosum*); for livestock, typical grasses such as orchard grass (*D. glomerata*), ryegrass (*Lolium* sp.), and plegadera (*Lachemilla* sp.) are maintained. *Páramo* zones with the lowest level of possible intervention were selected; the vegetation included Cortaderia (*Cortaderia selloana*), Reventadera (*Pernettya prostrata*), Buddleja (*Buddleja* sp.), Lupinus (*Lupinus albus*), Dendropanax (*Dendropanax* sp.), and Chusquea (*Chusquea* sp.). These areas of *Páramo* were characterized because they are isolated, and potato cropping or livestock rearing has never been performed. They are located at a minimum distance of 1 ha from the cultivation and livestock areas [3].

The soils were sieved through a 2 mm mesh before analysis. For microbial and enzymatic analyses, the samples were transported and stored at 4°C and 20°C, respectively.

2.3. Methodological Strategy for Analysing Microbial Communities

2.3.1. Soil Process, Physicochemical Parameters, and Enzymatic Activities. The following physical and chemical parameters were evaluated as part of soil processes according to IGAC methods (2006): (a) moisture: gravimetric method [28]; (b) bulk density: cylinder method [28]; (c) structural stability: Yoder mechanical sieving method [29, 30]; (d) texture: Bouyoucos method [28, 31]; (e) pH: potentiometric method using a soil : water ratio of (p/v) 1 : 1; (f) the percentage of CO: Walkley–Black method [32]; (g) cation, calcium, magnesium, and potassium contents and sodium exchange capacity: 1 N ammonium acetate extraction [33]; (g) exchangeable acidity: extraction with 1 M KCl [33]; (h) assimilable phosphorus: Bray II method [34]; (i) total nitrogen: micro-Kjeldahl method [35]; (j) N-NH_4 and N-NO_3:

Figure 3: Páramo's photography. Nevados National Natural Park 2: surroundings of Nevado Santa Isabel, taken by Lizeth Manuela Avellaneda-Torres.

Figure 4: Páramo's photography. Nevados National Natural Park 2: surroundings of El Edén, taken by Lizeth Manuela Avellaneda-Torres.

Figure 5: Photography of potato cultivation. Nevados National Natural Park, taken by Lizeth Manuela Avellaneda-Torres.

Figure 6: Photography of livestock. Nevados National Natural Park, taken by Lizeth Manuela Avellaneda-Torres.

extraction with 2 M KCl distilled with MgO and Devarda's alloy, respectively [36]); and (k) the total carbon (TC), total hydrogen (TH), and total nitrogen (TN) contents: LECO 1000 elemental analyser (Model CHN-1000; LECO Corp., St Joseph, MI) [3, 37]).

In addition, the following enzymatic activities associated with nitrogen cycles (urease and protease), phosphorus cycle (acid and alkaline phosphatase and phosphodiesterase), carbon cycle (β-glucosidase), and intracellular metabolism, including dehydrogenase, were determined according to the methods modified by Avellaneda-Torres et al. [27].

2.3.2. Functional Diversity of Microorganisms. The functional diversity of microorganisms associated with microbial functional groups of carbon, nitrogen, and phosphorus (arable functional groups) was determined using cultivation-dependent techniques. In this case, a recount of the colony-forming units (CFUs) for each of the soil samples was performed ($CFUg^{-1}$ soil) using the serial dilution method and plating. The selective media used for each group were as follows: (a) nitrogen-fixing microorganisms [38], as modified [39]; (b) phosphate-solubilizing microorganisms (bacteria and fungi) [40], as modified [39]; and (c) cellulolytic microorganisms (bacteria and fungi) [20].

Subsequently, the isolation, purification, and characterization of the different morphotypes obtained were carried out. For molecular characterization in the case of bacteria, *16S* rDNA was used using primers 1492R and 27F according to the procedures described by Avellaneda-Torres et al. [3]. In the case of fungi, the amplification of the internal transcribed spacer (ITS) was performed using primers ITS1 and ITS4. The sequencing and subsequent bioinformatic analysis were carried out according to the methods reported by Avellaneda-Torres et al. [3]. The diversity of microorganisms in the soil samples was described through two components: species richness and microbial structure (i.e., composition and abundance of each species) [3].

In addition, the abundance of the following microorganisms associated with the nitrogen cycle was determined by the most probable *number* method in accordance with a previous study [41]: ammonifier, proteolytic, ammonium-oxidizing, nitrite-oxidizing, and denitrifier microorganisms.

2.3.3. Microbial Diversity According to Cultivation-Independent Techniques. Microbial diversity was analysed according to cultivation-independent techniques that use the V5–V6 hypervariable region of the *16S rRNA* gene and pyrosequencing, as described [39] [42]. DNA from the soil samples was isolated and purified using the PowerSoil DNA Isolation Kit (MoBio). All of the PCR products were verified by 1% agarose gel electrophoresis and EZ-Vision® staining. The reads obtained were quality-controlled and clustered using QIIME v1.8.0 [43]. Potential errors of sequencing were minimised as reported previously [44–46]. The quality of the sequences was tested and filtered with quality thresholds as

reported by Bohórquez et al. [42]; the minimum sequence length selected was 150 bp after the removal of the bases corresponding to barcodes, adapters, and primers. The OTU clusters and representative sequences were determined through reference OTU picking using UCLUST at 97% identity [47], followed by abundance filtering (OTU cluster >0.1%), and taxonomic classification was carried out using the RDP Classifier [48] and applying a bootstrap confidence threshold of 70%. Statistical analyses and data visualization were conducted in the R statistical programming environment [49] using the OTU absolute abundance table and mapping file. These tasks were facilitated through the phyloseq package [50].

Based on the overall results of microbial diversity at the genus and representative OTU levels, each sample was defined considering two factors of diversity: species richness and microbial structure (i.e., composition and abundance). For the first factor, the number of (genus-level) phylotypes found in each sample and a mixed-effects analysis of variance, such as use, farm, sampling period, and observation window, were used. For microbial structure, the identity and abundance of each phylotype and representative OTU per sample were preserved. The data were grouped into two matrices, including one global matrix in which a sequence analysis at the genus level was included (*16S*-454 genus) and the second matrix in which data corresponded to the most representative operational taxonomic units were included (OTUs; *16S*-454 OTU).

2.4. Statistical Analysis

2.4.1. Relative Influence of Land Use on Microbial Structure. Univariate and multivariate statistical analyses were performed on the data matrices for the different methodological strategies listed earlier. A total of six matrices were generated, including two soil process matrices (physicochemical parameters and enzymatic activities) and four microbial matrices (arable functional groups, most probable number, *16S*-454 genus, and *16S*-454 OTU). Variables in the soil process matrices (physicochemical parameters and enzymatic activities) were normalised, and the Euclidean distance between each pair of samples was calculated. Information on the abundance of microorganisms associated with functional groups related to the N cycle, using the most probable number method, was organised into separate matrices by *functional group* × *sample*; these entries corresponded to the abundance values of each group in the respective sample. In addition, microbial diversity information for each of the arable functional groups related to nitrogen, phosphorus, and carbon cycles was organised into *phylotype* × *sample* matrices separated by size; these entries were the abundance values for each microorganism in the respective sample. Similarly, data from the cultivation-independent methods (V5–V6 *16S* rRNA region and 454) were organised into *phylotype* × *sample* matrices. In each case, similarity in the composition and abundance of microorganisms was analysed using the Bray–Curtis similarity index [3, 51].

Multivariation in each matrix was partitioned according to a linear model of mixed effects and three factors. In the linear model, land "Use" corresponded to a fixed factor with three levels (i.e., *Páramo*, crop, and livestock) that generated first- and second-order interactions. "Farm-elevation" was also considered a fixed factor with two levels (i.e., Buenos Aires and La Secreta) for cultivation-independent matrices and three levels for the remaining matrices evaluated (i.e., Buenos Aires, El Edén, and La Secreta). "Season" was considered a fixed factor because of its two levels. Observation windows or quadrants were considered a random factor with three levels nested within the second-order interaction term Farm × Use × Season. The components of variation associated with each term in the linear model were estimated to rank the relative importance of each source of variation and to identify the type of matrix that better recorded the response to land use.

The significance of each source of variation was analysed using permutational multivariate analysis of variance (PERMANOVA) [52]. In all cases, the probabilities of null hypotheses were estimated using 9999 permutations of residuals under the reduced model. Finally, to assess potential correlations between the microbial matrices (arable functional groups, most probable number, *16S*-454 genus, and *16S*-454 OTU), Spearman's correlations between each pair of matrices were calculated.

2.4.2. Explanatory Variables and Microbial Phylotypes as Indicators of Land Use. To model the relationship among microbial phylotype assemblages (arable functional groups, most probable number, *16S*-454 genus, and *16S*-454 OTU) and soil process matrices (physicochemical parameters and enzymatic activities), distance-based linear models [53–55] were generated. Previously, to avoid overparameterization in the construction of models, a procedure with preselected relevant environmental variables was applied using a computationally intensive algorithm known as *stepwise search* [56]. In this algorithm, random combinations of subsets of variables of each matrix are selected, and a similarity matrix is built for each preselection and then correlated with the original similarity matrix. The procedure is performed thousands of times until the subset of variables that best fits the information in the original matrix is obtained. This simplification procedure was applied to microbial matrices to identify indicators and redundant phylotypes.

Once the redundant variables corresponding to soil processes were filtered, a single matrix that included several physicochemical variables and enzymatic activities was generated. In each case, the inclusion of variables in the models was based on *forward, backward, and stepwise* procedures. In all cases, the Akaike information criterion with second-order correction (AICc) was estimated, and the model with the lowest AICc value was selected [57].

To reflect the relative importance of the variables chosen in each model, canonical analysis based on principal coordinates (CAP) was performed for the most probable number abundance matrices and arable functional group diversity and cultivation-independent methods, with land

use as the discriminant criterion. For each ordination, vectors of the variables with the highest correlations (≥0.6) with PCP1 and PCO2 were projected onto the discriminant axes. All analyses were performed with PRIMER v6 and PERMANOVA add-on [57, 58].

3. Results and Discussion

Generally, the soils under investigation in this study belong to the Andisol order. At the Buenos Aires farm, they are classified as Typic Haplocryands, while at the La Secreta and El Edén farms, they are classified as Thaptic Hapludands [3, 27, 59]. These soils exhibit high phosphorus fixation capacities due to their volcanic ash-derived origins [27, 60, 61]. The combination of a humid and cold climate results in slow organic matter decomposition within these soils [27, 62–65]. Furthermore, Andean Andisols have a high carbon content and are susceptible to erosion due to agricultural activities and deforestation [27, 66]. The organic carbon stored in these soils plays a crucial role in mitigating atmospheric carbon increases associated with climate change [27, 66].

The detailed physicochemical properties of the study soils can be reviewed in Avellaneda-Torres et al. [27] in which the following ranges are reported in each of the cases: soil gravimetric moisture, 70–80%; apparent density, 0.7–0.9 g cm^{-3}; mean weighted diameter, 10.4–12.8 mm; pH, 5.2–5.4; organic carbon, 5.7–8.0%; nitrogen, 0.51–0.65%; and phosphorus, 6.9–34.5 cmol kg^{-1}.

The impact of potato crops and livestock was detected by two land process matrices (physicochemical parameters and enzymatic activities) and two cultivation-dependent strategy matrices (most probable number and arable functional groups), with statistically significant results. However, no significant differences were observed with the cultivation-independent strategy, in which microbial community diversity using the *16S rRNA* gene (region V5–V6) and pyrosequencing was evaluated at the genus level and at the OTU level. These results indicate that at the sequence level (DNA), the diversity of microorganisms in the evaluated soils was not altered by agricultural practices, which is inconsistent with the results obtained through the evaluation of the functional diversity of the community using other strategies. These differences may have been the result of early changes in agricultural practices and land use that can be detected at the functional and physicochemical levels but not at the DNA level via analysis of genetic material. In addition, this behaviour can be understood as an early indicator of the impacts of potato cultivation and livestock and agricultural practices used by peasant farmers. In the village of El Bosque, fallow periods exceeding seven years between each potato crop were established, and there was a low ratio of cattle to land area, which mitigates the impact of these practices on microbial biodiversity, as determined by the *16S rRNA* gene V5–V6 region in soil.

Investigating the influence of agricultural practices on microbial communities using independent cultivation techniques has yielded noteworthy findings. In a study by Ibañez et al. [67], a metataxonomic approach employing the *16S* rRNA V4 marker region was used to assess the impact of 19 years of minimum tillage (MT) and no-till (NT) practices, in conjunction with crop rotation, on the soil bacterial community in the Mediterranean Basin. The results revealed significant alterations in bacterial community structure attributable to both sampling time and tillage management, while no discernible effect was attributed to crop type. In particular, long-term NT application resulted in a noteworthy reduction in bacterial diversity, although no significant impact on alpha diversity was observed due to tillage practices. Furthermore, variations in phylum composition were not observed based on crop type; instead, primary distinctions were identified among legume crops, particularly those associated with different sampling times [67].

In a study conducted by Xiao et al. [68], the effects of fertilization, cultivation practices, and rainfall on nitrogen (N) metabolism-related microorganisms and key functional genes were evaluated across various depths in a vegetable field located in Yueyang City, China. Using the amplification of *16S* rRNA genes in distinct regions (*16S* rRNA V4–V5), the researchers assessed the microbial community dynamics. The findings underscored the heightened sensitivity of soil microbial community structure to changes in soil properties. In particular, fertilization was found to promote the proliferation of nitrogen-fixing bacteria. Additionally, soil carbon-to-nitrogen ratio (C/N) emerged as a critical determinant influencing the abundance of soil N metabolism-related microorganisms. The study recommends fertilization prior to rainfall and cultivation at a depth of 30 cm to optimize agricultural practices in this context [68].

Guo et al. [69] reported significant alterations in the composition and structure of bacterial and fungal communities in subtropical seasonal wetlands due to upland land-use intensification and two common agricultural disturbances. The V3–V4 region of bacterial *16S* rRNA and fungal ITS4 genes was amplified for analysis. Their findings revealed several key points: (1) upland land-use intensification emerged as the most consistent and influential factor driving changes in bacterial and fungal community composition, surpassing grazing and fire disturbances in impact; (2) at the operational taxonomic unit (OTU) level, interactions between land-use intensity, grazing, and fire influenced fungal diversity, while bacterial diversity remained unaffected by these factors; (3) regarding functional diversity, land-use intensification led to an increase in both bacterial and fungal functional richnesses, whereas grazing and fire collectively affected bacterial functional richness; and (4) notably, the effects of wetland management on microbial communities, at both taxonomic and functional levels, were found to be mediated through alterations in specific soil physiochemical properties [69].

However, although some studies have been developed on the impact of agricultural practices on enzymatic activities [27] and functional groups of microorganisms (by dependent cultivation techniques) [70], as of the date of publication of this article, no studies of this type have been reported using independent cultivation techniques, which is why this publication becomes more relevant.

Significant changes were detected by the cultivation-dependent strategies (most probable number and arable functional groups) at the soil process level (physicochemical parameters and enzymatic activities), which indicates the need to introduce sustainable agricultural techniques that mitigate the impact of practices associated with farming and livestock. As discussed previously [39], these agricultural practices should be introduced within the framework of conservation policies that apply the following: (1) community management plans in protected areas, (2) agroecological models, and (3) changes in the agrarian structure to integrate potential and effective land use.

The diversity strategy of arable functional groups applied to nitrogen fixers, phosphate solubilizers, and cellulolytics detected the greatest differences in land use (Use factor; $p < 0.05$), as determined by the percentage of variation components for each of the evaluation strategies used to determine the impacts of potato cultivation and livestock on soil microbial community characteristics (Figure 7). However, when comparing the two sampling seasons, considerable differences in the microbial structure of soils were observed according to the strategy of arable functional groups. Nevertheless, the potential effect of land use was consistent between both seasons and among the three farms evaluated (Season × Farm × Use; $p > 0.05$).

Additionally, significant changes related to sampling season were observed in four of the six matrices (physicochemical parameters, most probable number, arable functional groups, and *16S*-454 genus), and significant differences were detected for all farms (elevation), with the exception of the most probable number matrix. This may indicate that phenomena associated with climate and elevation may have an equal or greater influence on microbial communities with respect to land use, which would increase the relevance of studies on the impact of climate variability and climate change on microbial communities, especially if these impacts were detected by cultivation-independent strategies that assess sequence changes associated with the V5–V6 variable region of the *16S rRNA* gene (Figure 7).

The matrix that detected the greatest changes according to sampling use, season, farm, and the different interactions between these factors was the arable functional group matrix. These results indicate that the main changes in the microbial communities of *Páramo* soils caused by agricultural use, sampling season, and farm elevation are best observed when evaluating the richness and structure of the microorganism community isolated from selective media to determine arable microbial groups associated with a particular function (nitrogen fixation, phosphate solubilization, or cellulolysis), even though the most probable number and soil process matrices also provided valuable information. Different investigations also suggest that the cultivation-dependent strategy is a valid indicator for detecting impacts on soils, reporting that analyses of the main functional groups of soil microorganisms are appropriate for detecting changes caused by productive practices, xenobiotics, and land use [71–74].

Table 1 shows the number of selected variables that have greater representation in terms of behavioural data in each of the matrices. For physicochemical parameters, 13 of 20 variables were selected, and the same number of variables was used to evaluate enzymatic activities and abundance using the most probable number matrix. The largest selection of indicator phylotypes was observed in the matrices for the abundance and diversity of arable microorganisms belonging to functional groups and matrices for diversity determined by analysis of the *16S rRNA* gene (region V5–V6). Annex D shows the microorganisms that were selected as indicators for the different methodological strategies.

Figure 8(a) shows that the functional groups included in the total counts prior to morphotype identification (fungi, bacteria, nitrogen fixers, phosphate solubilizers, and cellulolytics) were those with the highest frequencies as indicator groups and frequencies higher than those of phylotypes at the individual level. This result is important because it indicates that global abundance data on functional groups provide more information for individual impacts caused by agricultural practices than do diversity analyses of molecular information for each strain. However, compared with fungi, bacteria were a better indicator of impacts related to potato cultivation and livestock (Figure 8(a)).

In the case of functional groups, the frequency of indicator phylotypes showed the following trend: cellulolytics > nitrogen fixers > phosphate solubilizers. This trend indicates the greatest impacts of changes associated with the carbon cycle (reflected by cellulolytic microorganisms) on the evaluated factors.

It has been indicated that cellulolytic organisms are the most responsive and can immediately detect changes after an intense disturbance, such as working the land [75]. This is a relevant finding because these microorganisms are directly related to the transformation of soil organic matter and may indicate changes in native vegetation and changes in root exudates [75–77]. In addition, organic matter is an emerging property and response indicator because it is altered by the decomposition rates of crop residues and changes in the physical protection of the soil and different processes of soil tilling [66, 78]. The indicator phylotypes at the order level include Eurotiales, Sphingobacteriales, Actinomycetales, Bacillales, Pseudomonadales, and Burkholderiales.

Figure 8(b) illustrates the following indicator phylotypes (at the order level) included in the diversity matrix derived from the cultivation-independent assessment of the *16S rRNA* gene (region V5–V6): Sphingobacteriales, Gemmatales, Rhizobiales, Sphingomonadales, Rhodocyclales, Myxococcales, and unidentified phylotypes. With the exception of unidentified phylotypes, none had a greater frequency than any other; the maximum frequency was 1, which indicated low redundancy in the indicator behaviour of these phylotypes.

Figure 8(c) illustrates the following indicator phylotypes (at the order level) included in the representative OTU matrix derived from diversity analysis of the *16S rRNA* gene: Rhizobiales, Acidobacteriales, Spartobacteriales, Rhodocyclales, Planctomycetales, Verrucomicrobiales, Burkholderiales, Xanthomonadales, Flavobacteriales, Acidimicrobiales, Sphingobacteriales, and unidentified phylotypes. With

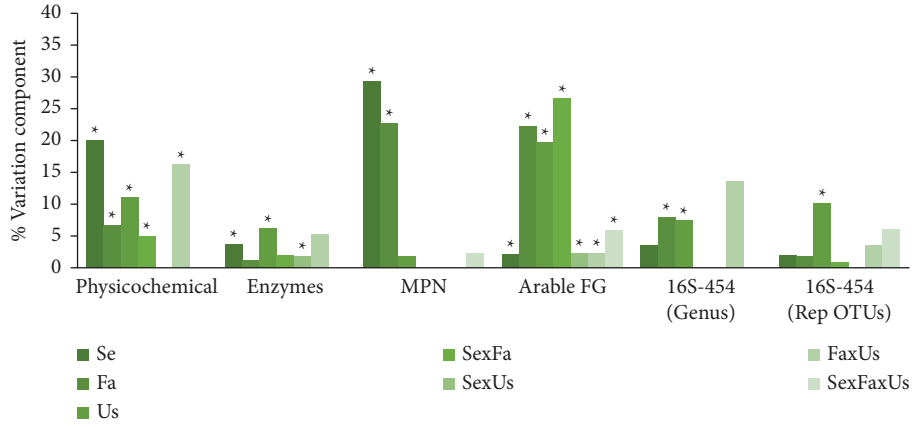

Figure 7: Percentage of relevant variation components used in PERMANOVA of each matrix. Us, use; Se, season; and Fa, farm. For graphical purposes, the interaction between the observation window (quadrant) and residuals was not included. The asterisks represent statistically significant differences.

Table 1: Variables selected from the BVSTEP analysis of several matrices.

Matrix	Total variables	Selected variables	Correlation
Physicochemical parameters	20	13	0.957
Enzymatic activities	7	7	NA
MPN	5	5	NA
Arable FG	197	24	0.951
16S-454 (genus)	557	9	0.954
16S-454 (representative OTUs)	81	23	0.952

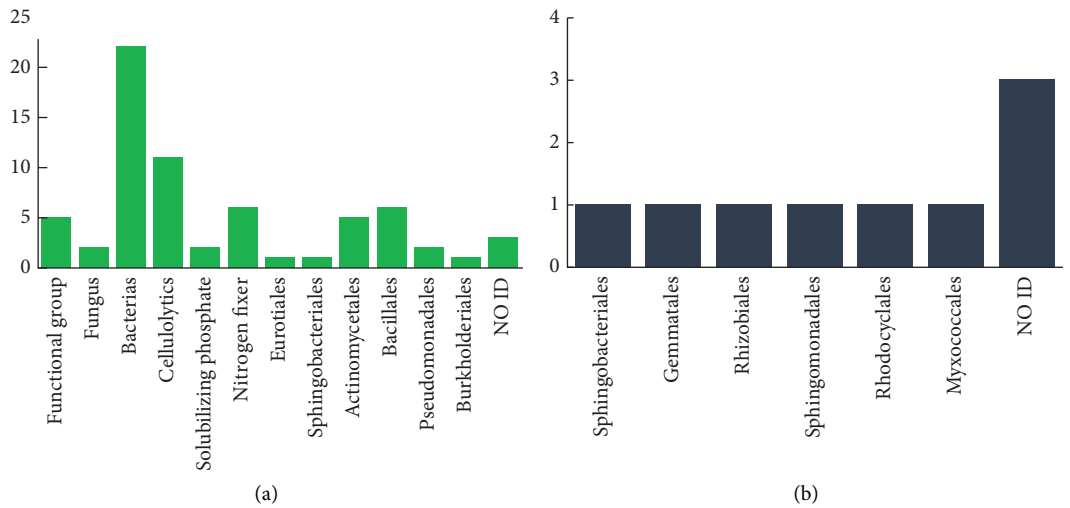

Figure 8: Continued.

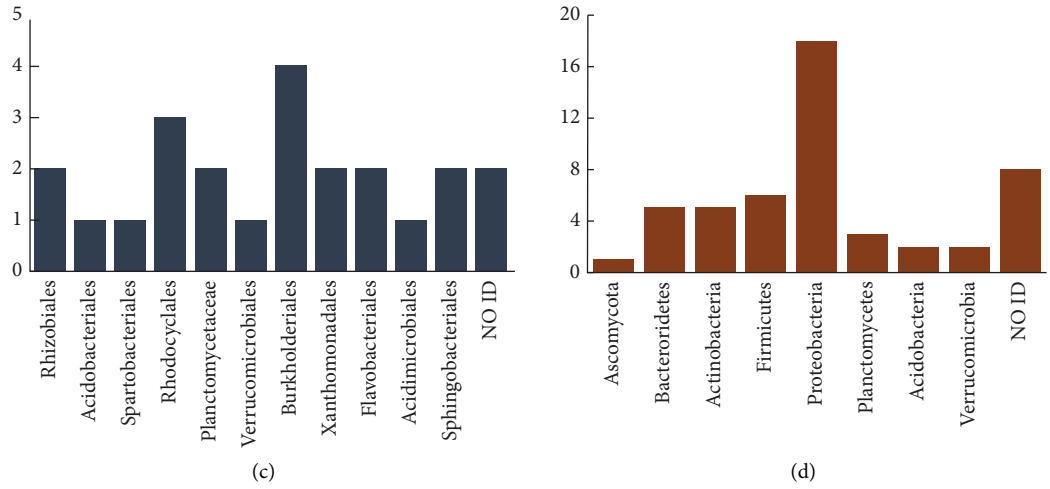

Figure 8: Frequency of indicator phylotypes in the different matrices. (a) Arable functional groups (order); (b) *16S*-454 (order); (c) *16S*-454 (representative OTU order); (d) total indicators at the phylum level.

a frequency of 3, Burkholderiales and Rhodocyclales stand out. The corresponding indicators in the three matrices are Sphingobacteriales, Rhodocyclales, and Rhizobiales.

Figure 8(d) shows the global distribution of indicator phylotypes in the different strategies at the phylum level. Proteobacteria predominate, followed by Bacteroidetes, Actinobacteria, and Firmicutes. These results are consistent with those reported for the restoration of forest soils degraded by mining, where the indicator phylotypes belonged to the phyla Proteobacteria (which were dominant), Bacteroidetes, and Actinobacteria [79]. Four dominant bacterial phyla (Proteobacteria, Acidobacteria, Actinobacteria, and Bacteroidetes) have been suggested as occurring in the soils of the majority of biomes on the planet [79–81].

Upon analysis of the results obtained by the most probable number technique with soil processes (physicochemical parameters and enzymatic activities) using CAP (Figure 9), the variables in *Páramo* lands that most correlate with functional group abundance using the most probable number method are organic carbon content, cation exchange capacity, Mg content, NH_4 activity, and urease activity. In addition, a positive correlation was observed between potato crops and livestock samples and between increased protease activity and higher apparent soil bulk density. Moreover, the observed inverse relationship between protease and urease activities was consistent with previously reported results for soils under potato crop and cattle farming, in which it was asserted that these enzymes do not act synergistically but rather are inhibited when the other is activated (and vice versa). Indeed, protease and urease have been reported to be inversely proportional in the consortia of bacterial morphotypes isolated from potato cultivation and livestock soils [82].

In the analysis of the diversity of nitrogen-fixing, phosphate-solubilizing, and cellulolytic functional groups that considered variables with soil processes represented by CAP (Figure 10), groupings occurred in the three agrosystems by land use (potato crop, cattle farming, and *Páramo*), indicating statistically significant differences

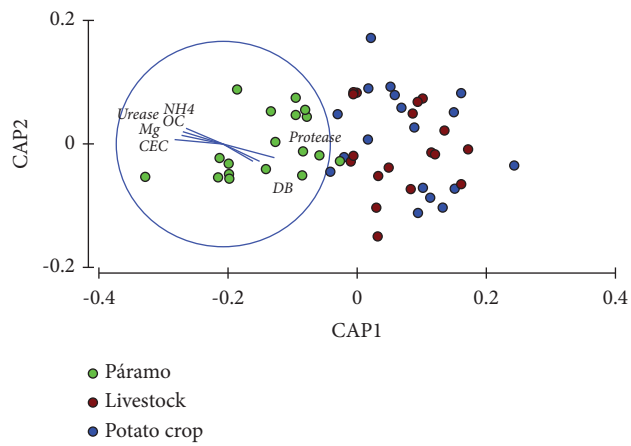

Figure 9: Most probable number principal component analysis strategy for Nevados NNP soils and parameters of soil processes. DB: bulk density; CEC: cation exchange capacity; OC: organic carbon. Cumulative variance CAP1 + CAP2: 79.7%.

according to PERMANOVA. These results suggest that agricultural soils are heterogeneous environments, that microbial diversity and activity are affected by different conditions [14], and that the structure of the soil microbial community and its activity are influenced by many factors, including climate, soil type, cover, and edaphic factors [79, 83–85]. This represents a fusion of the ecosystemic and cultural conditions of agroecosystems.

In this study, the microbial community present in soils under potato cultivation and livestock farming and analysed under the arable functional group strategy was influenced by practices such as the removal of native vegetation from the *Páramo*, the application of agrochemicals and mechanical soil tillage, and trampling from livestock. In addition, plant type has been reported as one of the main determinants of soil microbial communities because leaf litter and rhizodeposition are the primary suppliers of specific sources of carbon and energy [76, 86]. Moreover, plant species differ in their biochemical compositions [77, 87], and studies have

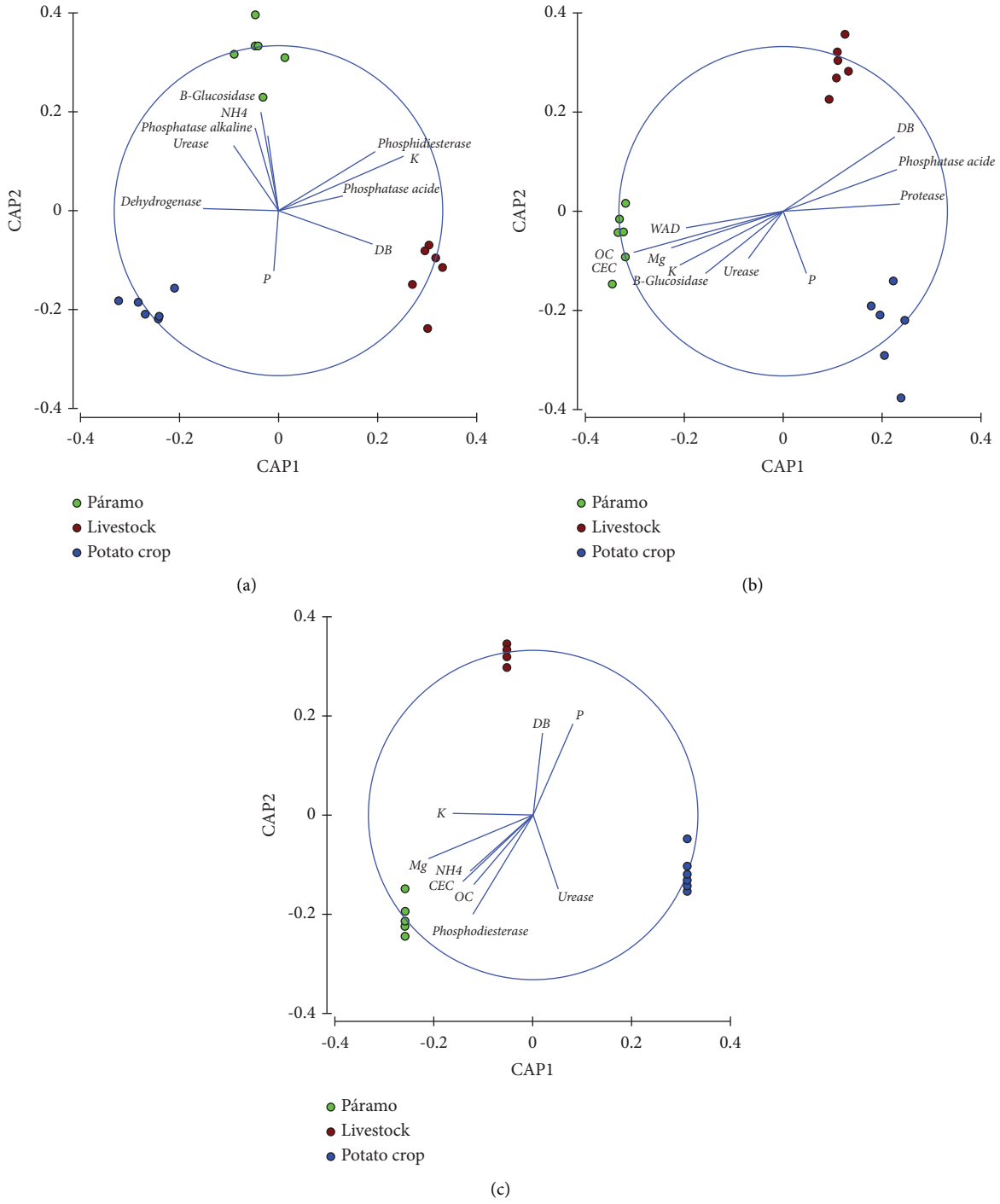

Figure 10: Principal component analysis of the arable functional group strategy of Nevados NNP soils and soil process parameters. DB: bulk density. A. Buenos Aires; B. El Edén; C. La Secreta. Cumulative variance (a) CAP1 + CAP2: 69.9%. (b) CAP1 + CAP2: 73.7%. (c) CAP1 + CAP2: 66.7%.

reported that soil microbial communities may change after repeated cultivation of the same crop over many seasons [75].

In the arable functional group strategy (Figure 10), the highest correlations of organic carbon content, cation exchange capacity, and magnesium content and β-glucosidase and urease activities were observed in the *Páramo*, which is consistent with the results of the most probable number technique. Thus, the carbon cycle may be greatly impacted by potato cultivation and livestock. The higher organic carbon content and cation exchange capacity and β-glucosidase activity in *Páramo* soil are consistent with a greater number of cellulolytic indicator phylotypes in the arable functional group matrix. This may also indicate that in the *Páramo*, the transformation processes of β-glucoside into glucose by the action of β-glucosidase and urea-type

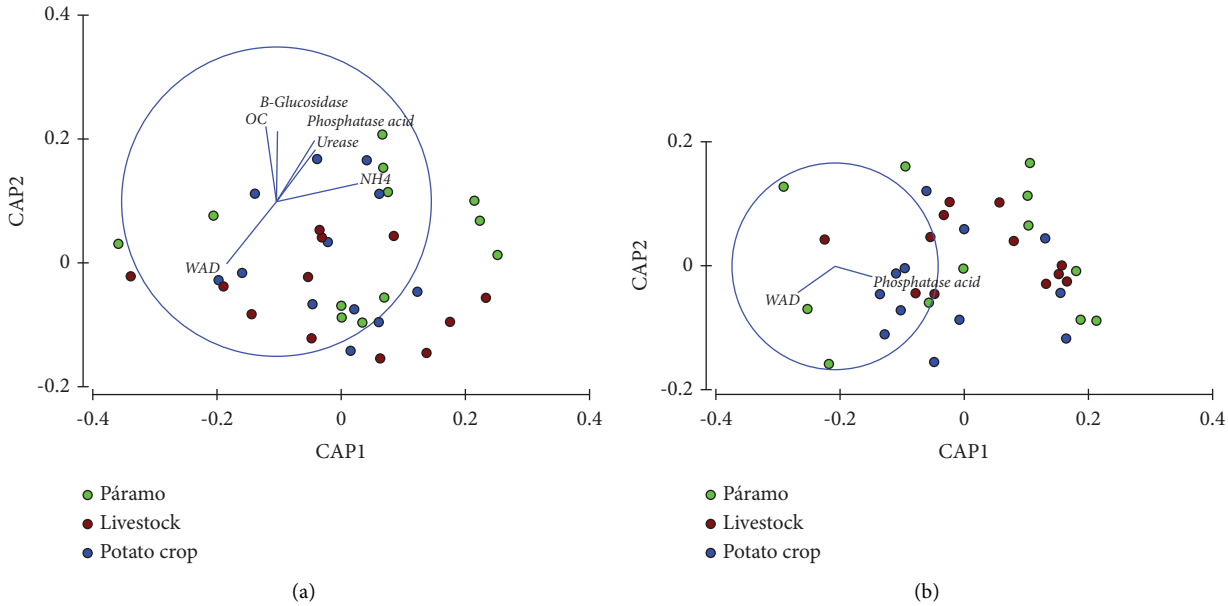

Figure 11: Principal component analysis of the *16S*-454 strategy and soil process parameters. A. Global matrix (genus); B. representative OTU matrix. DB: bulk density; CEC: cation exchange capacity; OC: organic carbon. A. Global matrix (genus); B. Representative OTU matrix. Cumulative variance (a) CAP1 + CAP2: 62.1%. (b) CAP1 + CAP2: 66.1%.

compounds into NH_4 via urease activity occur naturally throughout *Páramo* soils and do not occur in potato cultivation and livestock soils. Other authors have also reported that organic matter content is related to variations in the structure of the soil bacterial community [79], which indicates the importance of peasant farmers in developing processes to convert their agroecosystems towards ecologically sustainable farming practices that minimise the impacts of fertiliser and pesticide application and the removal of soil organic matter.

Bulk density and acid phosphatase were significantly correlated with soil microbial communities found under livestock pasture. The greater bulk density of livestock soils reflects the compaction of the soil as a result of livestock trampling, which alters the porosity of the soil and the quantity of oxygen and water that can be transported and affects microbial communities. These results are consistent with those reported by other authors, who detected a relationship between bacterial community structure and parameters such as soil aggregation and porosity [79, 88]. Phosphorus was positively correlated and potassium was negatively correlated with the functional groups of microorganisms associated with potato cultivation. The higher contents of phosphorus under potato cultivation reflect fertilization with N : P : K compounds and the possible release of retained phosphorus when organic matter was disturbed during cultivation. Some studies have reported that phosphorus from fertilisers increases microbial biomass and diversity [89], whereas others found that it had a significant effect on the composition of soil microbial communities [90–92]. However, despite the application of synthetic fertilisers with potassium, this element was decreased in soils under potato cultivation. The potassium in these soils may be lost as a result of the demand for nutrients by the crop itself, whereby nutrients leave the soil and do not return after the harvesting process, as well as by leaching. This phenomenon may also be a reflection of the lower organic carbon content and cation exchange capacity, leading to lower retention of potassium in soils under potato cultivation, and it should be studied further. Such previous trends are shown in Figure 9 (CAP for most probable number), which corresponds to the information in Figure 10.

As indicated in the methodology and given that the present research was developed in high mountain ecosystems, the slope of the three farms, as well as the different land uses, was similar in all cases, being between 50 and 75%. Although it has not been the objective of the present study, it is important to highlight that some authors have reported that soil bacterial and fungal alpha diversity was similar across different slope gradients or positions, but, likewise, that it has also been a factor that alters the composition of the microbial community [93].

In the CAPs shown in Figure 11, clear groupings did not occur by diversity (gene *16S rRNA* region V5–V6) because of potato cultivation, livestock, and *Páramo* in the diversity matrix at the genus level or representative OTU level. These results are consistent with the information shown in Figure 7, in which the components of variation do not present statistically significant differences as a result of land use. The physicochemical and enzymatic variables presenting the greatest correlation with the indicator phylotypes of the cultivation-independent strategy are organic carbon content, cation exchange capacity, β-glucosidase activity, urease activity, acid phosphatase activity, NH_4 activity, and weighted average diameter (DAW), consistent with the results of previous CAPs.

Table 2 shows low correlations between data from different abundance and diversity matrices. The strongest

Table 2: Correlations between the abundance and diversity matrices.

Matrix	MPN	Arable FG	*16S*-454 (genus)
MPN	—	—	—
Arable	0.270	—	—
16S-454 (genus)	−0.020	0.099	—
16S-454 (OTUs)	−0.070	0.109	0.356

correlations occurred at the arable strategy level between the most probable number and arable functional groups (0.270) and for independent cultivation between the genus level and representative OTU level (0.356), presenting low correlations with both the arable and nonarable strategies. However, global analysis of the correlations indicated that the diversity results obtained for each of the strategies were independent.

In general, four of the six methodological strategies detected changes caused by potato cultivation and livestock farming; however, at the microbial level, only the cultivation-dependent strategies detected these differences. Therefore, the results obtained with the molecular markers included in this study were able to detect changes in functional diversity (arable strategy) without detecting changes in genetic material at the *16S rRNA* gene level. Accordingly, changes in functional diversity may serve as an early indicator of changes caused by land use, which may be a useful tool in the development of proposals designed to implement more sustainable agricultural plans that consider both ecological and cultural factors within an area. Such plans can designate protected areas in the midst of agricultural practices that produce extensive natural degradation and design and implement mixed agroecosystems, where natural and anthropogenic dynamics approach ecological equilibrium [94].

Several interesting proposals have been developed to meet this end.

(i) Conservation tillage (reduced and zero tillage practices), which can increase organic carbon in the topsoil, improve aggregation, and preserve soil resources relative to conventional tillage [95–100]

(ii) Crop rotation, which can increase organic carbon in the soil, especially in rotation with legume species [96, 97]; in addition, soil fertilization rates should be reviewed because they can significantly affect soil quality [96, 97]

(iii) Ecological farming processes, such as natural pest control and compost and/or manure use, which could replace the use of synthetic fertilisers [101–103]

(iv) Silvopastoral systems, which have been shown to be a viable alternative for improving the quality of soil and metabolic functions, as reflected in the significant increase in microbial biomass and enzymatic activities [104]

Agricultural ecosystems are one of the main sources of income for communities and are associated with commercial activities at local and regional levels. Such systems are interwoven with socioeconomic variables, such as employment opportunities for the inhabitants of a given region. All the abovementioned factors and conflicts between humans and nature contribute to the reduction or imbalance of water-regulating functions in the Andean *Páramo* ecosystems of Colombia [94]. Therefore, community management plans have been proposed in which the communities inhabiting the *Páramo* in conjunction with environmental authorities will determine practices to diagnose, monitor, and conserve protected areas, apply these practices within a framework of ecological farming, and restore biocultural memory. Finally, the current agrarian structure must be changed to allow for fair land distribution and avoid communities from being displaced towards protected areas, which are considered uncultivated or unoccupied areas (Avellaneda-Torres [39]).

4. Conclusions

The strategy in which the diversity of nitrogen-fixing, phosphate-solubilizing, and cellulolytic functional groups was analysed revealed the greatest impacts caused by all evaluated factors (land use, season, and farm), especially those caused by potato cultivation and livestock.

Four of the six evaluated matrices (physicochemical parameters, enzymatic activities, most probable number, and arable functional groups) detected significant differences according to land use, which may indicate that potato cultivation and livestock in the village of El Bosque initially impact functional diversity before affecting *16S rRNA* gene V5-V6 region abundance.

This reduced effect on microbial diversity identified using the V5-V6 region of the *16S rRNA* gene may have been caused by the fallow periods between each potato crop, which can be greater than 7 years, as well as the low proportion of livestock with respect to the total land area of the village. However, these results can be understood as an early indicator of the impacts of potato cultivation and livestock on microbial diversity, and they can be employed in the design and implementation of community management plans, the application of agroecological models, and the implementation of changes in agricultural structure. In addition, other factors may also be used to mitigate future changes.

The best indicators were global counts of functional groups of microorganisms, among which cellulolytic organisms had the greatest redundancy at the group level. These aspects, which are related to indicators with the greatest influence on microbial communities (organic carbon content, cation exchange capacity, and β-glucosidase activity), indicate that the carbon cycle leads to the largest

transformations as a result of potato cultivation and livestock; this finding may suggest primary management recommendations. In addition, changes associated with the nitrogen cycle were also observed and reflected in aspects such as the activity of NH_4, urease, and protease.

Changes caused by sampling season and elevation provide relevant information on the behaviour of the microbial community, and these factors are of equal or greater magnitude than that of land use. As a result, further studies evaluating the impact of elevation and sampling season on microbial communities are suggested and are relevant in light of climate variability and climate change.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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