



Nutrient contents and microbial communities as mediators of the effects of land-use in ecosystem functioning in alpine ecosystems from Central Mexico

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Abstract

Purpose The interplay between microbial taxonomy, functional diversity, and land-use changes across diverse ecosystems must be better understood. This study explores, through a statistical modeling approach, the intricate relationships between microbial communities, soil properties (nutrient contents and physicochemical parameters), and land-use changes, using stable isotopes ratio ($\delta^{13}\text{C}/\delta^{15}\text{N}$) as ecosystem function indicator.

Methods Using Structural Equation Modeling (SEM) this study aims to investigate how soil microbial communities, soil properties and ecosystem function are interconnected in three ecosystems undergoing land-use change in Nevado de Toluca, Mexico.

Results Multivariate analyses of microbial communities and soil parameters for the different land-uses reveal significant variation in both microbial composition and soil properties (i.e. nutrient contents) mainly explained by the land-use history of the studied ecosystems. Through the SEM approach, it was possible to disentangle the relative contribution of land-use change, microbial community composition and nutrient contents to the ecosystem function defined as the $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio.

Conclusions Microbial communities are the main drivers of soil ecosystem functioning. However, through SEM approach it is possible to formally test direct and indirect relationships impacting microbial communities and the resulting functional consequences, contributing to a mechanistic understanding of the ecological implications of land-use change, aiding in sustainable land-use decision-making.

Keywords Soil microbial ecology · Statistical modeling · Stable isotopes · Structural Equation Modeling

1 Introduction

Microbial communities, as both the architects and operators of ecosystems, play a fundamental role in the mineralization of plant residues, thereby significantly influencing

the cycling and storage of carbon and nitrogen (Wang et al. 2020). In recent years, the interplay between microbial taxonomy and functional diversity has been recognized as a pivotal factor steering soil nutrient cycling across various ecosystems, such as crop fields, grasslands, and forests (He et al. 2023; Singavarapu et al. 2023). Nevertheless, the nuanced responses of microbial community structure and function to the complex mosaics of land-use changes across varying temporal scales remain elusive (George et al. 2019). In this context, the utilization of stable isotopes in soil ecology emerges as a powerful instrument for exploring the historical activities of microbial communities. Stable isotopes, such as ^{13}C and ^{15}N , serve as robust indicators of previous environmental changing conditions in soil over time, through intricate biogeochemical processes (Craine et al. 2015; Zhang et al. 2018; Choi et al. 2023; Park et al. 2023). Changes in land-use from natural ecosystems to agriculture or forestry, can notably impact isotopic composition (Gerschlauer et al.

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2019; Ngaba et al. 2019). For instance, the replacement of native C3 plants with C4 crops and shifts in organic matter inputs – in terms of quantity, quality, and composition – can exert direct effects on the microbial communities engaged in organic matter decomposition, altering $\delta^{13}\text{C}$ values (Diels et al. 2004; Wynn and Bird 2008). In addition, altered nutrient inputs from fertilization practices and changes in primary productivity can shift $\delta^{15}\text{N}$ values (Craine et al. 2015; Park et al. 2023). Thus, in principle, $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio as isotopic tapestry of any ecosystem can be taken as an indicator of its historical functioning, offering invaluable insights into past alterations in vegetation, shifts in carbon and nitrogen sources, as well as, the microbial activity dynamics (Choi et al. 2023; Tang et al. 2023).

Microbial communities are pivotal not only in nutrient cycling and organic matter decomposition but also in the restoration of ecological interactions (Singh et al. 2019; Hu et al. 2022). However, understanding how these communities and ecosystem functioning recover after land-use change is complex and influenced by multiple factors such as severity and duration of the land-use alteration, ecosystem type, and microbial communities adaptability (Tosi et al. 2016; McKinley 2019; Pérez-Hernández and Gavilán 2021). These relationships involve ecological interactions and feedback mechanisms. In this context, Structural Equation Modeling (SEM) is a powerful tool to understand the complex processes and mechanisms such as trophic interactions (Shao et al. 2015; Jiang et al. 2020), community dynamics (Luan et al. 2020; Ma et al. 2022), and ecosystem stability (Chen et al. 2021; Xu et al. 2021; Zhang et al. 2021a, b) based in causal-effect relationships (Heck and Thomas 2020). SEM offers a valuable approach for testing multivariate hypotheses that integrate multiple variables and relationships in a quantitative and visual manner (Shao et al. 2015; Hair et al. 2021). Also, SEM allows evaluating different models and incorporate empirical data from diverse sources into a unified framework that considers direct and indirect cause-effect relationships, including measurement error, typically overlooked in traditional linear models, (Saborío-Montero et al. 2020; Lai and Hsiao 2022;). Moreover, SEM enables the modeling of composite variables as latent variables, which are not individually quantified but are inferred from multiple observed or measured variables, allowing for the identification of the most plausible and parsimonious explanation for the observed patterns (Fan et al. 2016).

In this study, employing a SEM approach, we identified the relative significance of the microbial community composition, environmental variables, and metabolic diversity focused on the $\delta^{13}\text{C}/\delta^{15}\text{N}$ isotopic signature as indicator of ecosystem functioning along an altitudinal gradient of land use conversion in Nevado de Toluca, Mexico. The bidirectional relationship between microbial composition and nutrient content has been extensively documented

(Jing et al. 2020; Jiao et al. 2021; Kang et al. 2021; Philippot et al. 2023). Moreover, the relationship between soil microbial composition and environmental variables (including management practices) in different functional outcomes has been investigated in previous research (Sun et al. 2020; Liu et al. 2022; Xue et al. 2022). In addition, previous studies have shown that soil microbial composition and nutrient dynamics are also influenced by some environmental variables such as pH, soil organic matter (SOM) quality and quantity, nutrient content, etc. (Lauber et al. 2008; Trivedi et al. 2016; Fierer 2017; Philippot et al. 2023). Finally, it has been documented that land-use change negatively influences biodiversity and nutrient content, (Felipe-Lucia et al. 2020; Hasan et al. 2020). Based on these findings, we hypothesized the reciprocal (bidirectional) interaction between soil properties and microbial communities, and microbial communities acting as mediators of the effects of land-use change in soil functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$). Testing these hypothetical relationships is crucial for understanding the consequences of land-use changes in the underlying mechanisms of functional ecosystem processes and provide valuable insights for a sustainable land-management decision making.

2 Materials and methods

2.1 Experimental design and study sites

The study was conducted at Nevado de Toluca, Central Mexico (Fig. 1), which is the fourth highest formation in Mexico reaching 4645 m above sea level (masl). The climate in the area is characterized by semicol-subhumid C(E) wig and cold E(T)Hwig conditions (García 1990). The main ecosystems observed are temperate forests, which include species such as *Abies*, *Pinus* and *Quercus*, situated between 3000 to 4100 masl. Additionally, there are high mountain grasslands consisting of *Festuca* and *Calamagrostis*, found at altitudes ranging from 4100 to 4500 masl (Calderón de Rzedowski and Rzedowski 2005).

To statistically evaluate the relationship between land-use change, soil properties (nutrient contents and physicochemical parameters), microbial diversity, and their influence on stable isotopes measures as ecosystem functioning indicators, soil samples were collected from six sites with different land-use at different altitudinal floors (3200, 3400 and 4000 masl). The corresponding pairs of sites with contrasting land-use were: grassland-burned grassland, pine-reforested pine and fir-agriculture. As contextual background for the sites, we include the C and N stable isotopes of the dominant vegetation (González Contreras 2019) in each ecosystem studied (Table 1).

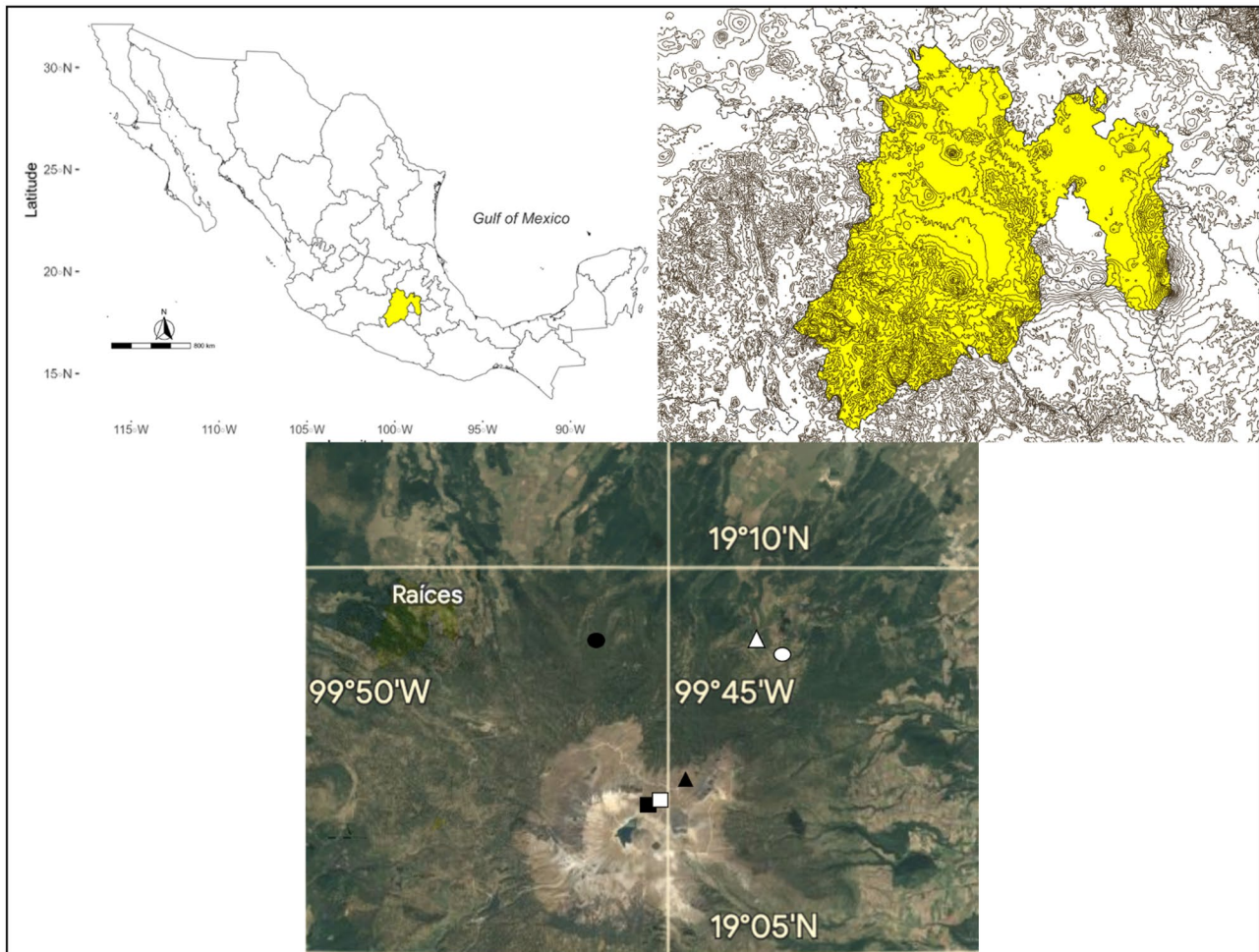


Fig. 1 Location of sampling sites at Nevado de Toluca, Mexico. Six types of land-use: Fir (solid circles), Agriculture (open circles), Pine (solid triangles), Reforested pine (open triangles), Grassland (solid squares) and Burned grassland (open squares) in Santiago Tlacotepec, Mexico

Table 1 Characteristics of land-use change pairs sites (original vs transformed)

Site	Status	Time of conversion (years)	Dominant vegetation (sp)	Altitude (masl)	$\delta^{13}\text{C}$ ‰(PDB) ^a	$\delta^{15}\text{N}$ ‰(ATM) ^a
Fir	Original	0	<i>Abies religiosa</i>	3275	-31.46 (0.91)	-3.484 (0.588)
Agriculture	Transformed	50	<i>Solanum tuberosum</i>	3268	NA*	NA*
Pine	Original	0	<i>Pinus hartwegii</i>	3959	-29.79 (0.72)	-4.65 (0.90)
Reforested pine	Transformed	30	<i>Pinus montezumae</i>	3284	-29.75 (0.91)	-3.23 (1.03)
Grassland	Original	0	<i>Festuca tolucensis</i>	4101	-26.35 (0.81)	-6.392 (1.17)
Burned grassland	Transformed	5	<i>Festuca tolucensis</i>	4059	-27.24 (0.69)	-5.105 (1.24)

*NA Not available at the time of sampling

^aGonzález Contreras (2019)

2.2 Sampling

At each site, a 30×30 m plot was established for sampling. Specifically, three equidistant rows (10 m apart) were drawn, and three soil cores (7.5 cm in diameter and 10 cm deep) were taken from three equidistant points (10 m apart) in each row. For the physicochemical and microbial diversity analyses, samples taken at each point were combined into one composite sample per row for a total of three composite samples per site (N = 18). Samples for microbial diversity analyses were added to 50 mL centrifuge tubes containing RNAlater™ (SIGMA) (1:1), RNAlater™ was removed before sample storage at -80 °C until DNA extraction. Samples for the physicochemical analyses were stored in high density black plastic bags at room temperature (RT) until processed. Samples for C and N isotopic measurements were taken individually (N = 36) and stored at RT.

2.3 Physicochemical analyses

Soil samples were sieved to 2 mm and air-dried for physicochemical characterization. Soil organic carbon (SOC) was analyzed using a ground subsample of 5 g of soil that passed through a 100-mesh screen by dry digestion at 900 °C in an automated C- analyzer (Shimadzu 5050A, Canada), concentration of total soil N (TN) was determined by acid digestion in H₂SO₄ with the Kjeldahl method (Bremner 1960), phosphorus content (TP) was determined using the Olsen Method (Sims 2000). Ca, Mg and Fe concentrations were determined by atomic absorption, K and Na by atomic emission. Particle size distribution (clay, silt, and sand) was analyzed using the modified hydrometer method (Gee and Bauder 1986). The pH was determined in water (1:2 w/v). Analysis of variance (ANOVA) and Tukey's HSD mean comparisons test at P < 0.05 were used to show differences of the soil physicochemical parameters among different land-use ecosystems.

2.4 DNA extraction and 16S rRNA sequencing

Genomic DNA from each sample was extracted using DNAeasy® PowerSoil® Kit (Qiagen, Valencia CA, USA) according to manufacturer instructions with the following modifications: 0.5 g of sample instead of 0.25 g was used and incubation periods at 4 °C were increased from 5 to 20 min. Genomic DNA samples were submitted to MacroGen laboratory for 16S rRNA gene sequencing of V3-V4 region (341F/805R primer set) (Herlemann et al. 2011) using an Illumina MiSeq instrument.

2.5 16S rRNA sequence data processing

Illumina raw sequences (7,720,761) were processed, and quality filtered using Quantitative Insights into Microbial

Ecology (QIIME 2). Chimera and amplicon sequence variants (ASVs) identification were performed using the DADA2 algorithm. Because of quality, only forward sequences were trimmed by 30 base pairs (bp) and truncated at 220 bp during the ASV clustering in DADA2. The filtered sequences (5,489,149) were assigned using SILVA database (v138.1). For the compositional nature of the metagenomic data, a center log ratio (CLR) normalization was performed with the Microbiome Analyst web-based tool (<https://www.microbiomeanalyst.ca>). The raw data were deposited in the NCBI sequence read archive (SRA) under the accession number PRJNA1037141.

2.6 Characterization C and N stable isotopes

Soil samples for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analyses were collected in the southwest corner of each plot: 0–15 and 15–30 cm-deep soil cores were collected with a 3.2 cm-diameter auger (Dorme Soil Samplers; Australia) and placed in sealed plastic bags and transported to the laboratory where they were kept refrigerated at 4 °C until they were processed. The processed samples were sent to the Environmental Isotope Laboratory, University of Arizona, USA for dC13 and dN15 detection with a continuous flow mass spectrometer (Finnigan Delta PlusXL; USA) coupled to an elemental analyzer (Costech, Analytical Technologies Inc; USA) using acetanilide as standard for C and N. The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were expressed in parts per thousand (‰) according to the international standard ($\delta^{13}\text{C}$, Vienna-PeeDee Belemnite; $\delta^{15}\text{N}$, atmospheric N₂) in the conventional δ -notation, where the X refers to ¹³C or ¹⁵N and R_{sample} and R_{standard} are the ¹³C/¹²C or ¹⁵N/¹⁴N ratios of sample and standard, respectively:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1,000\text{‰}$$

2.7 Microbial diversity analyses

Alpha diversity Chao1 diversity index was calculated from the ASV tables derived from the bioinformatic sequence analysis using the phyloseq R package (McMurdie and Holmes 2013). Post-hoc T-student tests were applied to detect differences between treatments using the R package (Constantin and Patil 2021).

2.8 Beta diversity

Aitchinson distance matrices were calculated and Non-Metric Multidimensional Scaling (NMDS) at order level were performed using the vegdist function of the vegan package in R (Oksanen et al. 2022). Permutational Analysis of Variance

analysis (PERMANOVA) and LDA Effect size (LEfSe) analysis were performed to test differences between land-use categories using the Microbiome Analyst web-based tool (Dhariwal et al. 2017).

2.9 Modeling approach

A structural equation modeling approach (SEM) was used to test the hypothesis that soil properties and microbial communities in reciprocal interaction act as mediators of the effects of land-use over the soil functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio). Based on the literature, we expected: (i) direct unidirectional relationships between land-use and nutrient content (negative), nutrient content and $\delta^{13}\text{C}/\delta^{15}\text{N}$ (positive and negative), microbial community and $\delta^{13}\text{C}/\delta^{15}\text{N}$ (positive and negative) (Qun and Geng-Xing 2010; Adugna and Abegaz 2016; Djodjic et al. 2021; Farooq et al. 2021; Jeong et al. 2023), (ii) direct, bidirectional and positive relationship between microbial community and nutrient content (Allison et al. 2007; Cui et al. 2018; Xu et al. 2022; Philippot et al. 2023), and (iii) indirect relationship, mediated by microbial community, between nutrient content and $\delta^{13}\text{C}/\delta^{15}\text{N}$.

The input variables for the model were selected and optimized in two steps. First, since the number of explanatory variables corresponding to microbial diversity was very large (> 50 Bacteria and Archaea orders), we performed an efficient branch-and-bound algorithm implemented on the `regsubsets` function in the `leaps` R package (Lumley 2020) to determine the ten best models that better explain $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio variances according with the Akaike Information Criterion (AIC). Second, to explain the variance of the outcome $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio as ecosystem function indicator, a SEM was constructed using the R package the `lavaan` (Latent Variable Analysis, v 0.6–17, (Rosseel 2012), where the following interactions were tested: 1) land-use as a latent variable (time of conversion and managed/not managed categorical variable), 2) nutrient content as a composite variable (N, NO_3 , SOM) and, 3) microbial community, as latent variable (only the three orders that exhibit significant differences with the response variable).

Statistical significance estimates were calculated by Maximum Likelihood and Non-Linear Minimization Subject to Box Constraints (NLMINB) was used as optimization method. Finally, the goodness of fit for the overall model was judged by the χ^2 -test ($p < 0.05$) and RMSEA (<0.05) (Root-Mean-Square Error Approximation) statistic for statistical significance (Gao et al. 2020) previous.

3 Results

3.1 Soil physicochemical analyses

Soil physicochemical properties within the pairs of land use changes (original versus transformed) indicate variations

from fir forest to agricultural land and, from pine forest to reforested pine (Table 2). Notably, the conversion from fir forest to agriculture presents the most substantial alterations, with 11 out of the 15 measured variables experiencing modifications. This conversion is marked by a shift to increased soil acidity, higher electrical conductivity, and elevated levels of organic matter, as well as, nitrogen, and calcium. Simultaneously, iron content decreased, while phosphorus, potassium, and, notably, nitrate increased. Conversely, the transition from pine forest to reforested pine showed an increase in organic matter, nitrogen, potassium, calcium, magnesium, and iron content.

In the case of the land use change from grassland to burned grassland, no statistically significant differences were observed ($P < 0.05$). Finally, ammonium, total phosphorus, and total carbon content remained unaltered in all sampled sites.

3.2 Microbial diversity

Microbial diversity measured as alpha diversity using the Chao1 index along different land use sites (original versus transformed) showed significant variations. Specifically, marked variations were evident in the transitions from fir forest to agricultural land and, from pine forest to reforested pine (Fig. 2). The conversion from fir forest to agriculture resulted in a reduction in diversity, while the transition from pine forest to reforested pine was associated with a diversity increase. Conversely, there were no differences in diversity between grassland and burned grassland sites.

Furthermore, these disparities were further underscored at beta diversity by the multidimensional ordination analysis, we observe differences associated with land-use in which geographic proximity also played a pivotal role, leading to greater similarity among nearby sites (Fig. 3a, b). In this context, factors such as soil texture (including sand, clay, and silt content) and altitude also emerged as influential determinants (Fig. 3a).

Also, these diversity shifts were consistent with the heatmap analysis conducted on the statistically associated groups identified through LefSe (Fig. 3b). The sites showing the most similarity were those within the grassland and burned grassland categories, followed by pine forest to reforested pine sites, while the fir forestland and agricultural sites displayed the most dissimilarity.

3.3 Isotope analyses and SEM

Stable isotope and $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio analyses across sampled sites revealed distinctive isotopic signatures per site (Fig. 4). Grassland and burned grassland sites exhibited the highest similarity, whereas pine trees (both natural and reforested)

Table 2 Soil properties of land-use change pairs (original vs transformed)

Site	pH	EC	SOM	N	P	K	Ca	Mg	Na	CIC	Fe	NO ₃	NH ₄	Pt	Ct
Agriculture	4.47** ^a (0.306)	0.467*** ^a (0.115)	10.8 * ^a (0.493)	0.367** ^a (0.058)	43.4 *** ^a (4.75)	0.667** ^a (0.153)	5.2*** ^a (0.7)	0.267*** ^a (0.058)	0	35.7* ^a (7.64)	24.7*** ^a (3.51)	341*** ^a (53.3)	12.7 (2.31)	0.2 (0)	9.87 (0.115)
Fir	5.7** ^a (0.346)	0.1*** ^a (0)	16.6** ^a (2.37)	0.633*** ^a (0.058)	23.5*** ^a (9.92)	0.267** ^a (0.115)	13.6*** ^a (5.53)	1.47*** ^a (0.493)	0	51* ^a (1.73)	59.7*** ^a (5.51)	58*** ^a (15)	23.3 (21.4)	0.133 (0.058)	15 (3.15)
Reforested pine	5.63 (0.058)	0.067 (0.058)	18.6*** ^b (1.46)	0.667*** ^b (0.058)	16.2 (1.96)	0.567*** ^b (0.153)	9.53*** ^b (0.764)	1.93*** ^b (0.208)	0	51.7*** ^b (4.62)	68.3*** ^b (0.577)	31 (16.5)	18 (6.56)	0.1 (0)	14.1 (0.577)
Pine	5 (0.173)	0.067 (0.058)	9.17*** ^b (4.14)	0.267*** ^b (0.153)	12.2 (3.68)	0.1*** ^b (0)	0.6*** ^b (0.265)	0.1*** ^b (0)	0.067 (0.115)	16.3*** ^b (5.77)	41*** ^b (13.2)	23.3 (10.8)	16.7 (2.52)	0.067 (0.058)	9.97 (3.79)
Burned grassland	5.07 (0.208)	0.067 (0.058)	4.53 (0.404)	0.2 (0)	6.2 (1.04)	0.033 (0.058)	0.433 (0.153)	0.033 (0.058)	0	14.3 (3.51)	9.67 (0.577)	23.7 (7.77)	12 (9.64)	0.033 (0.058)	6.4 (0.693)
Grassland	4.87 (0.404)	0 (0)	3.87 (0.896)	0.133 (0.058)	6.93 (1.19)	0.067 (0.058)	0.233 (0.153)	0.033 (0.058)	0.033 (0.058)	10.7 (1.53)	8.67 (1.53)	14.3 (5.13)	9.33 (2.31)	0 (0)	5.63 (0.493)

Values are means with standard error between parentheses. Only pairwise comparisons between pairs of land-use change where shown as different letters (ANOVA test) and asterisks indicate the statistical significance found; *p*-value <0.05 *, *p*-value <0.01 **, and *p*-value <0.001 ***

^aComparison of fir vs agriculture

^bComparison of pine vs reforested pine

diverged in opposite directions, and the fir's signature was closely resembling native pines. Conversely, the agricultural site showed a marked variation from the other sample sites, displaying the lowest intraspecific variation.

A Structural Equation Modeling approach (SEM) was used to test the hypothesis that soil properties and microbial communities act as mediators of the effects of land-use over the soil functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio). The model showed that land-use exerted a negative relationship over the functional response of $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio through nutrient content (path coefficient = -0.041, $p < 0.05$). Specifically, nutrient content had a direct and positive effect on the isotope signature ratio response, where the variables that explain variations in $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio were SOM (62.02, $p < 0.01$), N (-43.93, $p < 0.05$) and NO₃ (-25.04, $p < 0.05$). Moreover, microbial community was directly correlated with nutrient content and had a strong direct relationship to the functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio), through the three associated microbial orders *Chitinophagales*, (-19.59, $p < 0.05$), *Shingobacteriales* (25.63, $p < 0.01$) and *Tepidsphaerales* (29.13, $p < 0.05$). Finally, the multiple squared correlations highly explained (92.82%) the variation in the $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio as soil functional ecosystem indicator.

4 Discussion

4.1 Physicochemical soil properties changes are associated with land use change

Land-use changes can influence soil nutrient dynamics and microbial community composition, thereby impacting crucial soil functions and physicochemical processes (Kooch et al. 2016). Our results of soil properties (i.e. nutrient content) showed changes associated with land-use, for instance, the transition from fir to agriculture exhibited the most pronounced alterations, in particular changes in SOM, N, P, K, Ca, Mg, Fe, NO₃ (Table 2). This phenomenon is consistent with existing literature, highlighting that deforestation of entire ecosystems leads to nutrient depletion (Veldkamp et al. 2020; Bodo et al. 2021; Kumar et al. 2022). Specifically, the conversion of forests to agriculture has been shown to diminish organic matter (SOM), nitrogen, and calcium content—a trend reflected in our findings (Merloti et al. 2019; Peng et al. 2021). We also observe changes in agricultural field management, such as soil acidification (de Graaff et al. 2019) and elevation of nitrate and phosphorus levels, attributed to fertilizers commonly employed in potato cultivars (*Solanum tuberosum* L), including ammonium nitrate (AN), diammonium phosphate (DAP), and triple superphosphate (TSP) (Rosen et al. 2014).

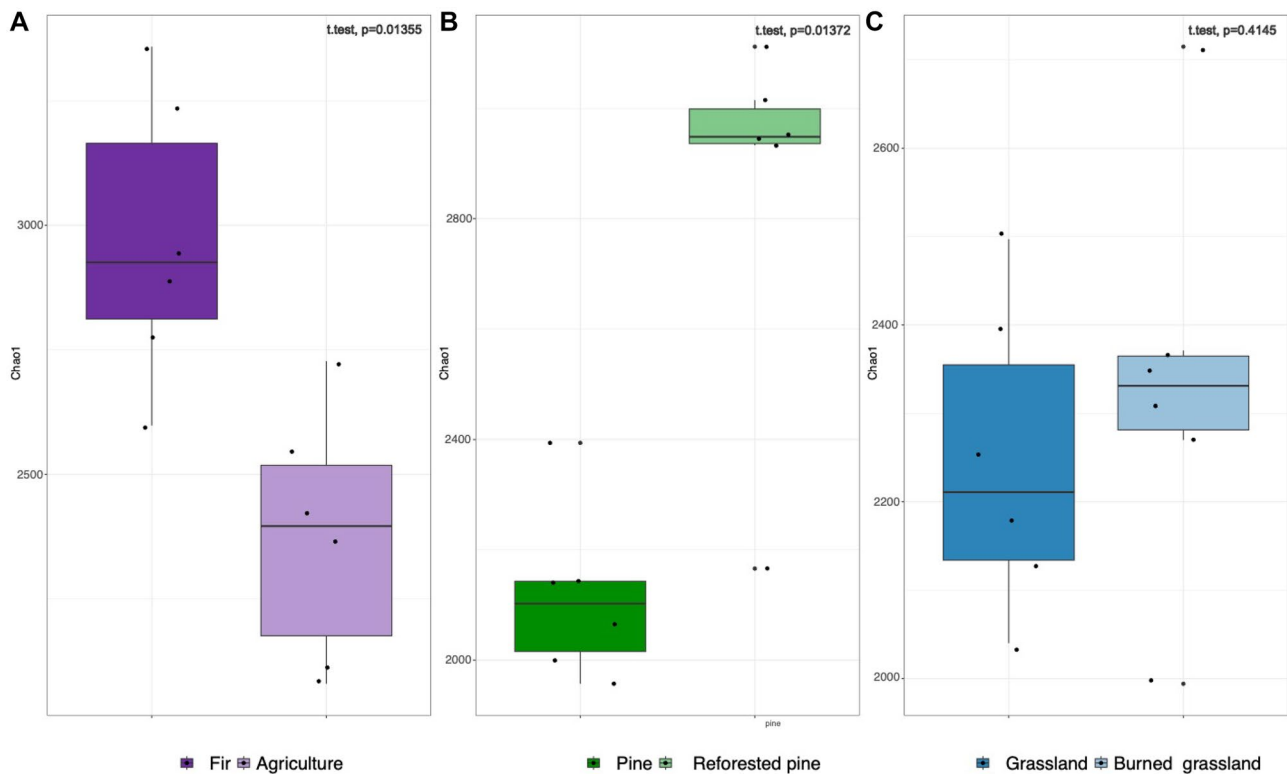


Fig. 2 Bacterial community diversity of land-use change pairs (original vs transformed). **A** Comparison of Fir vs. Agriculture **B** Comparison of Pine vs. Reforested Pine **C** Comparison of Grassland vs. Burned Grassland. Box plots of alpha diversity calculated as Chao1 index. Box represents the interquartile range (IQR) between the first and third quartiles (25th and 75th), middle line inside the box repre-

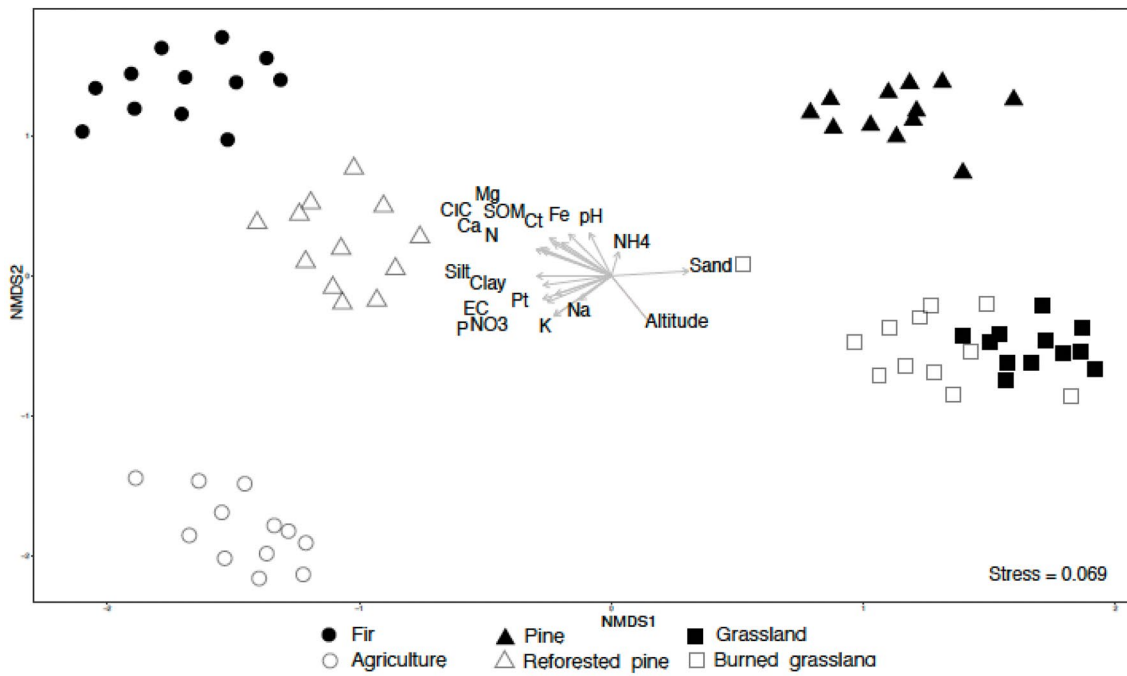
sents the median and whiskers represent the lowest and highest values within 1.5 times the IQR. t-tests for significant differences between pairs were conducted and the P values are indicated on the top right corner of each comparison and significance is indicated as asterisk ($P < 0.05$)

Moreover, native forest conversion typically results in a net carbon loss in the soil (Yang et al. 2011). In contrast, conversion from pine to reforested pine showed an increase in soil organic matter and nutrient contents (N, K, Ca, Mg, and Fe). This increase could be attributed to the selection of frost-resistant *Pinus montezumae* for reforestation, exhibiting rapid growth, higher survival rates, and greater biomass compared to *Pinus hartwegii*, the native vegetation species in Nevado de Toluca (Viveros-Viveros et al. 2007; Rojas-García et al. 2019). Moreover, the transition from grassland (*Festuca toluensis*) to burned grassland (due to firebreaks and controlled burning practices) did not result in significant soil properties alterations. This lack of statistical changes may be attributed to the lesser disturbance introduced to the environment where the burning is controlled and the soil structure is not affected (Neary et al. 1999), as opposed to the complete vegetation depletion observed in the fir-to-agriculture transition that affects soil structure, porosity, organic carbon, and nutrient availability or the plant species change observed in the native pine-to-reforested-pine scenario as reported in previous studies (Mueller et al. 2014; Goss-Souza et al. 2020; Hüblová and Frouz 2021).

4.2 Changes in microbial communities are associated with land-use change

Microbial diversity, or alpha diversity, is associated with land-use change and the specific management practices. Our study revealed that, land use change not only modified various soil properties but also resulted in changes in microbial diversity (alpha diversity) (Fig. 2). These changes in microbial diversity can be an increase or decrease in diversity, we observe both scenarios that also have been reported previously. In particular, reduction in microbial diversity was observed in the fir to agriculture land-use transition, potentially due to a reduction in soil organic carbon availability (Yu et al. 2012). Specifically in our case, the agricultural practices in the fir to agricultural transition are associated with potato agriculture that involves a complete depletion of any vegetation and natural input of organic carbon, being limited to intentional inputs that may not be sufficient to maintain microbial diversity. In contrast, an increase in microbial diversity was observed in the pine to reforested pine transition which could be attributed to the increased nutrient availability through intended nutrient

a



b

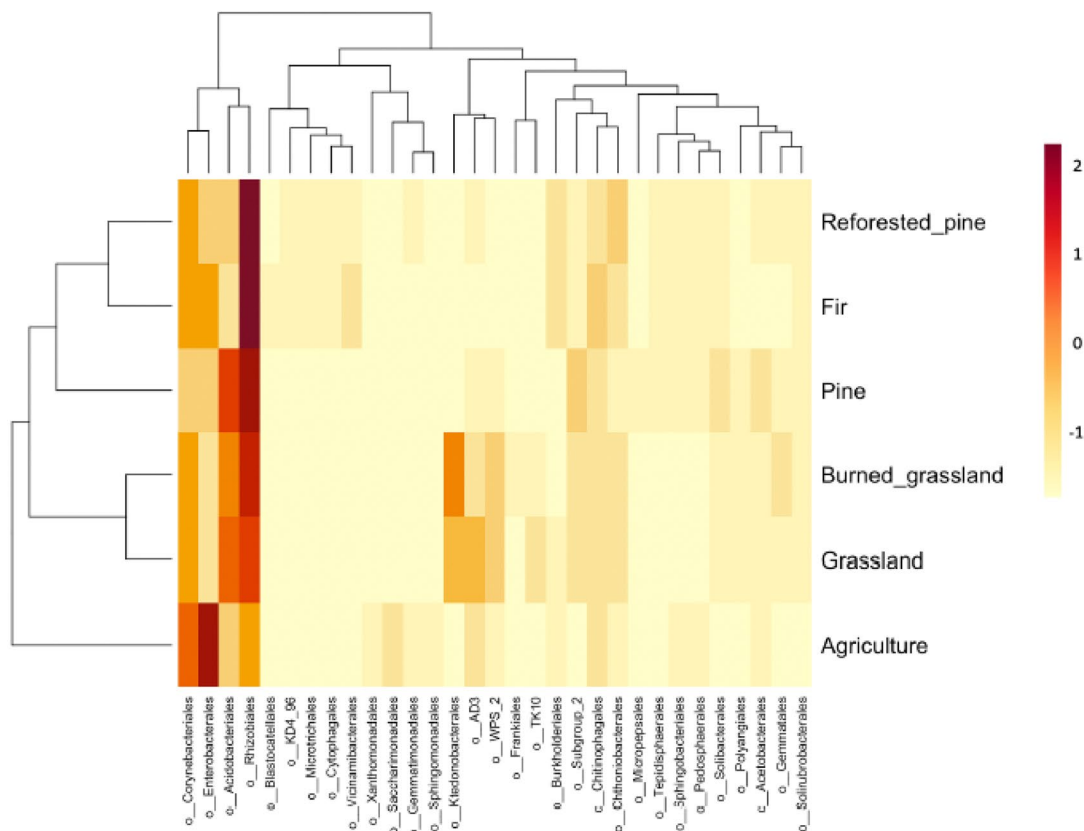


Fig. 3 Bacterial beta-diversity across land-use types. **a** Non-metric multidimensional scaling (NMDS) plot for bacterial community composition dissimilarity across samples. Dissimilarity was estimated as Aitchinson distance with soil physicochemical parameters vectors generated using envfit. Shapes correspond to pairwise land-use change comparisons, and colors correspond to original and transformed environments (solid color, original; open-color, transformed environment). **b** Heatmap of the most significant bacterial orders per land-use type obtained throughout LEfSe analysis. Abundance corresponds to the z-score values of each microbial group

inputs (Verchot 2010; Philippot et al. 2023). In particular, we attribute the increase in alpha diversity in the transition from pine to reforested pine to the enhanced nutrient supply from the leaf litter of *Pinus montezumae* compared to *Pinus hartwegii* (Lopez-Escobar et al. 2017).

Microbial community composition differences (beta diversity) are associated with specific management practices and the time elapsed since first intervention. We observe microbial composition differences associated with land-use and related factors such as soil properties (Fig. 3a, b). In this regard, it has been well documented that environmental perturbation (either natural or anthropogenic) can change microbial communities composition and if maintained, this change can be permanent (Hartmann et al. 2014; Yang et al. 2020). It is worth mentioning that the magnitude of differences in microbial communities can be associated with the time elapsed since the land-use change occurred. In particular, microbial communities that presented the greater differences are those in the transition from fir-dominated ecosystem-to-agricultural land, where land-use modification occurred ~ 50 years ago, while, intermediate compositional differences are observed in sites with a 30 year transition from pine to pine reforested. Finally the lowest compositional difference is observed in the grassland-to-burned grassland transition.

Land use transitions and associated management practices can be related to specific microbial groups. Bacterial orders whose abundances were statistically correlated with different land-uses and soil properties (Fig. 3b). For example, the highest abundance of *Rhizobiales* in fir and reforested pine where high nitrogen content is also observed. These orders have been reported as abundant in temperate forests and are key taxa contributing to the overall carbon decomposition process (Tláškal et al. 2017). Another example is *Acidobacteriales*, found in the pine forest site (*P. hartwegii*) and grasslands sites (natural and burned), this bacterial order has been reported to be abundant in coniferous forests and grasslands (Naether et al. 2012; Li et al. 2019). Previous studies have found that *Acidobacteriales* order is ubiquitous and abundant, mainly in soils with low carbon availability (constituting around 20% on average in soil microbiome surveys) (Janssen 2006), suggesting, adaptation to low substrate availabilities (oligotrophs) (Naether et al. 2012), and being

consistent with the grasslands, and pine forest sites that exhibit the lowest total carbon contents (Table 2). Finally, *Enterobacteriales* and *Corynebacteriales* were abundant in the agriculturally managed site, these orders have been historically associated with fecal pollution associated with agricultural inputs (i.e. manure) and anthropogenic influence (Tkhakakhova et al. 2016; Devane et al. 2023).

4.3 Understanding the ecosystem functional response through isotopic signatures: soil properties and microbial community as mediators of land-use change

There is currently insufficient emphasis on utilizing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ records as indicators of biochemical C and N cycling processes in microbial ecology studies (Park et al. 2023), despite their widespread application in plants and animals as indicators of trophic interactions (Wilkinson et al. 2022). While it is feasible to identify trophic pathways of C and N inputs into an ecosystem through stable isotope measures (De Clercq et al. 2015; Bieluczyk et al. 2023), the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratio can offer an aggregate overview of all interactions and transformations involving C and N in an environment (Layman et al. 2007; Liu et al. 2018; Soldatova et al. 2024).

In this study, $\delta^{13}\text{C}/\delta^{15}\text{N}$ was used as a reference of the C and N interactions and transformations in the environment (Layman et al. 2007; Liu et al. 2018; Soldatova et al. 2024), in order to show that each ecosystem and land-use possesses a unique isotopic signature (Fig. 4), reflecting different interactions among the components of the systems such as nutrient contents and microbial communities (Fig. 5). These relationships were formally tested using the Structural Equation Modeling (SEM) framework. Through this approach, we identified the effect of land-use over the soil functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio), acting in cascade with the nutrient content and microbial community (Fig. 5). Furthermore, we found a negative relationship between land-use change and nutrient content, in particular Soil Organic Matter (SOM), total nitrogen and nitrates. This relationship has been previously documented on forest soils, where the concentration of these nutrients decreases when land-use changes (Merloti et al. 2019; Peng et al. 2021). Additionally, specific bacterial orders emerge as potential bioindicators, with their abundance being a factor linked to the functional ecosystem parameter $\delta^{13}\text{C}/\delta^{15}\text{N}$ (Fig. 5). For instance, the abundance of *Sphingobacteriales* is positively related with the $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio, previous studies associated the abundance of *Sphingobacteriales* with high soil organic carbon (SOC) mineralization (Whitman et al. 2016; Zhang et al. 2021a, b; Carrasco-Espinosa et al. 2022). *Chitinophagales*, recognized as chitinolytic and hydrocarbon degraders, also show a strong influence with $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios (Kaoping

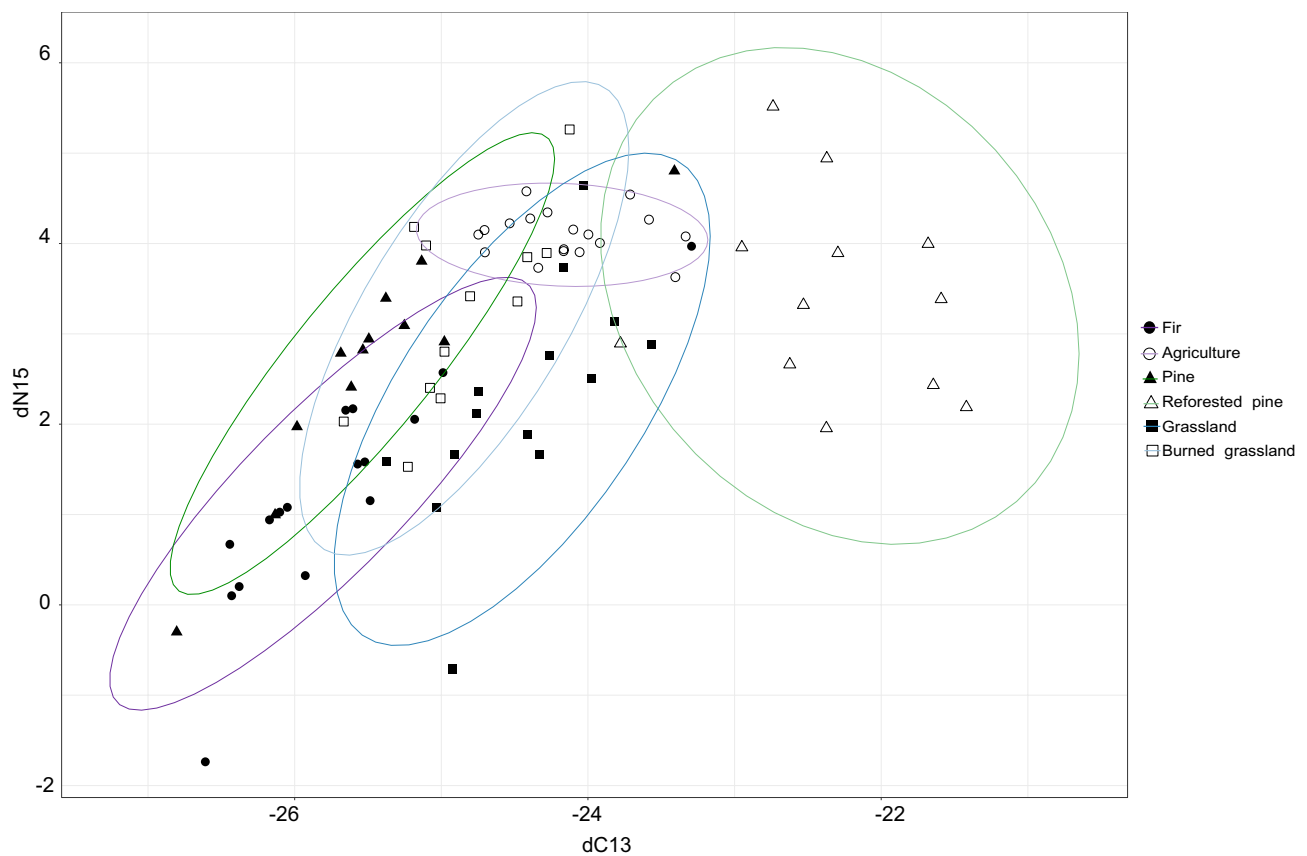


Fig. 4 Biplot of N and C stable isotope ratios for all land-use change samples (original and transformed). For each land-use type standard ellipses are drawn with confidence intervals at 95%: Fir (solid cir-

cles), Agriculture (open circles), Pine (solid triangles), Reforested pine (open triangles), Grassland (solid squares) and Burned grassland (open squares)

et al. 2023) and exhibit reduced abundance in afforestation and land-use change processes (Lan et al. 2022). *Tepidiphraerales*, displaying good tolerance to low temperatures (4–15 °C) (Ivanova et al. 2016), experience changes in abundance following restoration-fertilization processes (Cheng et al. 2021) and soil warming experiments (Parada-Pozo et al. 2022). Altogether the evidence here presented suggests potential roles for specific bacterial groups as biological indicators and soil nutrient drivers of C and N transformations, warranting further investigation through specific experiments such as mesocosms or complementary metagenomic analyses.

Finally, through SEM we tested the hypothesis of the role that nutrient content and microbial communities in reciprocal interaction play as mediators of the impact of land-use on ecosystem functioning. The resulting model shows that, in fact, land-use change (as environmental perturbation), directly impacts nutrient contents, and in turn nutrient contents interact reciprocally with microbial community composition that impact ecosystem function (Fig. 5). Although, in some sense, all these interactions have been previously documented

(Chadwick et al. 2015; Adetunji et al. 2020; Thakur et al. 2020; Philippot et al. 2023), it has not been common to formally test not only the interactions but the strength and direction of such interactions. In particular, it is worth noticing, that although the effect of specific management practices or land-use transitions in ecosystem function is obscured by summarizing all sampled sites in a single model, we do demonstrate that interventions do change ecosystem function in idiosyncratic ways (Fig. 4), and that microbial communities act as strong mediators of such effects, also identifying specific microbial groups involved (Fig. 5), which represents a generalizable model for different ecosystems and/or environmental perturbations (Jorgensen 2021; Vispoel et al. 2023). Also, although the interaction between nutrient contents and microbial community is highly significant, its magnitude is not very high, may be due to the specific measurements included in this latent variable that can overlook functionally relevant nutrients in this interaction, including specific carbon substrates that could be better correlated with the abundance of specific microbial groups (Abraham et al. 1998; Boschker and Middelburg 2002; Trivedi et al. 2016).

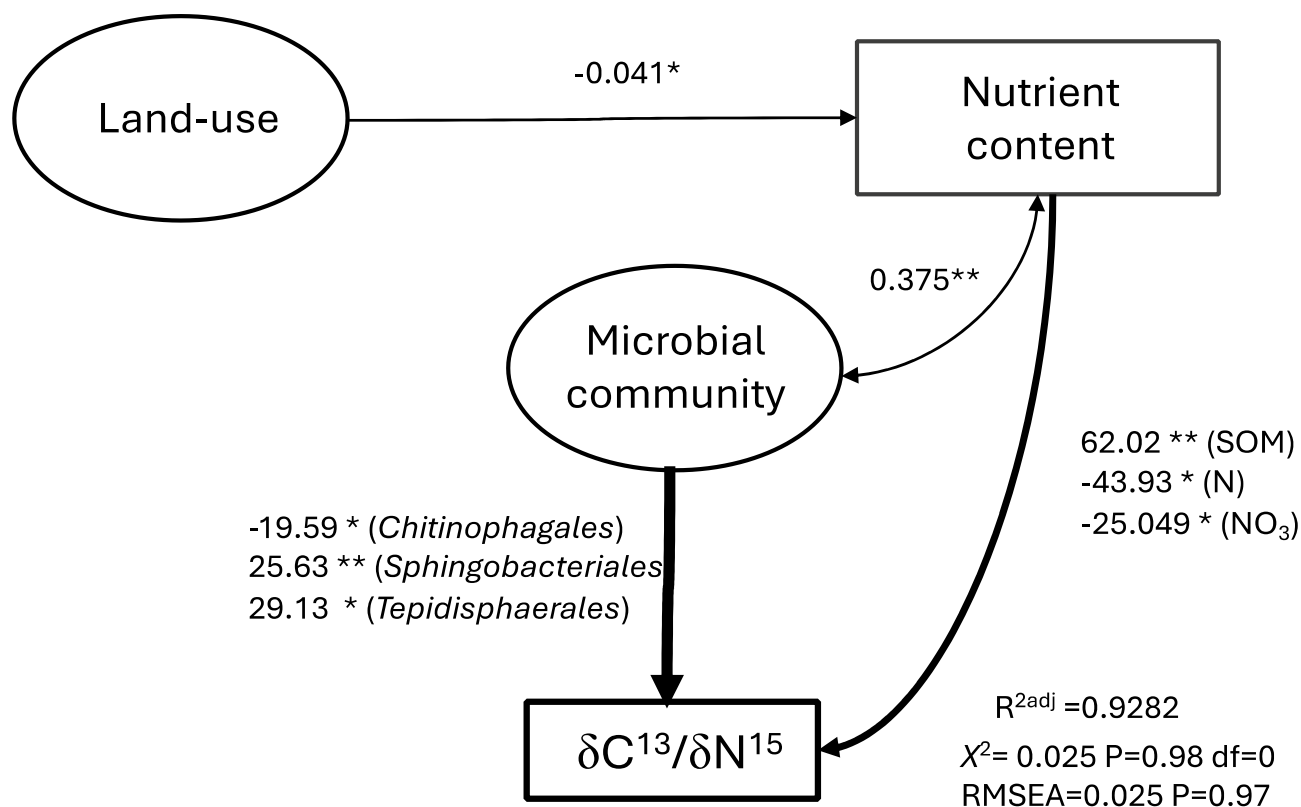


Fig. 5 Structural equation model describing soil properties and microbial communities in reciprocal interaction as mediators of the effects of land-use over the soil functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio). The model diagram depicts the different variables interactions (nutrient content, microbial communities and land-use) and their effect in the functional response ($\delta^{13}\text{C}/\delta^{15}\text{N}$). Latent variables (land-use and microbial communities) are represented as ellipses and compos-

ite variables (nutrient content) are represented as rectangular boxes; arrows depict the relationships (effects) between variables, their width is proportional to their coefficient path and its significance p-value (* P < 0.05, ** P < 0.01 and *** P < 0.001), which values are also included in the diagram. The model fit parameters correspond to the Root-Mean-Square Error Approximation statistic (RMSEA), Chi-square (χ^2) value and the significance probability of p-value

5 Conclusions

Our findings underscore the intricate relationship between microbial community structure, soil properties, and environmental transformations, particularly land-use change. Specifically, we demonstrate the cause-effect relationships among land-use, nutrient contents and microbial communities, where soil properties and microbial communities interact reciprocally, acting as mediators of the effects of land-use change in soil functional responses (i.e. $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio). This understanding highlights the potential use of bioindicators (microbial communities) by decision-makers regarding forest management in Mexico to facilitate the development of sustainable management strategies.

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Data availability Raw data and metadata were deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA1037141.

Declarations

Research involving human participants and/or animals No studies with human participants or animals were performed by any of the authors to obtain data for this paper.

Conflicts of interest The authors hereby declare that they have no conflicts of interest.

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