Evaluation of Nitrogen Dynamics in Páramo Soils under Green Onion (*Allium fistulosum*) Cultivation

Evaluación de la Dinámica del Nitrógeno en Suelos de Páramo con Cultivos de Cebolla de Rama (*Allium fistulosum*)

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Thesis submitted in fulfillment of the requirements for the degree of Doctor in Chemical Engineering

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This work is dedicated to the loving memory of Vidan Cicic Rey.

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Abstract

Title: Evaluation of Nitrogen Dynamics in Páramo Soils under Green Onion (*Allium fistulosum*) Cultivation*
Author: Daniela Cristina Rey Romero**
Keywords: soil nitrogen, Andes, agriculture, non-point source pollution, small-scale farmers.
Description:
The Santurbán-Berlín páramo is a strategic high-mountain ecosystem, vital for water regulation, biodiversity conservation, carbon sequestration, and the livelihoods of rural communities in northeastern Colombia. However, the intensification of green onion (*Allium fistulosum*) cultivation threatens its socio-ecological integrity by promoting nitrogen (N) losses and associated environmental impacts. Despite growing evidence of N losses from agricultural activities in páramo regions, there is a critical lack of integrated, field-based knowledge on how N is stored, transformed, and mobilized in páramo soils under intensive green onion cultivation, particularly regarding vertical soil N distribution, microbial N-cycling dynamics, and their links to specific farming practices. This doctoral thesis aimed to fill this knowledge gap by evaluating N dynamics in páramo soils under green onion cultivation to support the transition toward more sustainable agricultural practices. The research was structured into four phases: (i) identification of agricultural practices and soil properties influencing soil N concentrations; (ii) assessment of the vertical distribution of soil N; (iii) analysis of soil microbial biodiversity linked to the N cycle; and (iv) proposal of strategies to reduce N losses. The findings revealed that long-term green onion cultivation reduced total nitrogen (TN) by up to 40% and increased nitrate nitrogen ($N–NO_3^-$) concentrations up to eightfold, while ammonium nitrogen ($N–NH_4^+$) remained unchanged in surface soils (0–20 cm) compared with native grasslands. Cultivation also altered the vertical distribution of mineral N, particularly $N–NO_3^-$, which reached concentrations of 114 mg kg^{-1} in subsurface soils (20–60 cm) versus only 1.76 mg kg^{-1} in non-cultivated soils. Microbial communities shifted markedly, with cultivated soils favoring copiotrophic organisms associated with the priming effect and soil organic matter decomposition, while native grasslands were dominated by taxa with higher N fixation potential. Microbial functional changes were also evident, as nitrification potential increased up to ~75-fold in cultivated soils. Key agricultural drivers included the continuous use of fresh chicken manure, associated with the neutralization of soil acidity, inappropriate application of agrochemicals without fertilization planning, excessive irrigation, and limited stakeholder coordination to promote sustainable practices. The current management increased risks of N losses through leaching, volatilization, and greenhouse gas emissions. The pedotransfer functions developed in this research provide additional tools to estimate TN and $N–NO_3^-$ concentrations from more readily measurable soil properties, offering practical support for implementation. To address these issues, context-specific strategies were proposed, including improvements in fertilization and irrigation, diversification and rotation of cropping systems, management of soil biodiversity, and development of public policies aimed at reducing N losses. Overall, this thesis advances understanding of N dynamics in understudied Andean páramos and contributes evidence-based strategies to guide sustainable soil and nutrient management in high-mountain agroecosystems critical for Colombia's water and food security.

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*Doctoral Thesis

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Resumen

Título: Evaluación de la Dinámica del Nitrógeno en suelos de Páramo con Cultivos de Cebolla de Rama (*Allium fistulosum*)*
Autora: Daniela Cristina Rey Romero**
Palabras clave: nitrógeno del suelo, Andes, agricultura, contaminación difusa, pequeños agricultores.
Descripción:

El páramo de Santurbán-Berlín es un ecosistema de alta montaña vital para la regulación hídrica, la conservación de la biodiversidad, el secuestro de carbono y los medios de vida de comunidades campesinas. Sin embargo, la intensificación del cultivo de cebolla de rama (*Allium fistulosum*) amenaza su integridad socio ecológica, promoviendo pérdidas de nitrógeno (N) e impactos ambientales adversos. A pesar de la creciente evidencia sobre pérdidas de N derivadas de las actividades agrícolas en páramos, existe una brecha de conocimiento integrado, derivado de observaciones y mediciones de campo, sobre cómo se almacena, transforma y moviliza el N en los suelos de páramo bajo el cultivo intensivo de cebolla de rama, en particular en lo que respecta a la distribución vertical del N en el suelo, la dinámica del ciclo microbiano del N y su relación con prácticas agrícolas específicas. En ese contexto, esta tesis doctoral tuvo como objetivo evaluar la dinámica del N en suelos de páramo cultivados con cebolla de rama como aporte a la transición hacia prácticas agrícolas sostenibles. La investigación se estructuró en cuatro fases: i) identificación de prácticas agrícolas y propiedades edáficas que influyen en la concentración de N en el suelo; ii) estimación de la distribución de concentraciones de N del suelo; ii) análisis de la biodiversidad microbiana del suelo que interviene en el ciclo del N; y iii) propuesta de estrategias para reducir pérdidas de N. Los resultados demostraron que el cultivo de cebolla de rama a largo plazo llevó a una reducción de hasta 40% del N total (NT) y al incremento de hasta ocho veces la concentración de N en nitrato ($N\text{-}NO_3^-$), mientras que no generó cambios en los contenidos de N en amonio ($N\text{-}NH_4^+$) en el suelo superficial (0–20 cm) en parcelas cultivadas respecto a los pastizales nativos. Además, el cultivo alteró la distribución vertical del N mineral, particularmente del $N\text{-}NO_3^-$, que alcanzó concentraciones de hasta 114 mg kg^{-1} en el suelo subsuperficial (20–60 cm) en contraste con solo 1.76 mg kg^{-1} en los suelos no cultivados. Los suelos cultivados favorecieron el desarrollo de organismos copiotróficos asociados con el efecto *priming* que impulsa las pérdidas de materia orgánica nativa del suelo, frente a la dominancia de organismos copiotróficos con alto potencial de fijación de N en pastizales nativos. La funcionalidad microbiana evidenció cambios notables, dado que el potencial de nitrificación se incrementó hasta 75 veces en el suelo cultivado. Los principales factores agrícolas que impulsaron estos cambios fueron el uso continuo de estiércol de pollo fresco que contribuyó a neutralizar la acidez del suelo, el uso inadecuado de agroquímicos sin planes de fertilización, el riego excesivo y la falta de articulación entre actores para apoyar la transición hacia mejores prácticas agrícolas. El manejo actual incrementa los riesgos de pérdidas de N por lixiviación, volatilización y emisiones de gases de efecto invernadero. Las funciones de pedotransferencia desarrolladas en esta tesis son una herramienta práctica que pueden favorecer la implementación de las estrategias, mediante la estimación de las concentraciones de NT y $N\text{-}NO_3^-$ a partir de propiedades edáficas más fácilmente medibles. Se propusieron estrategias adaptadas al contexto, incluyendo mejoras en la fertilización y el riego, reducción de la dependendica del monocultivo mediante la diversificación y rotación de cultivos, el manejo de la biodiversidad del suelo y el desarrollo de políticas públicas enfocadas a reducir las pérdidas de N. En general, esta tesis aporta al avance del conocimiento sobre la dinámica del N en regiones de alta montaña poco estudiadas y críticas para la seguridad alimentaria e hídrica de Colombia.

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*Tesis Doctoral

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1. Contextual Background, Rationale, and Research Design

1.1 Introduction

Nitrogen (N) is a vital nutrient for all living organisms, essential for synthesizing cellular components such as proteins and nucleic acids. As N is typically a limiting nutrient in ecosystems (FAO, 2021), adding external N inputs, such as organic and synthetic fertilizers, has become a widespread practice to support agricultural productivity and socioeconomic development. However, over 50% of applied N is lost from soils (Houlton et al., 2019), leading to a cascade of negative consequences as the surplus N reaches different environmental compartments (Galloway et al., 2021). These impacts include the acceleration of climate change and stratospheric ozone depletion due to nitrous oxide (N_2O) emissions (Tian et al., 2020); acidification of soils due to atmospheric N deposition (Stevens et al., 2010); biodiversity loss and reduced ecosystem resilience (Erisman et al., 2015); eutrophication and hypoxia in aquatic ecosystems (FAO and IWMI, 2018); and threats to human health due to water and air pollution from N compounds and particulate matter emissions (Nieder and Benbi, 2022).

The environmental consequences of N losses are particularly severe in high-mountain regions with agricultural land use. Hillslope areas are more prone to soil, water, and nutrient losses (Lai et al., 2020; W. Zhang et al., 2020) and these regions also provide essential ecosystem services, including freshwater supply, to both upland and lowland populations (Grêt-Regamey et al., 2012; Immerzeel et al., 2020). Moreover, high-mountain ecosystems are considered among the most fragile on Earth, as they exhibit low recovery rates after disturbance (Bierman-Lytle, 2015). These regions include páramos ecosystems, unique tropical high-mountain landscapes spanning

approximately 25,000 km^2 across Venezuela, Colombia, Ecuador, Peru, Costa Rica, and Panama (Mosquera et al., 2023). Páramos are typically located between the upper forest limit (3,000 – 3,800 m a.s.l.) and the perpetual snow cover (4,400 – 4,800 m a.s.l.) (Hofstede, 1995). These ecosystems are widely recognized for their exceptional water supply and regulation capacity and for being biodiversity hotspots (Hofstede et al., 2014; Madriñán et al., 2013). Despite this importance, páramos are subject to increasing anthropogenic pressures, particularly in Colombia, where agricultural expansion and intensification have significantly altered these ecosystems (Sarmiento et al., 2017).

Previous studies have shown that intensified agriculture in Colombian páramos contributes to N losses and water quality degradation. For instance, in the Puracé páramo (Cauca Department), surface runoff from soils under potato (*Solanum tuberosum*) cultivation resulted in significantly greater N losses than in soils with natural vegetation or livestock pasture (Otero et al., 2011). In the Las Piedras River basin (Cauca Department), which includes páramo area, high surface water nitrate (NO_3^-) concentrations (2.20 mg NO_3^- L^{-1}) were linked to the presence of relatively small cultivated areas (Ruiz et al., 2017). Similarly, in the Lake Tota basin (Boyacá Department), the intensive use of fresh chicken manure in green onion (*Allium fistulosum*) fields contributed to the transformation of the lake from oligotrophic to moderately eutrophic (Aranguren-Riaño et al., 2018), with nitrate concentrations in tributaries ranging from 2.3 to 5.6 mg N-NO_3^- L^{-1} (Barrera et al., 2019). In the Santurbán-Berlín páramo (Santander Department), an exploratory study conducted in a relatively small hydrographic unit (i.e., 19 ha) reported a sharp increase in surface water nitrate concentrations (from 0 to 1.40 mg N-NO_3^- L^{-1}) attributed to agricultural activity (green onion and potato cropping, and fallow land) in only 15% of the catchment area, raising

concerns over irrigation water quality and the conservation of high-altitude aquatic ecosystems (Rey-Romero et al., 2022b).

Reducing N losses in páramo agroecosystems is therefore a priority for advancing sustainable agricultural practices. Although various strategies exist to improve plant N use efficiency and reduce losses (Mahmud et al., 2021), their implementation is often limited by the diffuse nature of agricultural pollution, characterized by non-point sources and complex contaminant transport and transformation mechanisms (Giri and Qiu, 2016). Thus, it is necessary to deepen the understanding of the agricultural and environmental factors that govern soil N dynamics to propose effective strategies for mitigating diffuse pollution in context-specific settings. Although the factors influencing soil N dynamics have been widely studied across different geographic regions, research in páramo ecosystems remains limited. Furthermore, the complex interactions among these factors prevent the direct application of generalized findings to specific locations. This issue is particularly relevant in páramos, where N transport and transformation are shaped by unique hydrological processes, resulting from their soils characteristics (e.g., high levels of acidity, porosity, organic matter, and water retention capacity) (Buytaert et al., 2006; Patiño et al., 2021), extreme climatic conditions that slow organic matter decomposition (Gutiérrez-Salazar and Medrano-Vizcaíno, 2019), and endemic soil microbial communities adapted to this environment (Lizarazo-Medina and Gómez-Vásquez, 2015).

Despite the growing evidence of N losses from agricultural systems in páramo regions and their impacts on water quality and ecosystem functioning, critical knowledge gaps remain regarding how N is stored, transformed, and mobilized in páramo soils under intensive green onion cultivation. In particular, there is limited understanding of (i) which soil properties and farming practices most strongly control N concentrations in these systems, (ii) how N pools are distributed

vertically along the soil profile under long-term cultivation, and (iii) how soil microbial communities involved in N cycling respond to this land-use change. This lack of integrated, field-based information constrains the design of context-specific strategies to reduce N losses while maintaining the livelihoods of small-scale farmers who depend on green onion production. Consequently, current management practices in Colombian páramos continue to operate under high uncertainty regarding their long-term environmental sustainability and impacts on critical ecosystem services such as water regulation.

Given this context, the present doctoral research aimed to evaluate N dynamics in páramo soils under green onion cultivation. To achieve this, three specific objectives were defined: i) to determine the agricultural practices associated with green onion cultivation and the soil properties influencing N concentrations in páramo soils; ii) to estimate the effect of green onion cultivation on the vertical distribution of N concentrations in a páramo soil profile; and iii) to analyze the dynamics of microbial biodiversity involved in the N cycle in páramo soils under green onion cultivation. This doctoral thesis is structured into seven chapters. Chapter I presents the justification for the study, fundamental concepts of soil N dynamics, the political, agricultural, and environmental context of the study area, the general methodological approach, and the research outputs. Chapters II and III address the first specific objective, while Chapters IV and V correspond to the second and third objectives, respectively. Chapter VI discusses potential strategies for reducing N losses in the study context. Finally, Chapter VII presents the general conclusions and perspectives for future research.

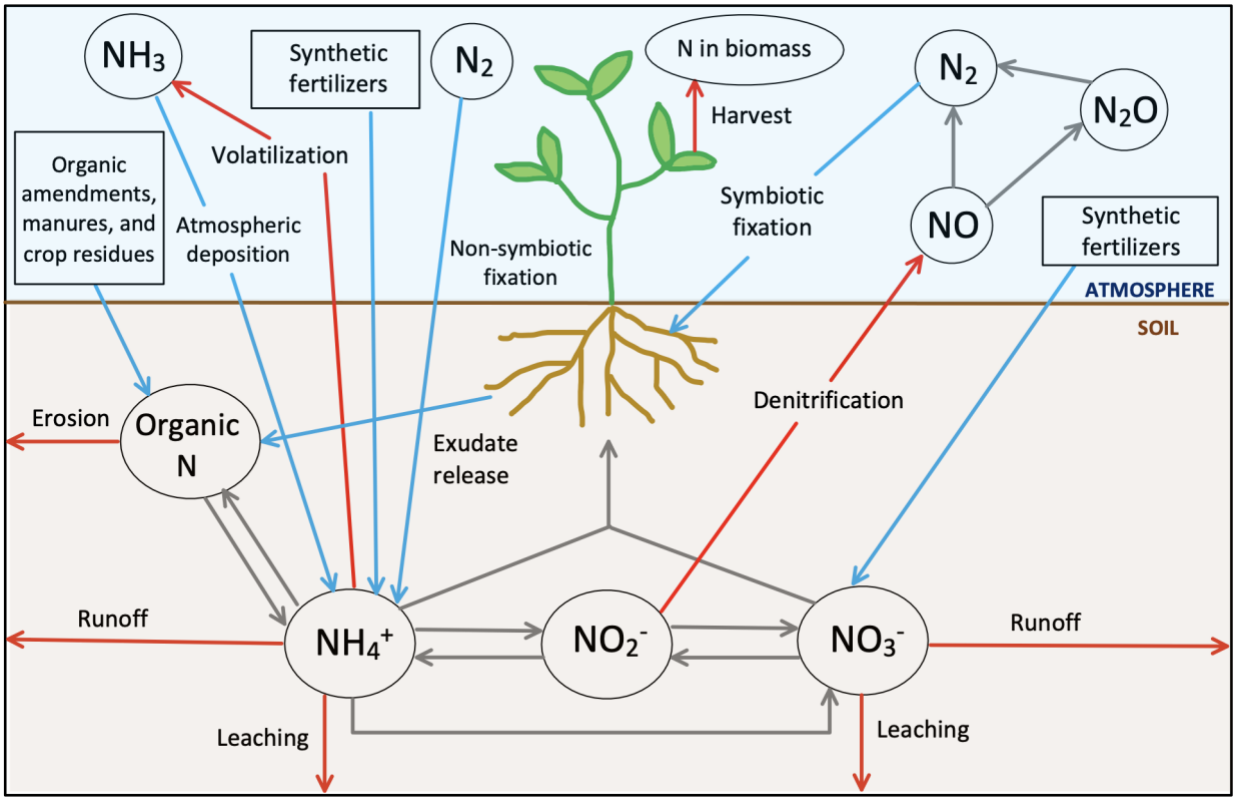
1.2 Overview of Nitrogen Dynamics in Soils

Soils are one of the main N reservoirs in the biosphere, containing this element in both organic and inorganic forms. Organic N, the dominant form in soil (Xin et al., 2019), is present in complex compounds such as free amino acids, peptides, and proteins (Yu et al., 2002). Inorganic forms include ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-). Plants primarily assimilate N as NH_4^+ and NO_3^-, with the preference for one form over the other depending on species-specific traits and environmental conditions (Ahmed et al., 2022). NO_2^- is generally considered a transient intermediate in the nitrification process and is found in very low concentrations in soil (Daza, 2018). Soil N dynamics are governed by a range of transformation reactions and transport processes involving exchanges with the atmosphere and water bodies, as schematically illustrated in Figure 1. N inputs to soil include: i) atmospheric deposition; ii) N fixation; iii) plant exudates that add organic N compounds; iv) agricultural sources such as synthetic fertilizers, organic amendments, crop residues, and animal manure; and v) non-agricultural sources, particularly relevant in urban settings, such as the improper disposal of solid and liquid waste (Xin et al., 2019). N losses occur via gaseous emissions, volatilization, erosion, leaching, runoff, and biomass removal through harvesting (Cameron et al., 2013).

Soil N undergoes complex transformations primarily driven by microbial activity. For instance, NH_4^+ can be produced through biological N fixation or the Dissimilatory Nitrate Reduction to Ammonium (DNRA) pathway (Stein and Klotz, 2016). Biological N fixation is carried out by bacteria and archaea that encode nitrogenase enzyme complexes, typically molybdenum-iron dinitrogenase, vanadium-based, or iron-only variants (Hoffman et al., 2014). DNRA, on the other hand, can be mediated by bacteria, diatoms, archaea, and fungi (Kuypers et al., 2018), and can substantially limit NO_3^- leaching in soils (Pandey et al., 2020). Another key

source of NH_4^+ in soils is mineralization, the microbial conversion of organic N into inorganic forms. Microorganisms use various enzymatic strategies to break down compounds like proteins, carbohydrates, and nucleic acids, releasing free amino groups ($-NH_2$) that are subsequently transformed into NH_4^+ (Myrold and Bottomley, 2015).

Figure 1. Nitrogen dynamics in agricultural soils



Note: Red arrows indicate nitrogen present in harvested agricultural products and losses from the soil; blue arrows represent nitrogen inputs to the soil; and gray arrows indicate transformation processes that do not involve transfer to other environmental compartments (i.e., the atmosphere or water bodies).

Once in the soil, NH_4^+ may follow several pathways: immobilization, assimilation, oxidation via aerobic or anaerobic microbial respiration, adsorption onto soil colloids, or fixation

by expansive clays such as vermiculite (Daza, 2018). Immobilization refers to the transformation of inorganic N into organic forms, while assimilation is the incorporation of N into microbial or plant biomass (Myrold and Bottomley, 2015). Nitrification, the aerobic oxidation of NH_3 to NO_3^-, involves three microbial groups (Stein and Klotz, 2016): i) NH_3 oxidizers producing NO_2^- (nitritation); ii) NO_2^- oxidizers producing NO_3^- (nitration); and iii) complete ammonia oxidizers (comammox), capable of converting NH_3 directly to NO_3^-. The anaerobic oxidation of NH_4^+, known as the anammox process, was first described in the late 20th century (Mulder et al., 1995), challenging the earlier notion of NH_4^+ stability under anoxic conditions. In this pathway, NH_4^+ and NO_2^- are converted into dinitrogen gas (N_2) through intermediates such as nitric oxide (NO) and hydrazine (N_2H_4) (Kartal et al., 2011).

Finally, denitrification is the reduction of NO_3^-, NO_2^-, NO, and N_2O to N_2 during anaerobic respiration. This process is typically mediated by heterotrophic bacteria and archaea. However, many of these microorganisms possess only partial denitrification pathways, lacking one or more genes required for complete reduction to N_2, resulting in the accumulation of intermediate gases such as NO and N_2O, or, in some cases, the absence of NO_3^- reduction altogether (Stein and Klotz, 2016). In natural and managed ecosystems, N transformation pathways are influenced by soil physicochemical properties, microbial communities, and environmental conditions. In high-altitude agroecosystems such as páramos, the interplay of these factors makes soil N dynamics particularly complex, underscoring the importance of integrated studies that consider biotic and abiotic processes.

1.3 Political, Agricultural, and Environmental Context of the Study Area

Colombia contains 36 páramo complexes, which account for 42.5% of the world's páramo ecosystems extension (Mosquera et al., 2023). Over the past seven decades, public policy in these areas has undergone substantial transformation. Murillo-Martín (2022) identifies three distinct periods in Colombian páramos governance. The first period (1959–1993) lacked regulations specifically targeting páramo management, although some areas were incorporated into broader environmental protection schemes. During this time, policies were mainly shaped by natural sciences knowledge, rendering human populations in páramos largely invisible. The second period (1993–2009) included explicit recognition of páramos as key environmental assets, especially for water supply, through co-management approaches that involved local communities, yet still lacked clear management direction. In the third period (2010–present), the state formalized the need to delimit and protect páramo ecosystems.

Páramos delimitation in Colombia was primarily framed as a national strategy to restrict mining and preserve water resources, while also being presented internationally as a climate change adaptation measure (Méndez Polo, 2019). This process is currently guided by two key policy instruments: Resolution 886 of 2018, which provides zoning guidelines and strategies for agricultural reconversion (Ministerio de Ambiente y Desarrollo Sostenible, 2018), and Law 1930 of 2018, which establishes the legal framework for integrated páramo management and includes specific restrictions on mining and agriculture (Congreso de Colombia, 2018). However, both the delimitation process and its regulatory framework have faced criticism from scientists and local communities. Critics argue that delimitation was based exclusively on biophysical mapping, without consideration of social, cultural, or territorial aspects (Ungar, 2021). This technocratic

approach has sidelined the cultural identities and livelihoods of páramo inhabitants, reinforcing a disconnect between environmental policy and lived realities (Méndez Polo, 2019).

Moreover, this lack of recognition of local communities undermines the development of participatory strategies that could more effectively address the conflicts arising from conservation policies (De Pourcq et al., 2017). Nonetheless, social mobilizations have led to meaningful proposals from páramo communities aimed at advancing sustainable territorial management. These include transitions toward agroecology, the creation of agro-parks and peasant reserve zones, intercultural agri-food territories, and organized farming initiatives promoting food sovereignty (Van der Hammen et al., 2015). Although social aspects are increasingly acknowledged in páramo conservation discourse, they continue to be treated as secondary to environmental concerns (Méndez Polo, 2019; Murillo-Martín, 2022). As such, it is essential to generate knowledge about the cultural diversity, identities, and livelihoods of páramo inhabitants. Moreover, given the diverse contexts across páramo regions, conservation policies must be tailored to the specific realities of each landscape and its inhabitants.

In line with these evolving policy frameworks, a recent milestone was the issuance of Resolution 963 by the Instituto Geográfico Agustín Codazzi (IGAC, 2025), which establishes a methodology for the environmental assessment of the degree of conservation of real estate located in delimited páramos. The tool incorporates an environmental component into commercial appraisals, assigning economic value to properties with significant coverage of natural páramo ecosystems or areas under restoration. The methodology evaluates aspects such as the extent of natural páramo cover, the presence of water bodies and springs, and evidence of erosion or land movement caused by human activity. While this represents a step toward recognizing the ecological value of páramos in land valuation and government procurement processes, the

assessment framework is primarily based on biophysical criteria and applies broad categories for anthropogenic intervention, without differentiating between types or intensities of land use. As such, its capacity to integrate and incentivize sustainable livelihood practices remains uncertain.

This research was conducted in the Santurbán-Berlín páramo complex, which is part of the eastern mountain range of the Colombian Andes. This region hosts the country's largest population of páramo inhabitants and has undergone the most significant transformation of natural land-cover due to anthropogenic activities (Sarmiento et al., 2017). The Santurbán-Berlín páramo is the scene of complex interactions between actors and human activities of various kinds, with economic activities including small-scale agriculture, ancestral mining, and tourism. Regarding agricultural activities, as outlined by Amorocho Pérez et al. (2024), potato cultivation was the primary crop in the study area until the 1950s. Green onion cultivation was introduced thereafter, initially using traditional practices, but agriculture modernized in the 1970s with Green Revolution technologies. These new management approaches included utilizing machinery for tillage, mechanized irrigation systems, and the use of chemical pesticides and herbicides, and synthetic fertilizers. During the past years, the local economy has become increasingly dependent on green onion cultivation (Figure 2), making this region one of Colombia's main green onion producers (DANE, 2017).

The study was conducted within a 207-hectare hydrographic unit (HU) located between 3,390 and 3,618 m.a.s.l., spanning parts of the Parra Juan Rodríguez and Arenales villages in the municipality of Tona, Santander Department (Figure 3). This HU includes 36 households, 32 of which are small-scale farming families. The area is characterized by extreme climatic conditions, including low temperatures, high solar radiation, elevated humidity, and strong winds, with average values of 8.9 °C, 487.9 MJ m^{-2} $month^{-1}$, 86.4%, and 4.28 m s^{-1}, respectively (Gómez, 2022). It exhibits pronounced daily temperature fluctuations and a distinct spatial gradient in

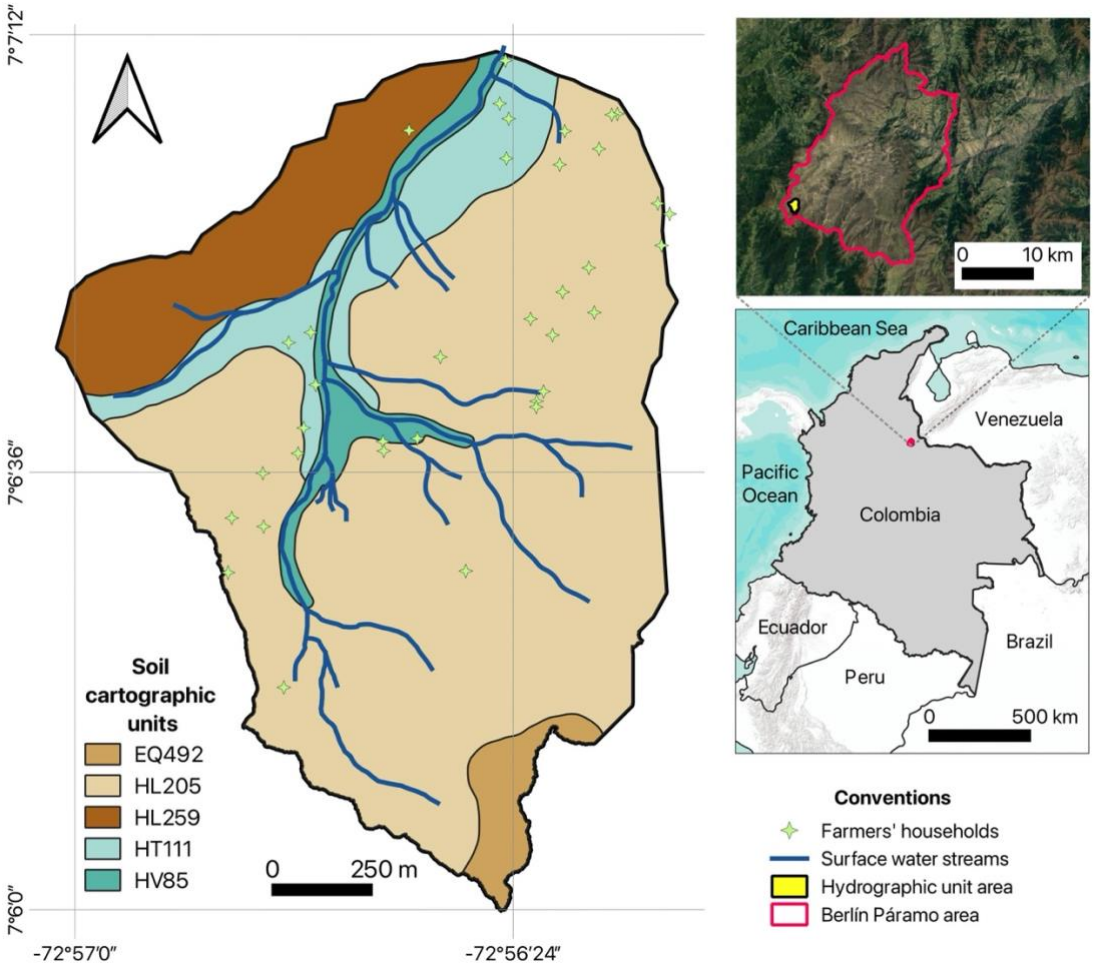
rainfall, following a bimodal distribution with higher rainfall amounts during March to May and September to November. During the field study period (2021–2024), the HU recorded an average annual precipitation of 1,258 mm.

Figure 2. Green onion cropping in Santurbán-Berlín páramo

Geologically, the HU is situated on the Santa Bárbara Monzogranite, a plutonic formation composed primarily of potassium feldspar, plagioclase, and quartz, with minor biotite and hornblende, and accessory minerals including opaque minerals, apatite, zircon, titanite, garnet, and allanite (Rodríguez et al., 2018). The weathering of this monzogranite contributes to its porosity and classifies it as an unconfined aquifer (Cetina, 2019). According to Ponce (2024), three major weathering profiles have developed in the HU: (i) fresh outcrops of the Santa Bárbara Monzogranite (the parent rock), (ii) completely weathered igneous rock, and (iii) Quaternary deposits. The parent rock is composed of orthoclase, quartz, albite, and biotite, with secondary

alteration products including chlorite, sericite, and kaolinite. In contrast, the weathered rock profile contains kaolinite, sericite, gibbsite, vermiculite, and brucite, while the Quaternary deposits include similar clays along with gypsum and phlogopite. These mineral assemblages can contribute to the availability of ions such as NO_3^-, NH_4^+, dihydrogen phosphate ($H_2PO_4^-$), potassium (K^+), sodium (Na^+), and calcium (Ca^{+2}) in soil.

Figure 3. Geographical context of the study area



Note. Soil cartographic units were defined according to IGAC (2015). EQ492: Andic Humicryepts with inclusions of Typic Humicryepts and Lithic Melanocryands; HL205: Entic Humudepts with inclusions of Acrudoxic Melanudand; HL259: Typic Humudepts with inclusions of Pachic

Humudepts and Fluventic Humudepts; HT111: Typic Dystrudepts with inclusions of Typic Humudepts; HV85: Complex of Aquíc Humudepts, Entic Humudepts and Fluventic Humudepts.

According to the semi-detailed soil survey (scale 1:25,000) conducted by the IGAC (2015), the dominant soils in the HU are classified as Inceptisols and Andisols under the USDA-NRCS system (Soil Survey Staff, 2022). These soils are characterized by high organic matter content, strongly acidic pH, elevated aluminum saturation, low bulk density, high total porosity, and inherently low fertility.

Despite the absence of allophane, the significant presence of organic colloids in soils imparts hydrophysical properties comparable to those of allophanic Andisols (Buytaert et al., 2006). Five soil cartographic units (SCUs) are identified within the study area (Figure 3). According to IGAC nomenclature, these include the HL205, HL259, and HT111 consociations, and the HV85 complex, which occur under very cold, humid to very humid climatic conditions, and the EQ492 consociation, found in extremely cold and very humid environments. Appendix A summarizes the characteristics of each SCU in terms of landform, terrain morphology, parent material, soil features, and taxonomic components.

This combination of environmental conditions and land use patterns has important implications for ecosystem functioning and sustainability, particularly in relation to nutrient cycling and N losses. While green onion farming provides vital income for rural households, its current development has raised concerns regarding environmental sustainability. For instance, a previous study in the HU monitored surface water quality at eight points along three streams crossing areas with varying land uses and found significantly higher concentrations of $N\text{-}NO_3^-$ and total N (TN) in streams adjacent to agricultural areas compared to pristine zones (Rey-Romero et

al., 2022a). $N\text{-}NO_3^-$ concentrations ranged from 0.00 to 3.40 mg L^{-1}, while TN levels varied from 0.15 to 5.50 mg L^{-1}. These elevated concentrations, largely attributed to non-point sources from green onion cultivation, pose a risk of eutrophication in páramo water bodies and highlight the environmental impacts of current N management practices. Consequently, this area reflects the broader challenges in Colombian páramos, where transition to sustainable N management is imperative.

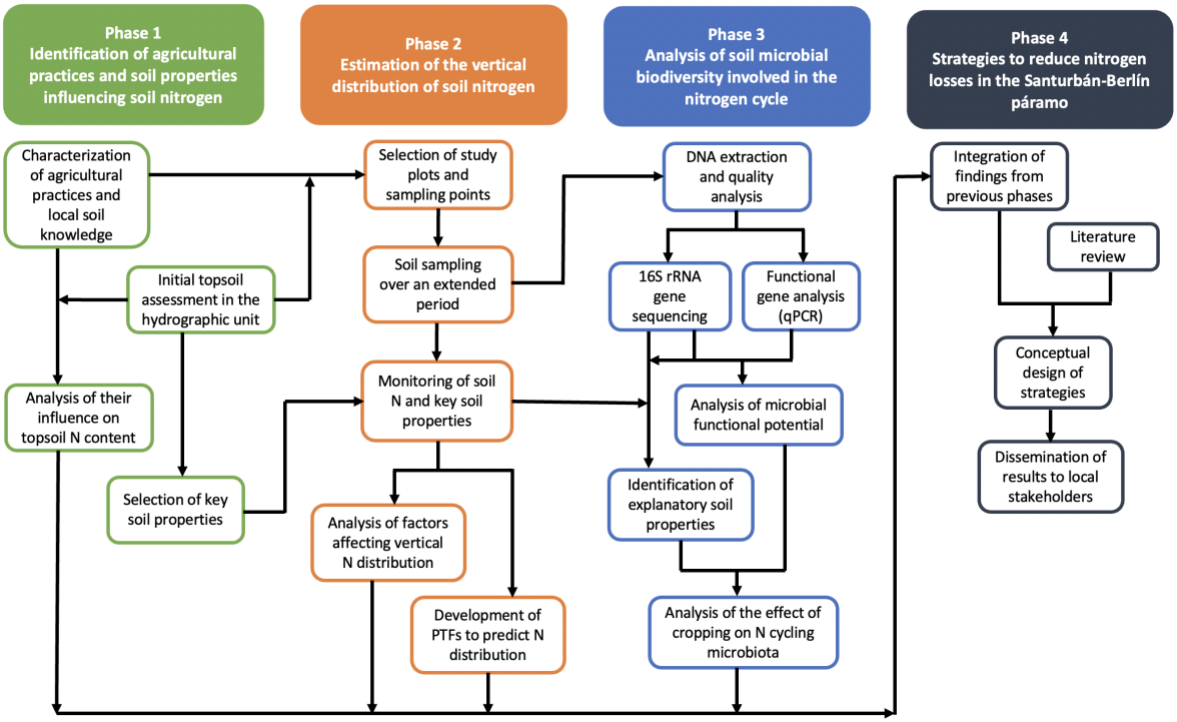
1.4 Methodological Framework

This doctoral research was structured into four methodological phases, as illustrated in Figure 4. Each of the first three phases corresponded to one of the specific research objectives. Phase 1 focused on identifying the agricultural practices and soil properties that influence soil N concentrations. Fieldwork for this phase was conducted across the entire HU. Agricultural practices and local soil knowledge were characterized through semi-structured interviews with farmers and field observations. Additionally, topsoil (0–20 cm) was sampled in plots under green onion cultivation and native grassland for an initial assessment of N content and related properties.

Phases 2 and 3 were carried out in parallel over an extended monitoring period in three sampling plots within the HU: two plots under green onion cultivation and one control plot with native grassland cover. Phase 2 aimed to evaluate the effect of green onion cultivation on the vertical distribution of N in páramo soils and to develop predictive models (i.e., pedotransfer funcions – PTFs) to estimate N concentrations, as a tool to improve N management in the study context. Phase 3 analyzed the influence of green onion cultivation on soil microbial biodiversity involved in N cycling; for this, two DNA-based molecular techniques were employed: 16S rRNA gene metabarcoding to assess bacterial community structure, and quantitative polymerase chain

reaction (qPCR) to quantify key functional genes associated with N transformation processes. Finally, phase 4 integrated the findings from the previous phases to propose potential strategies for reducing N losses in the soils of the Santurbán-Berlín páramo.

Figure 4. Methodological workflow for the doctoral thesis



1.5 Scientific and Outreach Contributions of the Research

This doctoral thesis contributed to the generation of new knowledge, the social appropriation of science, the development of human capital, and the strengthening of scientific collaboration networks. As part of the scientific output, the following research articles were published or are currently under peer review:

- Rey-Romero, D. C., Daza-Torres, M. C., Sanchez-Torres, V., & Oviedo-Ocaña, E. R. (2025). Effects of green onion (*Allium fistulosum*) cropping on topsoil nitrogen species contents in a Páramo ecosystem. *Geoderma Regional*, 41. https://doi.org/10.1016/j.geodrs.2025.e00958

- Rey-Romero, D. C., Méndez, Y. L., Daza-Torres, M. C., Domínguez, I., & Oviedo-Ocaña, E. R. (2025). Challenges and opportunities for the sustainable nitrogen management in a páramo agroecosystem: insights from local soil knowledge and farming practices. *Agroecology and Sustainable Food Systems*, 1-36. https://doi.org/10.1080/21683565.2025.2510357

- Rey-Romero, D. C., Varón-Ramírez, V. M., Daza-Torres, M. C., Sanchez-Torres, V., Márquez Molina, J. J., Guevara, M., & Oviedo-Ocaña, E. R. Nitrogen pools in páramo soils under green onion cropping: vertical distribution, prediction, and management implications. *Soil Research.* https://doi.org/10.1071/SR25162

- Rey-Romero, D. C., Zafra, G. Sanchez-Torres, V., Daza-Torres, M. C., Maeda, T. & Oviedo-Ocaña, E. R. Green onion cultivation restructures soil N-cycling microbiota toward N-loss pathways in Andean high-mountain ecosystem. Submitted to *Soil Ecology Letters.*

Research findings were also disseminated through presentations at national and international academic events:

- 1er Coloquio Queretano de Estudios Interdisciplinarios del Suelo. December 2024. Institute of Geosciences and University Program of Interdisciplinary Soil Studies of the Universidad Nacional Autónoma de México. Presentation: “Predicción de la concentración de nitrógeno total en suelos cultivados con *Allium fistulosu*m y pastizales nativos en un ecosistema de páramo en Colombia.”

- 17th International Symposium on Soil in Plant Analysis (ISSPA2023). March 2023. Concepción, Chile. Soil and Plant Analysis Council, Universidad Técnica Federico Santa María, Agriservice. Presentation: “Exploring the effect of land-use change in soil N dynamics in an Andean páramo.”

- Encuentro Internacional de Educación en Ingeniería 2022. September 2022. Asociación Colombiana de Facultades de Ingeniería. Cartagena, Colombia. Presentation: “Estudio de línea base sobre la variabilidad espacial de la concentración de nitrógeno en el agua superficial de un agroecosistema de páramo en Colombia”.

The author of this thesis participated as a supervisor or co-supervisor for the following undergraduate and master's research projects conducted within the framework of this doctoral research at the Universidad Industrial de Santander:

- Naranjo Barrios, Juan David. (2025). Análisis de la gestión del agua para riego de una unidad productiva de cebolla larga (*Allium fistulosum*) bajo las condiciones agroclimáticas del páramo de Berlín (Santander – Colombia). Trabajo de grado de Maestría en Ingeniería Civil.

- Ponce Betancur, Jose David. (2024). Caracterización de los perfiles de meteorización del Monzogranito de Santa Bárbara y depósito asociado en el Páramo de Berlín, Santander. Trabajo de grado de Geología.

- Villán Pacheco, Valentina; Ardila Aragón, Diana Paola. (2023). Análisis de la vulnerabilidad a la contaminación difusa por nitrógeno del agua superficial en una unidad hidrográfica del Páramo de Berlín (Santander, Colombia) mediante análisis espacial multicriterio. Trabajo de grado de Ingeniería Civil.

- Garzón García, Leydi Tatiana; Ríos Mercado, María Fernanda. (2022). Propuesta de alternativas y criterios para la selección de estrategias para reducir pérdidas de nitrógeno en agroecosistemas de páramo. Trabajo de grado de Ingeniería Civil.

- Barrera Cadena, María Juliana. Bautista Prada, Emily. (2022). Influencia de las actividades agropecuarias sobre el contenido de nitrógeno en el agua superficial de una unidad hidrográfica en el Páramo de Berlín (Santander, Colombia). Trabajo de grado de Ingeniería Civil.

- Páez Madariaga, Oliva Yineth. López Torres, Brayan. (2021). Revisión sistemática del estado del arte sobre la adición de inoculantes biológicos para reducir las pérdidas de nitrógeno en el suelo. Trabajo de grado de Ingeniería Química.

In terms of community engagement, the research results were shared with local stakeholders in the study area through a participatory workshop and field visits. Informational brochures were distributed to farmers to promote awareness of sustainable N management practices. Finally, this doctoral work facilitated the establishment of scientific collaborations between the Water Resources and Environmental Sanitation Research Group (GPH) and the Minerals, Biohydrometallurgy, and Environment Research Group (GIMBA), as well as with researchers from the Biochemistry and Microbiology Research Group (GIBIM) at the Universidad Industrial de Santander, the Integrated Water Resources Management for Agricultural Development and Food Security through Irrigated Agriculture Research Group (REGAR) at the Universidad del Valle in Colombia, the Institute of Geosciences at the Universidad Nacional Autónoma de México (UNAM), and the Kyushu Institute of Technology in Japan.

Finally, this doctoral research contributes to global efforts to achieve Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), by advancing Target 2.4 on

resilient agricultural practices. Additionally, it supports SDG 15 (Life on Land) by addressing Target 15.4, which focuses on conserving mountain ecosystems and their role in sustainable development.

2. Agricultural Practices and Local Soil Knowledge as Drivers of Soil Nitrogen Dynamics in a Páramo Agroecosystem

2.1 Introduction

Páramos are unique tropical high-mountain ecosystems in South and Central America deemed vital for sustainable development. These regions are subject to competing claims by various stakeholders due to the diverse activities they support, including water supply for rural and urban inhabitants, biodiversity conservation, agricultural production, coal and gold mining, and tourism (Duarte-Abadía and Boelens, 2016; Ungar, 2021). Consequently, multiple and often conflicting development visions have emerged regarding the future of the páramos. Some of these visions prioritize short-term extractive use over conservation, contributing to management practices that undermine the socioecological integrity of these ecosystems. The Colombian páramos are home to approximately 150,000 people, whose livelihoods have transformed 15% of the páramos' land cover into agricultural areas (Sarmiento et al., 2017). Although agriculture in these regions remains small-scale, neoliberal conservation policies and economic pressures have pushed páramo's farmers toward agro-extractivist practices (Blake et al., 2023).

This shift is characterized by monocropping, intensification and specialization, expansion of agriculture into non-cropped páramo areas, and reliance on external inputs (McKay et al., 2021), leading to biodiversity loss (Avellaneda-Torres et al., 2024), soil degradation (Patiño-Gutiérrez et al., 2024), and water pollution (Rey-Romero et al., 2022b). Agro-extractivism in páramos also forces peasants into precarious livelihoods and poverty cycles (Blake et al., 2023). Colombian legislation aims to harmonize the productive activities of traditional páramo inhabitants with the

provision of ecosystem services, but has exacerbated social tensions (Murillo-Martín, 2022). Many rural inhabitants feel excluded from dialogue and pressured to leave their lands under the proposed conservation framework (Ungar, 2021), and have also cited insufficient support from other stakeholders in transitioning to more sustainable agricultural practices (Blake et al., 2023; Leroy, 2020). Additionally, despite recognizing the páramo's environmental value, communities feel their conservation efforts are overlooked, especially by authorities (Amorocho Pérez et al., 2024).

To enhance understanding of the human dimension of páramos, earlier studies have examined the implications of local farming practices on páramo conservation, and small-scale farmers' responses to socio-environmental threats and conflicts. For instance, Avellaneda-Torres et al. (2014) analyzed farming practices in a rural páramo community in Colombia and found that livelihoods based on potato (*Solanum tuberosum*) cultivation and cattle ranching, influenced by cultural heritage, Green Revolution, and indigenous knowledge, create tensions between conservation and improving quality of life. Leroy (2019) examined Colombian páramo farmers' perceptions of water scarcity and adaptation strategies to climate change, emphasizing how these shape collective adaptation efforts. The study found that governance, capital access, public aid, and water availability strongly influence adaptation responses. In a subsequent study, Leroy (2020) examined pesticide use practices and perceptions in the Colombian and Venezuelan páramos, finding that while farmers are aware of the health risks, social-technical barriers and weak institutional support hinder their ability to change these practices.

Understanding how local communities perceive and engage with their environment is crucial for designing sustainable management strategies in the páramos. For example, Vergara-Buitrago and Murcia (2021) examined local environmental knowledge among páramo small-scale farmers, highlighting climate awareness, composting practices, and proper solid waste

management. Leroy and Barrasa García (2021) emphasized the importance of local perceptions in revealing what farmers truly value about their ecosystems. Chohan et al. (2024) assessed the socio-ecological contributions of small-scale agropastoralism in páramos, arguing for its role in conserving agrobiodiversity and strengthening rural autonomy. Moreover, Amador-Jimenez and Millner (2024) explored the self-identity of páramo inhabitants, showing how conservation-care practices are integral to their cultural life.

While these studies offer relevant perspectives on the sociocultural conditions of farmers in Colombian páramos, they do not directly address soil fertility and nutrient management implications. This oversight is critical, as inadequate nitrogen (N) management in cultivated soils has caused N losses leading to significant impacts on páramos' water sources. Those impacts are evidenced in eutrophication, biodiversity loss, and compromised water quality for various uses (Aranguren-Riaño et al., 2018). Furthermore, current N management poses a sustainability challenge for páramo farmers, who allocate approximately 60% of their resources to acquiring external inputs, including organic and synthetic fertilizers (Avellaneda-Torres et al., 2014). Additionally, available literature indicates that no previous studies have examined local soil knowledge (LSK) and its role in guiding sustainable N management in sensitive ecosystems like the páramos. As defined by Winklerprins (1999), LSK is 'the knowledge of soil properties and management possessed by people living in a particular environment for some period of time'. Recognizing and integrating LSK is essential not only for understanding farmers' adoption or rejection of agricultural practices, but also for selecting context-appropriate sustainability initiatives and strengthening participatory processes through improved communication among farmers, scientists, and extension workers.

Therefore, this chapter aimed to unravel aspects of local agricultural management that may favor or mitigate alterations in soil N dynamics and potential N losses. The findings of this study provide novel insights for fostering transitions to resilient agricultural systems that align with transformative environmental policies, promoting socio-ecological synergies in sensitive ecosystems.

2.2 Methods

This section addresses the methodology used to characterize LSK and farming practices for green onion cropping in the study area, including the use of qualitative methods and characterization of the organic amendments used by farmers. Additionally, it describes the approach to estimate a N partial balance across three representative farms, and the methods for data analysis.

2.2.1 Characterization of Local Soil Knowledge and Farming Practices

This activity involved the use of qualitative methods, including surveys, participant-observation, and a participatory community workshop, to characterize LSK and green onion cropping practices among small-scale farmers within the hydrographic unit (HU) described in Chapter I (section 1.3). Farmers' engagement in these activities were conducted with the approval of the Scientific Research Ethics Committee of the Universidad Industrial de Santander, and informed consent was obtained from all individual participants included in the study. Surveys were conducted through face-to-face semi-structured interviews in Spanish lasting between 30 and 60 min. With farmers' consent, interviews were recorded and transcribed. Twenty-three farmers were interviewed between November 2021 and May 2022. Given the sociocultural characteristics of the

rural population in Berlín Páramo, which limit accessibility, convenience and snowball sampling were used (Atkinson and Flint, 2001).

The survey questionnaire included both open- and closed-ended questions, organized into eight sections (Appendix B). The first section addressed general questions about the farm and farmer, such as time living on the páramo, years cultivating onions, how they learned onion farming, and the main challenges for cultivation. The second section explored LSK, including local fertilization terms, nutrient management criteria, and perceptions of soil fertility trends. Sections three through six focused on farming practices: soil preparation, fertilization, water management, and pests and disease control. The final sections addressed acquisition of external inputs, waste management, and perceptions of soil contamination.

Participant-observation was conducted through field visits, where the researcher actively engaged in farming activities, including soil preparation, sowing, harvesting, and onion peeling and packing. Researcher also accompanied farmers in tasks like operating irrigation sprinklers, preparing agrochemicals, and incorporating lime or fresh chicken manure (FCM) into the soil. This approach allowed for detailed follow-ups, providing a comprehensive perspective on green onion farming. Finally, a community workshop with participation of other members from the GPH-UIS research group and peasant families facilitated discussion and knowledge exchange on LSK and farming practices, validating the information from surveys and participant-observation.

To estimate the quantities and frequency of input applications, specific questions were incorporated into the farmer interviews. The cultivated area of each farm was determined using a portable GPS receiver with a precision of 3.65 meters to georeference plot boundaries and calculate their extent. This information was used to express input application rates relative to the cultivated

area. Due to the absence of formal record-keeping among many farmers, it was not possible to obtain precise quantities for all inputs in every case. For N input estimation, the focus was placed on the application of FCM. Farmers typically reported the number of FCM bags applied per plot, although they were often unsure of the exact weight of each bag. Based on interview responses, a weight range of 25 to 35 kg per bag was used to estimate the total quantity of manure applied. N inputs from FCM were then calculated using these estimates and the average total nitrogen content obtained from laboratory analyses. The calculations also accounted for the average moisture content of the locally used FCM, which is approximately 22% (Ríos, 2025). Additionally, FCM used by eight farmers was analyzed for their total nutritional content, following Colombian technical standards (ICONTEC, 2022).

2.2.2 Estimating Nitrogen Partial Balance

To better understand on-farm N flows and their implications for potential N losses, a rough partial N balance was conducted for three representative farms in the HU. N input was estimated using the previously calculated N application rates. As farmers from these three farms reported applying FCM once every two crop cycles, N input was halved to reflect the rate per crop cycle. N output through harvest was estimated using the N content of green onion dry matter for each farm, measured via spectrometry. Since farmers did not keep yield records, an average yield of 34.3 t/ha, estimated for the Santander Department, was used (AGRONET, 2022), and a dry matter content of 7.66%, measured for the regional green onion variety used in Aquitania (Boyacá Department, Colombia) (Galeano Mendoza et al., 2018), was employed for calculation. It is important to note that this N balance represents a first-order approximation. Several assumptions were necessary due to data limitations. Despite these constraints, this approximation provides

valuable insight into the likely magnitude of reactive N surpluses under current fertilization practices.

2.2.3 Data Analysis

The qualitative data from surveys, participant-observations, and workshop discussion were analyzed using a thematic analysis approach. This method involves familiarizing oneself with the data, generating initial codes, organizing these into themes, and presenting the findings through narrative descriptions (Nowell et al., 2017). Thus, it enabled the identification of recurring patterns and key insights regarding soil and crop management strategies. It also provided a deeper understanding of the factors that influence local farmers' willingness to adopting certain farming practices. Quantitative analysis was also performed on interview data. Percentages were calculated for multiple-choice responses, and categorical data on residence time, onion cultivation experience, and farming practices were grouped for analysis. Information on input doses was summarized in ranges and histograms, while data on farm area under onion cultivation and FCM composition were described using measures of central tendency and dispersion.

2.3 Results and Discussion

This section presents and discusses general aspects of land use, tenure, and local farmers' experiences. It then analyzes the influence of agricultural practices and LSK on N dynamics in the páramo agroecosystem. Finally, it examines aspects of local perceptions that influence nutrient management.

2.3.1 Land Use, Tenure, and Farmers' Experiences

All interviewed farmers grow green onion as their main livelihood. Other land uses within the study area include potato cropping (43%), extensive livestock farming (sheep and cattle) primarily for family milk production (26%), non-permanent crops (12%, e.g., faba beans, strawberries, carrots), trout farming (4%), and conservation (17%). Green onion field areas average 0.50 ± 0.27 ha. Regarding land tenure, 39% of the farmers are landowners, while 61% are tenants. Additionally, 65% of respondents have lived in the páramo for over 20 years; 20% between 10 and 20 years, and 15% for less than 10 years, indicating that agricultural activities are also developed by recent settlers attracted by increasing migration (Amorocho Pérez et al., 2024).

Farmers reported learning agricultural practices primarily through family tradition (37%), with others citing learning through observation (32%), advice from friends and neighbors (21%), and working for experienced farmers (10%). They perceive the onions grown in Santurbán-Berlín Páramo as high quality, attributing this to their thickness, greenness, and flavor. Farmers identified the main challenge in green onion cultivation as low temperatures causing frost, which negatively affects field productivity. They also noted that drastic temperature fluctuations over short periods promote the onset of diseases in the onions, while pest proliferation tends to increase during periods of high rainfall. Despite these difficulties, all farmers agree that green onion remains the only crop with significant economic potential for the region.

2.3.2 Influence of Farming Practices and Local Soil Knowledge on Nitrogen Dynamics

The main local agricultural practices characterized in this study are summarized in Figure 5. This subsection presents key aspects of farming practices and related LSK, grouping the

activities into three categories: i) soil preparation and sowing, ii) regular farming practices, and iii) harvesting and crop residues management. Given the relevance of recognizing and preserving local specific terms to foster the integration of LSK and scientific knowledge, as well as to improve communication between stakeholders (Reinhardt and Herrmann, 2017), this discussion includes relevant local terminology in Spanish (in italics) alongside its translation into English.

- Soil preparation:

Conventional tillage is employed on all studied farms for soil preparation (Figure 5A). Interviewees explained that the process begins with disk harrowing for vegetation removal and deep plowing, followed by rotary tillage to smooth the soil. Tractors are used due to the fields not being very steep, though most farmers hire tillage services as they lack machinery. While long-term conventional tillage can promote soil compaction, reduced infiltration, erosion, soil organic matter loss, and increased N runoff (Bosch et al., 2015), its impact in the study area may be limited, as it is only performed once before establishing green onion crops. These fields are then maintained for several years without crop rotation. However, farmers do perform manual hilling during the crop cycle, which contributes to continued soil disturbance. Among respondents, 22% implemented conventional tillage over 20 years ago, 17% between 10 and 20 years ago, and 31% within the last 5 years. The remaining farmers, being tenants, were unsure of when the fields were last tilled.

None of the interviewed farmers have conducted soil analyses, yet they widely perceived undisturbed páramo soils as acidic and low in fertility. This perception was reflected in their use of lime after tillage and before planting green onions, and in the common practice of cultivating potatoes beforehand (Figure 5B), based on the belief that potatoes, being more tolerant to acidity,

help improve soil conditions. This perception aligns with Patiño-Gutiérrez et al. (2024), who reported increased soil pH in onion fields compared to recently plowed soils used for potato cultivation in Santurbán-Berlín páramo. These practices suggest that farmers recognize and adapt to the limitations of native soils in the páramo, particularly related to acidity.

Figure 5. Main farming practices for green onion cropping in Santurbán-Berlín páramo

Note. Soil preparation and sowing include conventional tillage (A), a cycle of potato cropping (B), delimitation of subplots (C), and sowing seedlings (D). Regular farming practices involve fertilization with fresh chicken manure (E), spraying mixed foliar fertilizers and pesticides (F), sprinkler irrigation (G), and lime application (H). Harvesting and crop residues management

include manual harvesting (I), picking and peeling onions (J), packing into 32 kg bundles (K), and accumulating crop residues for decomposition (L).

After land preparation, the plot is divided into *cuadros* (subplots) using a rope as a guide (Figure 5C). This division allows onions at different growth stages within the same plot, ensuring continuous harvests year-round. *Tomas* (drainage channels) are dug between subplots, with their size determined by soil drainage. Sowing occurs on *surcos* (ridges) oriented along the contours, spaced 0.8 m apart. This orientation helps prevent soil erosion and may reduce N losses through runoff. *Calles* (furrows), are spaced 0.4 m apart, as marked by knots on a guiding rope. Holes are dug, and seedlings with about four *gajos* (pseudo-stems) are planted (Figure 5D). A few days after sowing, the first dose of fresh chicken manure (FCM) is surface applied, followed by the first hilling after a month. Farmers either select seedlings from their own high-performing plots or purchase them locally. Seedlings are stored in shaded areas and generally not treated with phytosanitary products. The first growth cycle lasts about 6 months, with subsequent cycles lasting 3 to 4 months, comprising stages of pseudo-stem development, thickening, and harvesting.

- Regular farming practices:

All interviewed farmers use FCM as fertilizer (Figure 5E), a low-cost input providing a major source of nutrients and trace elements that boost crop yields (Hoover et al., 2019). In Colombia, FCM use is widespread among small-scale farmers in páramos (DANE, 2017; Rodríguez-Robayo et al., 2022) and other rural areas where green onions are cultivated (Gómez Benítez et al., 2021; Gómez-Balanta and Ramírez-Náder, 2022). FCM typically contains higher concentrations of N, P, Ca, Mg, and S than other livestock manure (Kacprzak et al., 2023). Its application improves soil fertility by releasing nutrients through mineralization and enhancing

chemical properties such as soil organic matter (SOM), pH, EC, and cation exchange capacity (Rayne and Aula, 2020). Furthermore, FCM can improve the physical quality of degraded soils by enhancing soil aggregation and structure, reducing BD, and increasing water-retention capacity (Agbede and Oyewumi, 2022).

However, the use of FCM can have adverse ecological effects, primarily due to non-point source N pollution. Uric acid and undigested proteins, major constituents of FCM, decompose in soil, leading to N losses through ammonia (NH_3) volatilization and NO_3^- leaching (Nahm, 2003). Nitrous oxide (N_2O) emissions also increase due to enhanced nitrification and denitrification (Akiyama et al., 2023). FCM also adds unstable organic matter into the soil, increasing microbial biomass and activity (Urra et al., 2019) and accelerating the breakdown of native SOM through the priming effect (Kuzyakov et al., 2000), further promoting the formation of dissolved organic N and mineral N which are easily leachable (Quan et al., 2015; Wang and Li, 2024). Furthermore, the long-term use of FCM can lead to P accumulation in the plant-soil system, which may disrupt nutrient balance by limiting the uptake of iron and zinc (Gržinić et al., 2023). Additionally, concerns of using FCM exist due to its contamination with pathogens, antibiotics, antibiotic-resistant genes, heavy metals, pesticides, and growth hormones (Kyakuwaire et al., 2019).

In addition to nutrient release and soil conditioning, FCM also influences soil pH, which can affect both nutrient availability and N losses. While previous studies suggest that FCM can lead to soil acidification, resulting from SOM mineralization and nitrification (Rayne and Aula, 2020), this may not be the case in Santurbán-Berlín páramo. In fact, a previous study in the studied HU found the highest pH values in soils with green onion crops compared to other land covers (Patiño-Gutiérrez et al., 2024). Beyond its nutrient contribution, FCM's effectiveness in improving crop yields may be linked to its ability to raise soil pH, enhancing N and other nutrients'

bioavailability. According to Ano and Ubochi (2007), soil acidity is neutralized through microbial decarboxylation of calcium-organic matter complexes present in chicken manure, resulting in the release of Ca^{+2} that undergoes hydrolysis to produce calcium hydroxide ($Ca(OH)_2$) and hydroxyl ions (OH^-). These OH^- then react with soil hydrogen (H^+) and aluminum (Al^{+3}) ions to form water and insoluble aluminum hydroxide ($Al(OH)_3$), respectively. However, the rise in soil pH from FCM use may also increase NH_3 volatilization, as higher pH levels promote the conversion of ammonium (NH_4^+) into gaseous NH_3, potentially causing significant N losses (Sommer and Ersbøll, 1996).

Table 1 shows the composition of FCM used for fertilization in the study area, alongside values for poultry litter/manure from literature and Colombian regulatory standards for organic products used as fertilizers. Except for K, the minimum values for total organic carbon (TOC), N and P meet the required standards, reflecting the high nutrient content of locally used FCM. However, the Colombian regulation mandates that fresh organic matter undergo transformation to ensure agronomic stabilization before use (ICONTEC, 2022), a requirement not fully met. Significant variability in the chemical composition of these manures, reflected in the high CVs (≥ 20% for all elements except TOC), and the broad concentration ranges in the literature, is influenced by factors like breeding type, bedding material, animal density, and feed composition (Rogeri et al., 2016). This variability poses challenges for developing fertilization practices that match fertilizer composition with crop requirements, as local farmers purchase this input from various poultry farms across Santander Department, leading to inconsistency and uncertainty in the composition of the organic fertilizer.

Table 1. Characterization of locally used fresh chicken manure, including reference values measured in poultry litter/manure from literature, and Colombian regulatory standards

Element	Unit	This study (n = 8)				Reference values from literature[1]	Colombian regulatory standards[2]
		Average	CV	Min	Max		
TOC	g kg^{-1}	404.7	8%	371.7	467.5	128 – 292	> 150
N	g kg^{-1}	29.4	20%	21.6	39.13	13.1 – 54.6	> 10
P	g kg^{-1}	10.8	36%	7.7	18.52	6.9 – 37.5	> 4.4
K	g kg^{-1}	22.8	49%	6.28	41.78	9.8 – 37.9	> 8.3
Ca	g kg^{-1}	24.7	44%	13.59	44.9	3.0 – 41.3	–
Mg	g kg^{-1}	5.7	28%	3.70	8.62	0.59 – 6.4	–
Fe	mg kg^{-1}	2,422	80%	163.5	5,555	145 – 7,000	–
Mn	mg kg^{-1}	589.1	25%	450.2	884.1	46.7 – 624	–
Cu	mg kg^{-1}	345.8	70%	74.89	734.7	15 – 152.4	–
Zn	mg kg^{-1}	421.1	20%	326	543.2	53.5 – 516.2	–
B	mg kg^{-1}	37.4	33%	20.1	57.29	132	–
S	mg kg^{-1}	7,000	30%	3,457	10,940	160 – 5,700	–

Note. TOC: Total Organic Carbon. [1]Range of values from the review by (Gržinić et al., 2023) [2] Standards for organic products used as fertilizers (ICONTEC, 2022). CV: Coefficient of variation.

Farmers in the study area apply FCM on the soil surface at doses ranging from 15 to 48 t ha^{-1}, equivalent to 191 to 1,091 kg ha^{-1} of N per crop cycle, based on application frequency, the range of FCM bag weights (25 to 35 kg), and the average nutrient content of the manure (Table 1). Appendix C presents a histogram of N input doses, assuming an average bag weight of 30 kg. According to this distribution, 70% of respondents apply between 230 and 437 kg ha^{-1} of N per crop cycle, already elevated rates relative to crop needs (Zhao et al., 2020). However, the remaining 30% apply substantially higher doses with some exceeding 900 kg ha^{-1} of N, indicating that while excessive N application is a widespread practice, a significant portion of farmers may be contributing disproportionately to potential N surpluses. Additionally, 64% of interviewees apply manure once every two crop cycles, while 36% apply it once per crop cycle, using similar

amounts during both rainy and dry seasons. As reported in other Colombian páramos (Rodríguez-Robayo et al., 2022), manure application in the study area is largely guided by tradition rather than by soil nutrient testing or technical recommendations.

Table 2 presents the results of the partial N balance for three farms. The estimated N surplus ranged from 136 to 430 kg ha^{-1} of N. These values, even under the conservative scenario assuming 25-kg FCM bags, are remarkably high given the relatively low N removal rate by green onion (i.e., foliar N accounted for only 14 to 29% of applied N), and indicate a substantial surplus of reactive N remaining in the soil. It is also important to note that this partial balance does not consider additional N inputs such as synthetic fertilizers, N released through mineralization of native SOM, biological nitrogen fixation, or atmospheric NH_3 deposition, all of which may further exacerbate reactive N accumulation in soils. Depending on environmental conditions, this surplus is vulnerable to losses via leaching, denitrification, or surface runoff, contributing to diffuse pollution. For instance, runoff from overfertilized plots with FCM may contain N concentrations ranging from 10 to 70 mg L^{-1}, posing significant environmental risks (Gržinić et al., 2023). These findings are consistent with previous evidence of elevated nitrate concentrations in water streams in the study area (section 1.3), underscoring the urgent need to revise current fertilization practices.

In addition to FCM, 57% of respondents use synthetic fertilizers, known locally as *abonos químicos* (chemical fertilizers). Interviewed farmers commonly use 15-15-15 and 10-4-14 formulations (representing the percentages of total N, P_2O_5, and K_2O, respectively) available in local markets. Application is irregular and depends on budget, a common practice among smallholder farmers in the Andean region, who lack specific fertilization plans and often select the cheapest fertilizers based on advice from peers or store owners (Jiménez et al., 2022). All interviewees also use highly water-soluble synthetic foliar fertilizers mixed with fungicides and

insecticides. These products, known as *líquidos* (liquids), are mixed in 200 L tanks and diluted with water before being sprayed on the crop fields (Figure 5F). Farmers apply 370 to 1,600 L ha^{-1} of 5 to 6 mixed and diluted products every 8 to 12 days throughout the crop growth cycle (Appendix C). As in other rural regions of Colombia (Avellaneda-Torres et al., 2014; Gil et al., 2019), no records of the products, quantities, or application dates are kept. This lack of record-keeping hinders the adoption of sustainable agricultural practices in Colombian páramos (Rodríguez-Robayo et al., 2022).

Table 2. Partial nitrogen (N) balance for three farms in the study area

Farm ID	FCM applied (bags)	Cultivated area (ha)	N input per crop cycle[1] (kg ha^{-1})	N content in green onion dry mass (g kg^{-1})	N output via harvest (kg ha^{-1})	N surplus range (kg ha^{-1})
1	200	0.2995	191 – 268	21.1	55	136 – 213
2	150	0.1262	341 – 477	17.7	47	294 – 430
3	220	0.2667	236 – 331	12.9	34	203 – 297

Note. FCM: fresh chicken manure. [1]The minimum and maximum values were calculated assuming each bag of FCM weighed 25 and 35 kg, respectively.

Tank mixing of fertilizers and pesticides is a common agricultural practice (Kenfack Essougong et al., 2020) and has been evidenced in other Colombian páramos (Leroy, 2020). While this practice offers agricultural benefits, such as synergistic or additive effects, reduced soil compaction due to limited entry into the application area, and cost savings, it also poses several risks, including antagonistic interactions that can increase products' toxicity and reduce crop productivity (Martins Gandini et al., 2020). Safe use requires prior knowledge of the active ingredients and their compatibilities to be effective for improving soil fertility and minimize health and environmental impacts (Petter et al., 2013).

However, local farmers are generally unaware of these properties or the specific functions of the agrochemicals they use (e.g., fertilizers, fungicides, or insecticides). Instead, they categorize these products by the crop growth stage in which they are used (development and thickening). Input selection is largely influenced by recommendations from local sellers, leading to frequent changes in products and the use of a wide range of compounds and brands. In another rural area in Colombia with green onion small-scale agriculture, this lack of technical knowledge resulted in the application of up to 140 different products, including fertilizers and pesticides with varying toxicity levels, in doses up to 1,449 L ha^{-1} (Gómez Benítez et al., 2021), a practice similar to that observed in the study area. This misuse poses significant health risks and environmental pollution, and challenges efficient N management, especially when combined with the overapplication of FCM with inconsistent compositions and synthethic fertilizers applied without fertilization plans.

Additionally, 86% of interviewees use lime dissolved in water, spraying it at rates of 13 to 550 kg ha^{-1} (Figure 5H, Appendix C). Similar to other rural areas of Colombia (Gil et al., 2019), local farmers apply lime irregularly, without a dosing plan, often based on plants appearance and primarily during the rainy season when pest and disease issues are more prevalent. Although lime helps neutralize soil acidity, improving N and nutrient availability not all farmers recognize this benefit. Half of the interviewees apply lime solely for pests and diseases control, 12% to reduce acidity, and 38% for both. All farmers perform monthly manual weed removal and leave the weeds to decay in the field, based on the belief that this enriches the soil with nutrients. Additionally, 59% use herbicides, while others avoid them due to perceived negative effects on soil fertility. These practices reflect ecological knowledge relevant to sustainable N management. However, farmers showed limited recognition of the broader agroecological benefits of weed diversity, such as

supporting pollinators and biocontrol agents, and reducing weed competition (Storkey and Neve, 2018).

Local farmers use sprinkler irrigation, considering green onion a water-intensive crop (Figure 5G). Water application is based on farmers' experience and local soil classification. They neither estimate crop water requirements nor manage irrigation schedules, adjusting frequencies according to weather and perceived soil moisture. Irrigation occurs every 2 to 8 days on non-rainy days, with each plot being irrigated for 6 to 12 hours daily, using alternating sprinklers so that each subplot receives continuous irrigation for 1 to 3 hours. A previous study quantified irrigation inputs on a local green onion farm in the same HU, finding applications of 54 ± 26 mm per event for durations of 137 ± 67 min (Naranjo, 2025). Such high water inputs, applied over soils with high N surplus, increase the risk of nitrate leaching (Quemada et al., 2013). While some excess water likely results from rainfall events beyond farmers' control, especially given the high annual precipitation in the study area, much of the risk remains linked to on-farm management practices. Although 33% of farmers report water shortages during the dry season, suggesting limited leaching risk in that period, overwatering in other months remains a concern.

- Harvesting and crop residues management:

Most local practices are handled by a single farmer, typically the farm resident. However, onion harvesting involves hiring temporary local workers, mainly men (Figure 5I). Harvesting is done manually, leaving at least four *gajos (*pseudo-stems*)* to enable the next crop growth cycle. One month after harvesting, farmers perform manual hilling, a practice they consider vital for soil health, as they avoid mechanical plowing between crop cycles. Onion fields are generally cultivated for several years until productivity declines significantly, after which point farmers

either replant in existing furrows or use conventional tillage. This lack of crop rotation or fallow periods contributes to soil degradation and nutrient depletion, a challenge also observed in other high Andean systems where continuous land use without diversification has led to declining soil fertility (Fonte et al., 2012).

After harvest, onions are selected in the field to remove damaged or unmarketable plants. The selected onions are peeled and packed into 32 kg *ruedas* (bundles), typically by hired women or female family members (Figure 5J–K). Crop residues are piled near storage sheds to decompose naturally. This practice may create anaerobic hotspots that promote denitrification and N_2O emissions (Li et al., 2021). Although all farmers recognize the potential of residues as fertilizer, particularly for pastures, they avoid applying them to onion fields due to concerns about disease transmission and reduced productivity. These perceptions provide an opportunity for farmers to engage in studies on waste valorization, which could improve N management and soil fertility.

2.3.3 Local Perceptions and Indicators of Soil Fertility

This subsection discusses relevant findings related to LSK, including perceptions of soil fertility and degradation, given their relevance to the formulation of appropriate strategies to improve N management in agroecosystems in the study context. For instance, local farmers point out that it is not possible to determine soil suitability for cropping by sight alone. Instead, they believe that any soil type can be used for agriculture with the application of fertilizers. Nevertheless, they classify local soils into three categories: *tierra gredosa* (clay soil), *tierra polvosa* (powdery soil), and *tierra arenosa* (sandy soil), with the first considered low in fertility and the latter two as highly fertile (Table 3). This classification highlights the importance of texture in farmers' assessments of fertility. Farmers' classification of powdery soils reflects their

understanding of soil structure, as these soils have a fine granular structure with low development. Color is another key criterion, with farmers considering *carmelita* (brown-red) soils as ideal for green onions. Farmers primarily use color to classify soils due to its perceived relationship with plant yields or fertility, while texture is associated with plowing difficulty, soil compaction, and water-holding capacity (Huynh et al., 2020).

Local farmers have developed specific management considerations based on their soils (Table 3), including the use of lime, the quality of applied FCM, the complexity of cleaning harvested onion, and irrigation levels, with particular emphasis on managing waterlogging. This focus on waterlogging highlights irrigation practices with overapplication of water and underscores the need to prioritize water management to improve N management and move toward sustainable practices. Furthermore, local farmers are largely unaware of soil biodiversity. Except for one farmer, all interviewees identified only earthworms, grubs, and slugs, and denied the presence of bacteria in soil. Fungi were recognized by a few, but only as harmful to crops. Similar to other contexts (Pauli et al., 2012), local farmers acknowledge both the beneficial effects of earthworms on soil fertility and their harmful impact on plant roots, yet do not consider them as indicators of fertility.

Rather than reflecting nutrient availability or soil chemical properties, local definitions of fertility appear to prioritize soil workability and moisture management. This highlights an important divergence: scientific assessments focus on properties linked to nutrient cycling, while LSK emphasizes conditions affecting day-to-day crop management (Table 3). Therefore, these results underscore that LSK and scientific knowledge are complementary and integrating both can inform more culturally grounded strategies for achieving sustainable N management. For example, the prioritization of water management by farmers aligns with this study's findings on the risk of

N losses from leaching and surface runoff. Moreover, involving farmers in participatory trials tailored to their observations of soil behavior may enhance the adoption of sustainable N practices. As Habanyati et al. (2024) noted, farmers are more likely to engage in management changes when these are grounded in their lived experiences rather than prescribed externally.

Table 3. Local soil classification and management considerations for green onion cropping

Local soil name	Perceived color	Perceived fertility	Local agricultural management considerations
Tierra gredosa (clay soil)	*Café* (Brown)	Low	This soil type is the most difficult to work with due to its high clay content. Requires less irrigation as it tends to become waterlogged. Lime should not be used because it hardens easily.
Tierra polvosa (powdery soil)	*Negro* (Black)	High	This soil type is easier to work with than *tierra gredosa* but can also cause waterlogging. Lime can be used to control slugs or soil acidity. More cleaning is required during harvest as the pseudo-stems of the harvested onions come out with soil residue, which needs to be removed to meet market standards. Preferred to use organic fertilizer with a higher concentration of rice chaff and a lower concentration of manure.
Tierra arenosa (sandy soil)	*Carmelita* (Brown-red)	High	This soil type is the easiest to work with and produces the highest quality onions. Lime can be used to control slugs or soil acidity. Water drains easily and soil dries quickly, so more water is needed through irrigation. Cleaning tasks are easier because the pseudo-stems do not contain as much soil residue. Preferred to use organic fertilizer with a high concentration of manure and a low concentration of rice chaff.

Note. Terms in italics are local Spanish terminology, with translations provided in parentheses.

Finally, farmers' perceptions of soil degradation further support the need for improved N management. While 25% of the respondents believe that green onion cultivation does not deplete the soil, 75% recognized a decline in crop productivity due to intensive farming. Calcium loss was frequently cited as a sign of declining fertility (65% of respondents), and over half of the interviewees viewed agrochemical use as harmful to soil health. These insights reveal a growing

awareness of soil degradation, which can serve as a critical entry point for engaging farmers in conversations around sustainable N and soil management.

2.4 Conclusions

Green onion cultivation is deeply embedded in the local farming culture, yet farmers demonstrate openness to alternative practices that support ecosystem conservation while sustaining their livelihoods. Several existing practices already contribute to more sustainable N management, including contour planting, the use of lime to improve nutrient availability, and the incorporation of weeds as green manure. Farmers' knowledge regarding the value of crop residues, the disadvantages of excess soil moisture, and the role of physical soil properties in fertility provides opportunities to support the transition toward more sustainable systems.

However, the widespread application of fresh chicken manure with inconsistent composition, coupled with the improper use of agrochemicals, undermines sustainable N management. These practices contribute to nutrient losses that negatively impact páramo ecosystems and result in economic inefficiencies. Current irrigation practices further exacerbate these problems by increasing the risk of N leaching.

Importantly, local farmers define soil fertility primarily based on observable physical attributes, such as soil workability and water retention capacity, criteria that differ from scientific assessments focused on nutrient cycling and chemical properties. Bridging this gap through integrative approaches, such as participatory research that aligns farmers' perceptions with measured soil data, can improve fertilization practices and promote more sustainable N use.

Many of the agricultural factors influencing soil N dynamics and potential losses identified in this study are consistent with findings from other páramo regions and rural areas where green onion is cultivated on a small scale in Colombia. Therefore, the strategies proposed for improving nitrogen management in this context could be adaptable, at least in part, to other regions across the country.

3. Topsoil Nitrogen Content and their Edaphic Drivers in Páramo Soils under Green Onion Cultivation

3.1 Introduction

Anthropogenic alteration of the nitrogen (N) biogeochemical cycle is among the most severe human-driven disruptions affecting ecosystem sustainability worldwide (Fowler et al., 2013). Globally, the primary alteration involves the conversion of inert dinitrogen gas (N_2) into reactive nitrogen (Nr), i.e., all the other biologically, photochemically, and radiatively active N species found in the atmosphere and biosphere (Galloway et al., 2003). This transformation has triggered a cascade of both beneficial and adverse effects as Nr reaches different environmental compartments (Galloway et al., 2021). Among the positive impacts, food security benefits are remarkable, as almost half of the global population depends on the increased crop yields attained with N fertilizers (Erisman et al., 2008). On the other hand, the Nr not used by crops is injected into the environment, promoting soil, water, and air pollution, biodiversity losses due to soil acidification and eutrophication, threats to human health, and climate change (X. Zhang et al., 2020). Moreover, as Nr moves through multiple biogeochemical pathways, its negative impacts tend to amplify over time (Galloway et al., 2003).

Given that Nr losses contribute to the degradation of downstream water quality and compromise the sustainability of high-altitude ecosystems, it is critical to understand Nr losses in high-mountain agricultural land to develop strategies to mitigate them (W. Zhang et al., 2020). These losses are shaped by the distribution patterns and transport mechanisms of N in soil, which are spatially and temporally heterogeneous due to the combined effects of natural processes and

human activities. Multiple factors modulate soil N content and promote Nr losses in agricultural land, which include soil physicochemical properties (i.e., texture, pH, redox conditions, structure, soil organic matter (SOM) content, among others) (Cameron et al., 2013), soil water processes (Zhu et al., 2018), soil microbial diversity and activity (Myrold and Bottomley, 2015), topographic characteristics (W. Zhang et al., 2020), climatic and hydrological regimes (Liu et al., 2022; Zhao et al., 2022), and agricultural management practices (Di and Cameron, 2002; He et al., 2022). Therefore, understanding the local-scale drivers of soil N pools, transport, and transformation is essential for effective N management and evaluating associated environmental impacts.

Páramos are high-altitude ecosystems, strategic for sustainable development due to the wide range of ecosystem services they provide. Their biophysical characteristics make them a priority for conservation, as about 100 million people in the Andean region benefit from the water stored and provided by páramos ecosystems (Buytaert and Bievre, 2012). Given the central role of páramo soils in maintaining a sustained baseflow and water quality, there is a growing interest in understanding the impact of land-use change on edaphic parameters in these ecosystems. As reviewed by Patiño et al. (2021), most páramo studies to date have focused on land-use impacts on hydro-physical soil properties. In contrast, fewer studies have examined soil N pools, transformation processes, or transport dynamics in response to anthropogenic disturbances (e.g., Chacón et al., 2009; Farley and Kelly, 2004; Hofstede, 1995; Otero et al., 2011; Sarmiento and Bottner, 2002). Moreover, the limited studies on soil N in páramos have primarily addressed land uses such as pine plantations, potato (*Solanum tuberosum*) cultivation, cattle grazing, and fallow fields. The influence of green onion (*Allium fistulosum*) cropping on soil N dynamics remains largely unexplored. The lack of knowledge regarding N pools in soils under green onion cropping limits decision-making towards Colombian páramos management and conservation, as that is the

livelihood of a considerable proportion of small-scale farmers who have traditionally inhabited these ecosystems (Sarmiento et al., 2017).

Identifying the specific factors that regulate soil N species in páramo ecosystems is therefore critical. Such efforts would allow researchers to prioritize the most influential variables, optimize resource allocation, and inform the development of robust predictive models. These models, in turn, are valuable tools for evaluating the effects of agricultural practices on ecosystem functioning and promoting sustainable land management in páramo landscapes. Therefore, this chapter analyzes the effect of green onion cultivation on topsoil concentrations of total nitrogen (TN), ammonium-nitrogen ($N\text{-}NH_4^+$), and nitrate-nitrogen ($N\text{-}NO_3^-$), as well as the edaphic properties that influence these N pools in the Santurbán-Berlín páramo complex. The novelty of this research lies in addressing an anthropogenic activity with significant development within a unique ecosystem. It also addresses the scarcity of information on both the environmental impacts of agriculture and the specific biophysical context of this Colombian páramo complex. As Nr use is expected to increase in Latin America (Galloway et al., 2021), the findings of this study can serve as a baseline for future research on soil N dynamics in páramo ecosystems, ultimately contributing to efforts to mitigate Nr losses and promote sustainable N management.

3.2 Methods

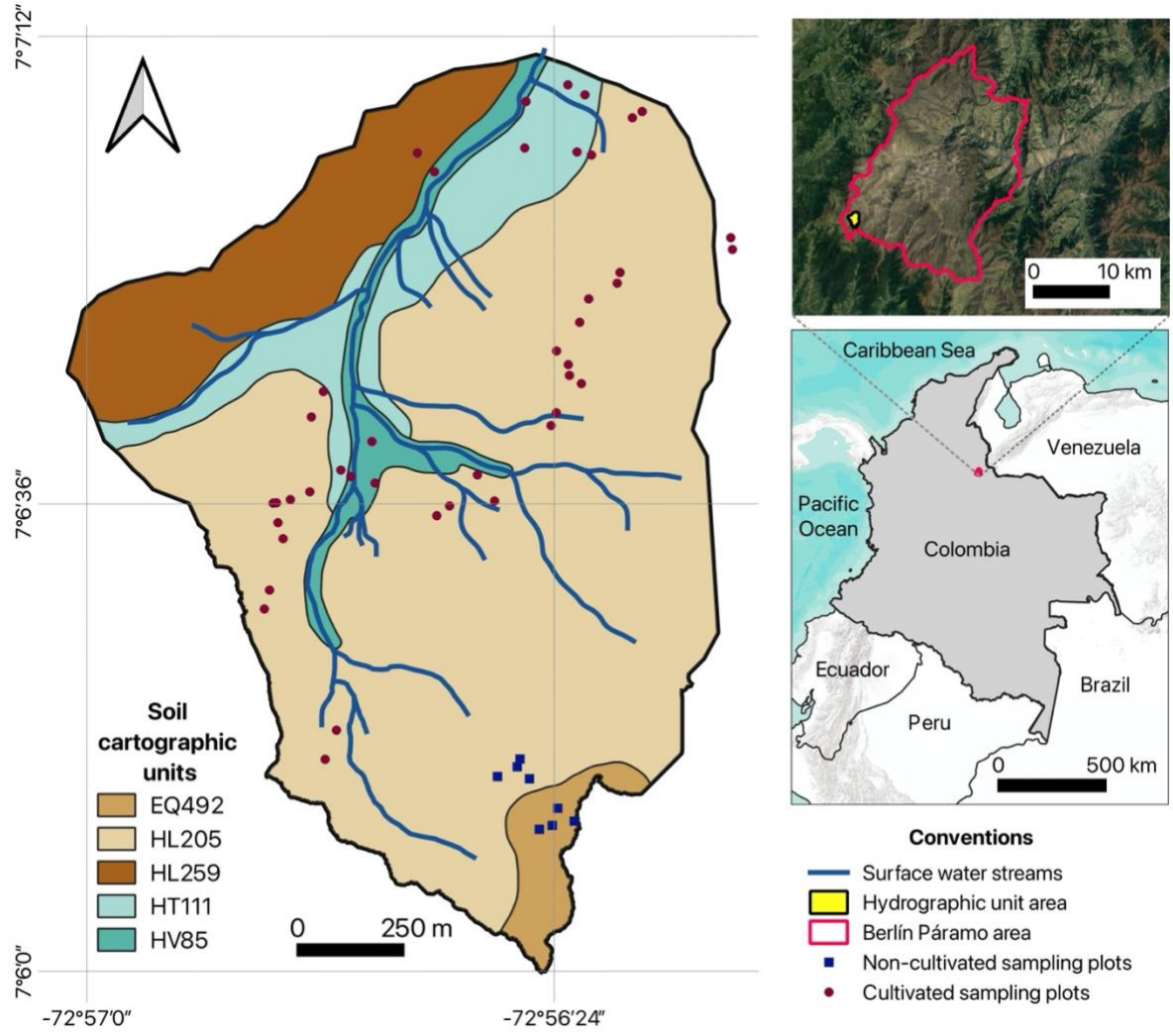
This section describes the activities conducted for the initial soil assessment within the study hydrographic unit (HU), the analytical methods used to determine soil properties, and the data analysis approach.

3.2.1 Soil Sampling

The initial soil sampling campaign for the first methodological phase of this thesis was conducted during the dry season, from December 2021 to February 2022. A total of 50 plots (~100 m^2 each) were sampled, including 42 cultivated plots and eight non-cultivated plots. The location of the sampling plots within the study HU is shown in Figure 6. Plot size was defined to ensure homogeneity in land cover and agricultural management conditions within each farm. Cultivated plots were distributed across 21 farms (two plots per farm), and all associated farmers had previously participated in the characterization of agricultural practices presented in Chapter II. Non-cultivated plots were established on native grassland dominated by plant species such as *Arcytophyllum nitidum*, *Sporobolus lasiophyllus*, *Agrostis mertensii*, *Calamagrostis effusa*, *Cortaderia nitida*, *Diplostephium spp*., and *Lycopodium clavatum* (Löwer, 2020). Soil textural class for each plot was determined using the texture-by-feel method (Thien, 1979). The soils were predominantly coarse-textured, with the following classifications: loamy sand (n = 21), loam (n = 13), sandy loam (n = 13), and clay loam (n = 3).

As detailed in Chapter II, fresh chicken manure (FCM) is used as the main nutrient source across all farms, applied at rates ranging from 15 to 48 t ha^{-1}, equivalent to 191 to 1,091 kg ha^{-1} of N per crop cycle. FCM application frequency varied from every three to six months, depending on farmer preference. Therefore, the period between the last application of FCM and soil sampling varied across plots, ranging from one week to six months. This variability provided a broad representation of soil N content under local fertilization practices. In addition, foliar NPK fertilizers were consistently applied every eight to 12 days during the crop growth cycle. These were mixed with conventional pesticides and diluted in water, with application volumes ranging from 370 to 1,600 L ha^{-1}.

Figure 6. Location of the sampling plots for the initial soil assessment



Note. Soil cartographic units were defined according to IGAC (2015). EQ492: Andic Humicryepts with inclusions of Typic Humicryepts and Lithic Melanocryands; HL205: Entic Humudepts with inclusions of Acrudoxic Melanudand; HL259: Typic Humudepts with inclusions of Pachic Humudepts and Fluventic Humudepts; HT111: Typic Dystrudepts with inclusions of Typic Humudepts; HV85: Complex of Aquíc Humudepts, Entic Humudepts and Fluventic Humudepts.

To assess bulk density (BD) and soil water content (SWC), duplicate undisturbed samples were randomly collected from each plot at two depths (0–5 cm and 10–15 cm) using a soil core sampler and 100 cm^3 metallic cylinders. Paired sample t-tests were performed to evaluate differences between depths. Since no statistically significant differences were observed (Appendix D), the average values from both depths were used to represent topsoil BD and SWC in subsequent analyses. For the analysis of other edaphic properties, 20 disturbed subsamples were collected from the top 20 cm of each plot using a soil auger, following a zigzag transect. These subsamples were homogenized to form a composite sample (1 kg) per plot. The 0–20 cm depth was selected in this methodological phase to capture the effective rooting zone relevant to the study crops.

A portable GPS receiver (3.65 m precision) was used to georeference undisturbed sampling points and delineate plot boundaries. The resulting coordinates were processed into shapefile format using DNRGPS 6.1.0.6 software developed by the Minnesota Department of Natural Resources (https://gisdata.mn.gov/es/dataset/dnrgps). Mean slope for each plot was estimated in QGIS (QGIS Development Team, 2021) using the field-delineated polygons and a digital elevation model (DEM) with a 12.5 m resolution, acquired from an ALOS PALSAR satellite image dated May 10, 2010 (https://search.asf.alaska.edu). Based on this analysis, the mean slope of cultivated plots ranged from 3.0% to 21.0%, while non-cultivated plots ranged from 3.9% to 40.7%.

3.2.2 Laboratory Analysis

Soil samples were air-dried, passed through a 2-mm sieve, and analyzed for TN, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, soil organic carbon (SOC), pH, electrical conductivity (EC), BD, SWC, P, available sulfur (S), exchangeable cations, i.e., sodium (Na^+), potassium (K^+), calcium (Ca^{+2}), magnesium (Mg^{+2}), and aluminum (Al^{+3}), effective cation exchange capacity (ECEC), and textural class. Soil TN, N-

NH_4^+, and $N\text{-}NO_3^-$ were analyzed with a San++ automated wet chemistry analyzer from Skalar Analytical B.V. For TN measurement, soil samples were digested with sulfuric and salicylic acids (Bremner, 1996), while for $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$, measurement, samples were extracted with 1 M potassium chloride (Kachurina et al., 2000). P was analyzed using the Bray II method (Bray and Kurtz, 1945). S was determined by the extraction of soil samples with monocalcium phosphate (Hoeft et al., 1973), precipitation with barium chloride, and measurement with atomic absorption spectrophotometry.

SOC was determined with the Walkey-Black method (Nelson and Sommers, 1996). Soil pH and EC were measured with sensors in 1:1 and 1:5 soil-water mixture, respectively. BD was estimated with the core method (Blake and Hartge, 1986a). SWC was determined by gravimetry, drying the samples in an air oven at 105°C for 24 h, and measuring the soil mass before and after drying. Exchangeable cations were determined by atomic absorption spectrophotometry. For soil pH > 5.5, samples were extracted with ammonium acetate for determination of Ca^{+2}, Mg^{+2}, K^+, and Na^+, while for soil pH < 5.5, samples were extracted with potassium chloride for determination of Mg^{+2}, Ca^{+2}, and Al^{+3} (Shuman and Duncan, 1990); and with hydrochloric acid and ammonium fluoride for determination of K^+ (Zebec et al., 2017). ECEC was calculated as the sum of exchangeable cations.

It is important to mention that although there are potential effects of air-drying samples on the measurement of soil inorganic N and other edaphic properties (Erich and Hoskins, 2011; Kaiser et al., 2015), it was necessary to use this sample preservation method due to logistical constraints that led to delays in analysis. Further, all samples were treated consistently under the same conditions, ensuring that any changes due to air drying affected all samples uniformly. Additionally, among various soil sample preservation methods, air drying is known to present the

least differences with respect to fresh samples, thereby minimizing potential alterations in the inorganic N measurements compared to other preservation protocols (Ma et al., 2005).

3.2.3 Data Analysis

Descriptive statistics were applied to characterize the edaphic properties of cultivated and non-cultivated soils. Principal component analysis (PCA) (Jolliffe, 2002) was used to identify patterns and relationships among variables and samples. The Shapiro–Wilk test and the Bartlett test were used to assess data normality and homogeneity of variances, respectively (Appendix E). Statistical comparisons were conducted to identify differences in soil N pools and other edaphic properties between cultivated and non-cultivated soils. For normally distributed variables (i.e., BD and EC), Welch's t-test was used, as it does not assume equal variances and is robust to unequal sample sizes between groups. Non-parametric Mann–Whitney U tests were applied to variables that did not meet parametric assumptions. Both Welch's t-test and the Mann–Whitney U test are widely recognized as robust approaches under conditions of variance heterogeneity and unequal sample sizes (Delacre et al., 2017; Nachar, 2008). The statistical analyses were performed in R (R Core Team, 2024) with a significance level of 0.05. To further evaluate the effects of green onion cropping on soil N pools, the results were compared with data from 25 studies conducted in páramo ecosystems, which examined the impacts of land-use change on physicochemical topsoil properties related to soil N dynamics.

The relationship between the edaphic variables was explored using the Spearman correlation analysis. Redundancy analysis (RDA) (Legendre and Legendre, 1998) was used to identify which edaphic properties could be used as explanatory variables for soil TN, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ concentrations. This unsupervised multivariate approach allowed examination of

significant relationships among a response dataset (e.g., soil N pools) and independent variables (e.g., edaphic properties). Further, stepwise forward selection was conducted to identify the subset of predictors that explained the most variance in the response dataset. The RDA and forward selection were performed with the package 'vegan' in R (Oksanen et al., 2023), using the 'rda()'and the 'ordiR2step()' functions, and their significance was determined with a permutation test using the 'anova.cca()' function.

3.3 Results

3.3.1 Descriptive Statistics, Exploratory Data Analysis, and Statistical Comparisons

Descriptive statistics for the measured variables and the results of statistical comparisons between cultivated and non-cultivated soils are provided in Table 4. Significant differences were found between cultivated and non-cultivated soils for most edaphic properties ($p < 0.05$), except for $N\text{-}NH_4^+$ ($p = 0.576$), BD ($p = 0.158$), and SWC ($p = 0.244$). Mean TN values ranged from 3.83 ± 0.95 to 6.34 ± 1.98 g kg^{-1}, with a noticeable decrease in soils with green onion crops. $N\text{-}NO_3^-$ also remarkably changed from 8.29 ± 13.20 to 75.22 ± 34.01 mg kg^{-1}, with the highest values observed in cultivated soils. This N form presented high variability in data, with a CV of 45.2% for cultivated soils and 159.2% for non-cultivated soils. In contrast, $N\text{-}NH_4^+$ content exhibited minimal variation, ranging from 32.80 ± 10.57 to 34.66 ± 21.51 mg kg^{-1}, with an average decrease of only 5% observed in cultivated soils.

The three first principal components (PC) from the PCA accounted for 82.7% of the variance in the data. These PCs were extracted for exploratory data analysis based on their proportion of explained variance and their adherence to the Kaiser-Guttman criterion (i.e., eigenvalue greater than one) (Zwick and Velicer, 1986).

Table 4. Descriptive statistics and statistical comparison results for the edaphic properties in the initial soil assessment

Variable	Unit	Cultivated soils (n = 42)					Non-cultivated soils (n = 8)					p-value
		Mean	SD	CV (%)	Min	Max	Mean	SD	CV (%)	Min	Max	
TN	g kg^{-1}	3.83	0.95	24.8	1.99	5.62	6.34	1.98	31.2	4.45	10.03	<0.001
$N\text{-}NH_4^+$	mg kg^{-1}	32.80	10.57	32.2	15.23	56.24	34.66	21.51	62.1	15.82	83.67	0.576
$N\text{-}NO_3^-$	mg kg^{-1}	75.22	34.01	45.2	19.64	161.00	8.29	13.20	159.2	1.03	37.82	<0.001
pH	-	5.66	0.56	9.9	4.61	6.63	4.67	0.17	3.6	4.39	4.86	<0.001
SOC	%	6.35	1.67	26.3	3.64	9.20	10.75	1.89	17.6	7.43	12.96	<0.001
Ca^{+2}	cmol(+) kg^{-1}	12.85	7.02	54.6	2.16	35.08	0.45	0.14	31.1	0.29	0.69	<0.001
Mg^{+2}	cmol(+) kg^{-1}	1.90	0.92	48.4	0.38	3.96	0.29	0.06	20.7	0.22	0.39	<0.001
K^+	cmol(+) kg^{-1}	2.34	1.02	43.6	0.74	4.67	0.27	0.04	14.8	0.22	0.32	<0.001
Al^{+3}	cmol(+) kg^{-1}	0.46	0.74	160.9	0.00	2.94	4.42	0.65	14.7	3.70	5.71	<0.001
Na^+	cmol(+) kg^{-1}	0.12	0.11	91.7	0.00	0.39	0.00	0.00	-	0.00	0.00	0.003
ECEC	cmol(+) kg^{-1}	17.67	8.07	45.7	6.98	41.24	5.43	0.80	14.7	4.54	7.06	<0.001
P	mg kg^{-1}	720.90	642.97	89.2	26.52	3052.50	19.92	20.55	103.2	3.93	48.79	<0.001
S	mg kg^{-1}	107.54	39.20	36.5	56.92	206.30	22.72	2.64	11.6	20.84	28.65	<0.001
SWC	%	41	13	31.7	17	68	56	26	46.4	33	112	0.244
BD	g cm^{-3}	0.66	0.11	16.7	0.47	0.87	0.60	0.13	21.7	0.46	0.87	0.158
EC	µS cm^{-1}	393.38	136.47	34.7	171.00	841.00	155.50	102.98	66.2	42.00	330.00	<0.001

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, Ca^{+2}: exchangeable calcium, Mg^{+2}: exchangeable magnesium, K^+: exchangeable potassium, Al^{+3}: exchangeable aluminum, Na^+: exchangeable sodium, ECEC: effective cation exchange capacity, P: available phosphorus, S: available sulfur, EC: electrical conductivity, SD: standard deviation, CV: coefficient of variation, n: number of sampled plots. p-values indicate the results from the statistical comparisons between cultivated and non-cultivated soils using two-sample T-tests, Welch's T-test or Mann-Whitney U test.

Table 5 displays the Pearson correlation coefficients between the original edaphic properties and the scores of the first three PCs. PC1, which accounted for 47.2% of the variance in the data, exhibited a strong and negative correlation with Al^{+3} and a weaker negative correlation with SOC, whereas it showed a strong and positive correlation with pH, exchangeable cations (excluding Al^{+3}), ECEC, P, N-NO_3^-, and EC. These relationships reflect two gradients accentuated by green onion cropping in a páramo agroecosystem: one associated with increasing soil fertility and the other with soil organic matter (SOM) loss. PC2, explaining 22.3% of the variance in the data, evidenced a strong positive correlation with BD and a strong negative correlation with TN, SWC, and SOC. PC3, accounting for 13.2% of the variance, was primarily negatively correlated with S and N-NO_3^-.

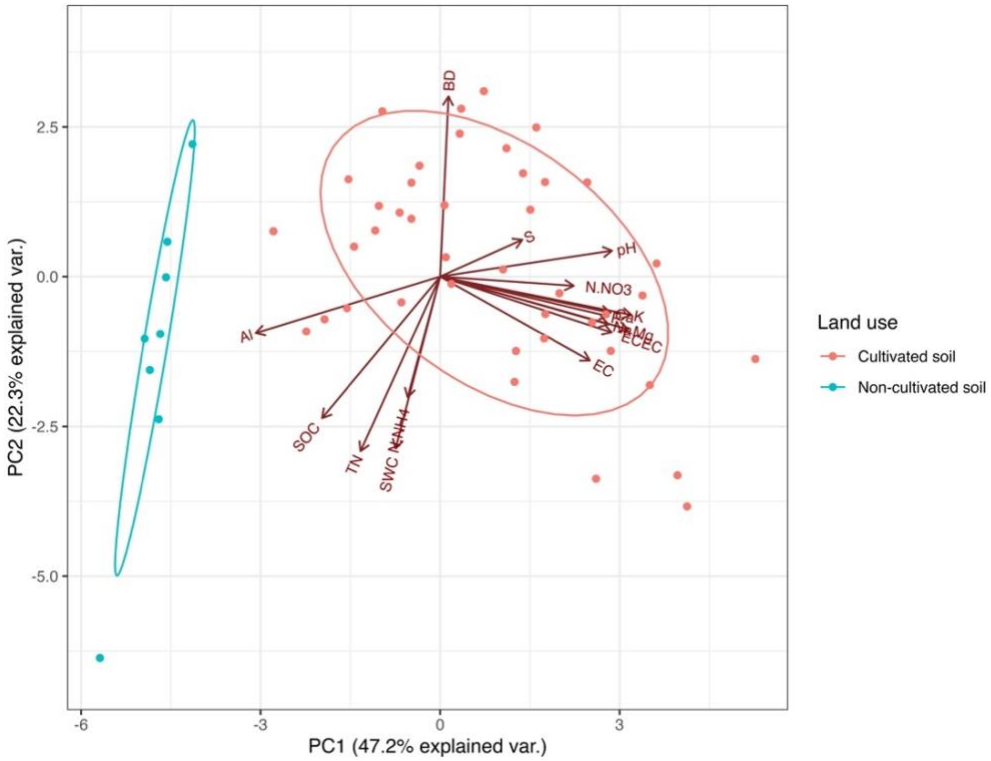
Table 5. Pearson correlation coefficients (r) between the data of the original variables and the scores of the first three principal components

Variable	Principal component 1	Principal component 2	Principal component 3
TN	-0.385**	-0.845**	0.132
N-NH_4^+	-0.157	-0.584**	-0.497**
N-NO_3^-	0.648**	-0.045	-0.673**
pH	0.833**	0.125	0.422**
SOC	-0.572**	-0.685**	0.200
Ca^{+2}	0.826**	-0.175	0.285*
Mg^{+2}	0.911**	-0.255	0.185
K^+	0.928**	-0.185	0.038
Al^{+3}	-0.893**	-0.273	0.045
Na^+	0.817**	-0.231	0.169
ECEC	0.832**	-0.270	0.298*
P	0.817**	-0.192	0.080
S	0.398**	0.178	-0.833**
SWC	-0.215	-0.832**	0.014
BD	0.040	0.872**	0.104
EC	0.726**	-0.406**	-0.482**

Note. TN: total nitrogen, N-NH_4^+: ammonium-nitrogen, N-NO_3^-: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, Ca^{+2}: exchangeable calcium, Mg^{+2}: exchangeable magnesium,

K^+: exchangeable potassium, Al^{+3}: exchangeable aluminum, Na^+: exchangeable sodium, ECEC: effective cation exchange capacity, P: available phosphorus, S: available sulfur, EC: electrical conductivity. Significant correlations are indicated * $p < 0.05$; ** $p < 0.01$.

Figure 7. Principal component analysis (PCA) biplot representing measured edaphic variables and observations across the hydrographic unit



Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, Ca^{+2}: exchangeable calcium, Mg^{+2}: exchangeable magnesium, K^+: exchangeable potassium, Al^{+3}: exchangeable aluminum, Na^+: exchangeable sodium, ECEC: effective cation exchange capacity, P: available phosphorus, S: available sulfur, EC: electrical conductivity. Correlations between the data for the original variables and the PCA scores are represented by vectors. The ellipses were drawn around centroids from 95% confidence intervals.

3.3.2 Relationships Among Edaphic Variables and Topsoil Nitrogen Concentration

Table 6 shows the Spearman correlation coefficients among the studied variables. These results showed that TN had a significant and very strong positive correlation with SOC ($\rho = 0.871$), a strong negative correlation with BD ($\rho = -0.739$), and a weaker positive correlation with SWC ($\rho = 0.581$). For $N\text{-}NO_3^-$, positive and significant correlations with some edaphic properties were also found. The concentration for this N form was very strongly correlated with EC ($\rho = 0.827$), strongly correlated with S ($\rho = 0.754$) and K^+ ($\rho = 0.630$), and weakly correlated with P ($\rho = 0.524$). The statistical analysis also showed significant correlations between $N\text{-}NH_4^+$ and some edaphic properties. However, these correlations were moderate, with coefficients with the highest absolute values found for S ($\rho = 0.400$) and BD ($\rho = -0.424$). On the other hand, the RDA evidenced that the constrained variance (i.e., the proportion of the variance in TN, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ concentrations explained by the other edaphic properties) was 71.91%. The permutation test revealed that the RDA was significant, with an F-statistic of 11.454 and a p-value of 0.001.

Table 7 presents the results of the RDA with the forward selection process used to identify the most influential edaphic properties driving the variation in soil N pools. Variables were added iteratively to the model based on their contribution to explained variance, as assessed through permutation tests and the Akaike Information Criterion (AIC). For each step, Table 7 shows the included variables, the AIC, the F-statistic, the p-value indicating statistical significance, and the cumulative proportion of variance explained. This analysis showed that SOC, EC, K^+, S, and SWC explained 70.83% of the variance in the response variables. These relationships are consistent with the Spearman correlation analysis, which reflected the strong relationship among those explanatory variables and soil N concentrations.

Table 6. Spearman correlations coefficients (ρ) among measured variables in the hydrographic unit for the initial soil assessment

	$N\text{-}NH_4^+$	**$N\text{-}NO_3^-$**	**pH**	**SOC**	**Ca^{+2}**	**Mg^{+2}**	**K^+**	**Al^{+3}**	**Na^+**	**ECEC**	**P**	**S**	**SWC**	**BD**	**EC**
TN	0.265	-0.189	-0.299*	0.871**	0.006	-0.011	-0.069	0.299*	0.039	0.018	-0.123	-0.229	0.581**	-0.739**	0.110
	$N\text{-}NH_4^+$	0.151	-0.221	0.210	-0.084	-0.004	-0.053	0.141	-0.086	-0.072	-0.005	0.400**	0.227	-0.424**	0.392**
		$N\text{-}NO_3^-$	0.233	-0.364**	0.454**	0.455**	0.630**	-0.369**	0.352*	0.491**	0.524**	0.754**	-0.024	-0.115	0.827**
			pH	-0.450**	0.809**	0.824**	0.746**	-0.906**	0.757**	0.796**	0.808**	-0.046	-0.179	0.223	0.304*
				SOC	-0.242	-0.279*	-0.319*	0.486**	-0.233	-0.239	-0.416**	-0.273	0.493**	-0.616**	-0.136
					Ca^{+2}	0.872**	0.873**	-0.789**	0.711**	0.992**	0.878**	0.133	0.144	-0.099	0.562**
						Mg^{+2}	0.884**	-0.891**	0.905**	0.895**	0.892**	0.166	0.090	-0.086	0.601**
							K^+	-0.790**	0.804**	0.902**	0.887**	0.266	0.089	-0.064	0.706**
								Al^{+3}	-0.855**	-0.791**	-0.836**	-0.144	0.166	-0.181	-0.427**
									Na^+	0.740**	0.808**	0.051	0.065	-0.066	0.502**
										ECEC	0.894**	0.149	0.166	-0.116	0.604**
											P	0.190	-0.012	0.011	0.653**
												S	-0.148	0.002	0.635**
													SWC	-0.639**	0.177
														BD	-0.326*

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, Ca^{+2}: exchangeable calcium, Mg^{+2}: exchangeable magnesium, K^+: exchangeable potassium, Al^{+3}: exchangeable aluminum, Na^+: exchangeable sodium, ECEC: effective cation exchange capacity, P: available phosphorus, S: available sulfur, EC: electrical conductivity. Significant correlations are indicated * $p < 0.05$; ** $p < 0.01$.

Table 7. Results of the forward stepwise selection of variables that explain variation in soil N concentrations.

Step of forward selection	Included variables	AIC	F-statistic	p-value	R^2
1	SOC	36.268	26.014	0.002	0.3379
2	SOC, EC	13.538	30.073	0.002	0.5877
3	SOC, EC, K^+	7.501	8.0213	0.004	0.6413
4	SOC, EC, K^+, S	1.695	7.6037	0.002	0.6863
5	SOC, EC, K^+, S, SWC	- 1.069	4.3978	0.016	0.7083

Note. S: available sulfur; SOC: soil organic carbon; EC: electrical conductivity; K^+: exchangeable potassium; SWC: soil water content; AIC: Akaike Information Criterion; R^2: adjusted R^2, representing the cumulative variance explained across total nitrogen (TN), ammonium-nitrogen ($N\text{-}NH_4^+$), and nitrate-nitrogen ($N\text{-}NO_3^-$) by the redundancy analysis model when the variable is included.

3.4 Discussion

This section is organized into two parts, corresponding to the objectives addressed in this chapter. First, the influence of green onion cultivation on topsoil N concentrations is examined. Then, the key edaphic properties driving variation in soil N pools are analyzed.

3.4.1 Influence of Green Onion Cropping on Topsoil Nitrogen Concentration

Previous studies in páramo ecosystems have shown that soil N contents and other edaphic properties related to them vary both within a single land-cover type and among different land-covers (see Table 8). In this study, a 40% average decrease in topsoil TN was observed for soils under green onion crops compared to native páramo grassland. As shown in Table 8, the mean value for TN in soils with green onion crops (3.83 g kg^{-1}) was almost at the lower limit reported

in other studies for soils with potato crops (i.e., TN ranged from 3.80 to 11.0 g kg^{-1}). Given that organic N is the most abundant N pool in soil (Xin et al., 2019), the decrease of TN evidenced in this study may be linked to the application of FCM. As discussed in Chapter II, this management practice enhances soil microbial biomass and activity (Urra et al., 2019), accelerating the breakdown of native SOM through the priming effect (Kuzyakov et al., 2000). Further, it promotes the depolymerization of soil recalcitrant organic N-containing compounds, forming dissolved organic nitrogen (DON) which can subsequently be transformed into inorganic N and leached through the soil profile (Wang and Li, 2024).

Furthermore, while FCM supplies organic N to the soil, its instability results in a high proportion of easily leachable DON (Quan et al., 2015), leading to further N losses and explaining part of the reduction of TN in cultivated plots. Moreover, the decline in soil organic N, and consequently TN, is exacerbated by SOM losses due to local agricultural practices such as plowing for soil preparation. These practice favors the direct exposition of soils to environmental factors, enhancing oxidation of SOM and its release to the atmosphere (Peña-Quemba et al., 2016). The SOM loss is reflected not only in the decreased TN concentration, but also in a reduction of SOC, from 10.75 ± 1.89% in non-cultivated soils to 6.35 ± 1.67% in cultivated soils. These changes in SOM directly affect C and N dynamics, compromising ecosystem services associated with their stocks in soils. These findings underscore the need for comprehensive assessments of soil nutrient alterations resulting from green onion cropping in the studied páramo.

A similar pattern of soil TN reduction was observed by Farley and Kelly (2004), who determined that afforestation with pine in páramo grasslands had large effects on soil TN with a tendency to deplete its content at intermediate depth. A significant TN depletion was also reported by Benavides et al. (2018) for páramo soils with anthropic uses (i.e., managed shrubs, cattle

grazing, and potato cropping) compared to undisturbed soils. In contrast, Hofstede (1995) found that TN did not differ significantly among grazed, burned, and undisturbed soils in a páramo ecosystem. Sarmiento and Bottner (2002) also concluded that TN was not altered by land-use, comparing páramo soils with fallow and potato crops. Avellaneda-Torres et al. (2018) reported the same behavior for TN, which did not change significantly among páramo soils with potato crops, cattle grazing, and native vegetation. The diverseness in those results for soil TN concentration indicates that agricultural management is a key driver of N pools, underscoring the importance of conducting a deeper evaluation of soil N contents in páramo soils under green onion cultivation.

On the other hand, long-term green onion cropping led to a 5% average decrease in N-NH_4^+, and an increase of almost 8 times in N-NO_3^- concentration. The contents of available N species found in this study contrast findings in other páramo soils. High values of soil N-NH_4^+ concentrations, relative to those reported in other studies for soils with natural and anthropogenic uses (Table 8), were observed in both cultivated and non-cultivated soils within the study HU (Table 8). The concentrations of N-NH_4^+ measured in this study were only lower than those reported by recent studies in undisturbed soils from Southern Ecuador. For instance, Carrión-Paladines et al. (2023) found high contents of N-NH_4^+ (70 to 140 mg kg^{-1}) in both burned and unburned grasslands, while Delgado-Fernández et al. (2024) found even higher levels (112 to 212 mg kg^{-1}) in soils under native páramo vegetation.

Table 8. Mean values for N dynamics-related properties for the surface horizons of páramo soils with various land-cover or land-use

Variable	Unit	Data from this study		Data from literature								
		Cultivated land (mean ± SD)	Non-cultivated land (mean ± SD)	Native páramo vegetation[1-19, 24,25]	Native Andean highlands forests*[3,20,21]	Pastures for livestock[1,5,10, 14-18,21,22]	Fallow[2,4,8-10,12,21,23,24]	Recently ploughed soil after a fallow period[4,8]	Pine plantations[3,6,7,16,20]	Burned areas[1,11,19]	Potato crops[4,8-10,14,15,17,18,22-24]	Green onion crops[24]
TN	g kg^{-1}	3.83 ± 0.95	6.34 ± 1.98	2.50 – 12.0	4.70	1.30 – 10.80	0.90 – 8.00	4.50	1.80 – 9.50	3.50 – 11.80	3.80 – 11.0	–
N-NH_4^+	mg kg^{-1}	32.80 ± 10.57	34.66 ± 21.51	1.08 – 212	21.14	10.29	0.39 – 2.85	–	0.94 – 29.40	75 – 140	1.33 – 11.78	–
N-NO_3^-	mg kg^{-1}	75.22 ± 34.01	8.29 ± 13.20	0.07 – 4.28	1.93	3.59	0.18 – 0.71	–	1.78 – 9.13	–	1.01 – 4.07	–
SWC	%	41 ± 13	56 ± 26	11 – 140	–	11.1 – 92.1	75	–	11 – 18	130 – 170	40 – 70	–
BD	g cm^{-3}	0.66 ± 0.11	0.60 ± 0.13	0.11 – 1.17	0.46 – 0.57	0.41 – 1.12	0.45 – 1.03	–	0.32 – 1.25	0.30 – 0.81	0.65 – 0.90	0.69 - 0.74
pH (H_2O)		5.66 ± 0.56	4.67 ± 0.17	3.60 – 5.70	4.70 – 5.40	4.70 – 5.50	4.27 – 6.35	4.82	4.73 – 5.20	4.30 – 5.37	4.30 – 5.30	5.8 – 6.1
SOC	%	6.35 ± 1.67	10.75 ± 1.89	6.82 – 25.10	2.80	4.00 – 17.00	2.90	–	–	4.96 – 12.16	6.00 – 11.00	–
Ca^{+2}	cmol(+) kg^{-1}	12.85 ± 7.02	0.45 ± 0.14	0.70 – 2.50	2.10	3.50 – 22.90	1.20 – 27.34	7.52	0.40	0.72 – 3.60	4.10 – 15.52	–
Mg^{+2}	cmol(+) kg^{-1}	1.90 ± 0.92	0.29 ± 0.06	0.20 – 4.96	3.50	0.65 – 6.41	0.31 – 4.16	0.62	0.70	0.23 – 1.10	0.20 – 1.06	–
K^+	cmol(+) kg^{-1}	2.34 ± 1.02	0.27 ± 0.04	0.18 – 1.54	0.68	0.17 – 0.45	0.16 – 1.03	0.12	0.32	0.16 – 0.60	0.20 – 1.15	–
Al^{+3}	cmol(+) kg^{-1}	0.46 ± 0.74	4.42 ± 0.65	0.06 – 11.58	1.90	0.01 – 1.65	9.60 – 11.70	12.33	3.00	1.10 – 1.67	1.10 – 11.34	–
Na^+	cmol(+) kg^{-1}	0.12 ± 0.11	0 ± 0	0.02 – 0.38	–	0.05 – 0.65	0.04 – 0.08	0.04	–	–	0.03 – 0.30	–
ECEC	cmol(+) kg^{-1}	17.78 ± 8.03	5.43 ± 0.80	3.27 – 18.71	7.90	–	–	–	4.50	2.90 – 6.50	–	–
P	mg kg^{-1}	720.90 ± 642.97	19.92 ± 20.55	3.30 – 43.0	1.90 – 11.80	10.0 – 20.0	10.2 – 39.2	12.90	1.0 – 43.9	1.89 – 47.0	26.9 – 130	–
S	mg kg^{-1}	107.54 ± 39.20	22.72 ± 2.64	6.60 – 10.20	–	–	–	–	–	–	–	–
EC	mS cm^{-1}	393 ± 137	155.5 ± 103	45 - 297	–	–	62 - 171	–	–	–	167 - 296	246 - 406

Note. Data extracted from [1](Hofstede, 1995), [2](Llambí and Samiento, 1998), [3](Hofstede et al., 2002), [4](Abadín et al., 2002), [5](Podwojewski et al., 2002), [6](Farley et al., 2004), [7](Farley and Kelly, 2004), [8](Cabaneiro et al., 2008), [9](Abreu et al., 2009), [10](Estupiñán et al., 2009), [11](Camargo-García et al., 2012), [12](Sarmiento et al., 2015), [13](Minaya et al., 2016), [14](Avellaneda-Torres et al., 2018), [15](Benavides et al., 2018), [16](Marín et al., 2018), [17](Farfán et al., 2020), [18](Avellaneda-Torres et al., 2022), [19](Carrión-Paladines et al., 2023), [20](Chacón et al., 2009), [21](Andrade Castañeda et al., 2014), [22](Otero et al., 2011), [23](Sarmiento and Bottner, 2002), [24](Patiño-Gutiérrez et al., 2024), [25](Delgado-Fernández et al., 2024). TN: total nitrogen, N-NH4+: ammonium-nitrogen, N-NO3-: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, Ca+2: exchangeable calcium, Mg+2: exchangeable magnesium, K+: exchangeable potassium, Al+3: exchangeable aluminum, Na+: exchangeable sodium, ECEC: effective cation exchange capacity, P: available phosphorus, S: available sulfur, EC: electrical conductivity. SD: Standard deviation.

High levels of assimilable N (particularly $N\text{-}NH_4^+$) originate from the decomposition of SOM, which is abundant in páramo ecosystems. However, in volcanic páramo soils, such as those included in most studies summarized in Table 8, the clay fraction is often dominated by allophane and imogolite. These minerals strongly adsorb organic matter, thereby protecting it from microbial degradation and slowing the mineralization of organic N to inorganic forms (Parfitt, 2009), a process less likely to occur in the non-allophanic soils of the present study. Additionally, clay minerals with permanent charge, such as vermiculites, can bind ammonium in a way that prevents its easy replacement by other cations (Nieder et al., 2011). The presence of vermiculite among the main components of the weathered rock profile in the study area, as described in Section 1.3 of Chapter I, may partially explain the elevated ammonium concentrations observed in the native soils. Therefore, the high $N\text{-}NH_4^+$ concentrations in non-cultivated plots likely reflect both the geological origin and mineralogical composition of the soils, which differ from those in páramos soils from volcanic origin. Moreover, these elevated ammonium levels may also be associated with a high potential for biological nitrogen fixation by the native microbiota, as discussed later in Chapter V.

Regarding $N\text{-}NO_3^-$ concentrations, interestingly, the soils with green onion crops largely surpassed the maximum values measured for other páramo soils with anthropic land-uses (Table 8). A remarkable difference was observed between $N\text{-}NO_3^-$ in soils with green onion crops (75.22 ± 34.01 mg kg^{-1}) and values reported in the literature for soils under potato cultivation (i.e., $N\text{-}NO_3^-$ levels ranged from 1.01 to 4.07 mg kg^{-1}). Consequently, it is evident that green onion cropping in páramos leads to a significant shift in soil N pools, increasing nitrate content, which could promote Nr losses by erosion, runoff, or leaching. This finding aligns with a previous study in the area, which identified high nitrate levels in surface water attributed to land-use changes from

native grasslands to green onion cultivation (Rey-Romero et al., 2022a). These results suggest that elevated nitrate concentrations may also extend to deeper soil layers and groundwater in cultivated areas, underscoring the need for deepen the assessment of nitrate distribution and transport and their implications for páramo ecosystems.

Páramo soils with native vegetation are characterized by high N stocks that are largely unavailable to plants (Hofstede, 1995). This behavior can be attributed to low mineralization and nitrification rates caused by low temperatures and high levels of Al^{+3} that inhibit the microorganisms involved in these processes (Zhao and Shen, 2018). In this study, the enhancement of nitrification, reflected in noticeable increase in $N\text{-}NO_3^-$ content, appears to be linked to significant changes in soil acidity resulting from agricultural management. Specifically, cultivated soils showed an increase in pH (from 4.67 ± 0.17 to 5.66 ± 0.56) and a reduction in Al^{+3} concentrations compared to non-cultivated soils (from 4.42 ± 0.65 to 0.46 ± 0.74 cmol(+) kg^{-1}). These significative changes can be attributed to the widespread use of FCM, which can promote acidification (Ano and Ubochi, 2007), as well as the frequent addition of lime to cropland. This is consistent with PCA results, which revealed strong gradients in soil pH and Al^{+3} concentration. Such alterations in soil acidity significantly affect soil biogeochemistry, enhancing nutrient availability (Neina, 2019). In addition, the increase in available N (particularly $N\text{-}NO_3^-$) suggests increased rates of mineralization and nitrification, likely driven by enhanced microbial biomass and activity following FCM application.

The neutralization of soil acidity, combined with the addition of FCM, and increased microbial biomass and activity, could also be expected to result in higher concentrations of $N\text{-}NH_4^+$ in cultivated soil from the mineralization of unstable organic matter. However, the similar $N\text{-}NH_4^+$ concentrations observed in both cultivated and non-cultivated soils suggest that elevated

nitrification rates may occur in cultivated soils, likely driven by an increase in nitrifying microorganism populations (Norton and Ouyang, 2019). Furthermore, the observed similarity in BD values between cultivated and non-cultivated soils suggests minimal alteration in macroporosity, thereby allowing oxidative processes from organic N to ammonium to proceed consistently. This could potentially explain the consistent concentration of soil $N\text{-}NH_4^+$ despite agricultural practices.

The increase in available N was also observed by Farley and Kelly (2004), who reported that afforestation with pine plantations in a páramo ecosystem promoted a significant rise in soil $N\text{-}NO_3^-$ concentrations, while a reduction in $N\text{-}NH_4^+$, compared to native grasslands. Contrastingly, Avellaneda-Torres et al. (2018) found that $N\text{-}NH_4^+$ content was significantly lower in soils with potato cropping and cattle farming, compared to undisturbed páramo soils, but did not find significant changes for soil $N\text{-}NO_3^-$ concentrations across land-covers. Although previous studies in páramos have not directly assessed the effect of land-use change on nitrification rates, the relatively low nitrate concentrations reported in the literature suggest that other anthropogenic land-uses may not have the same effect on soil N pools observed for green onion cropping in this study.

This distinction may be explained by findings from prior research in páramos, which concluded that soil pH remained largely unchanged under fallow (Sarmiento and Bottner, 2002), grazing (Farfán et al., 2020; Hofstede, 1995), burning (Carrión-Paladines et al., 2023; Hofstede, 1995), and potato cropping (Avellaneda-Torres et al., 2018; Farfán et al., 2020; Sarmiento and Bottner, 2002). In studies evaluating the impact of potato cultivation, lime application did not significantly neutralize soil acidity (as shown in Table 8, pH values ranged from 4.30 to 5.30). In contrast, in the study HU, green onion monocrops are permanently established without fallow

periods, unlike potato cropping in páramos, where fallow is commonly practiced to restore soil quality after a few years of cultivation (Fonte et al., 2012; Pestalozzi, 2000).

The absence of fallow periods in soils under green onion cultivation likely intensifies the impact of management practices, contributing to the observed significant changes in soil acidity. These changes are consistent with increased levels of cations such as Ca^{+2} and Mg^{+2} (Table 4), which are likely to result from the application of lime composed of calcium or magnesium carbonates by local farmers. When lime interacts with soil particles, the hydrolysis of carbonates releases OH^- ions which neutralize soil acidity, and the remaining Ca^{+2} and Mg^{+2} cations become available in the soil's exchange complex. This process not only raises pH but also contributes to an increase in EC, as the dissolved Ca^{+2} and Mg^{+2} ions enhance the ionic content of the soil solution. The altered pH and ionic environment likely enhanced nitrification rates, as evidenced by the elevated $N\text{-}NO_3^-$ concentrations in cultivated soils compared to non-cultivated soils.

Finally, while the soils in the study area share similar andic characteristics (IGAC, 2015), spatial variability in topography may have also contributed to the differences observed in soil N pools and other edaphic properties between cultivated and non-cultivated plots, as the cultivated plots were located in the middle and lower reaches of the HU. The physiographic position of these plots is particularly relevant, as it influences factors like moisture availability, drainage, and organic matter accumulation, all of which play a significant role in N dynamics. Additionally, the high variability in the measured variables among cultivated plots may be linked to heterogeneity in agricultural practices across farms. These findings underscore the need for further assessment of soil N contents in this ecosystem. Expanding sampling coverage across the study area, including measurements at different soil depths, and assessing variations over crop cycles and under diverse climatic conditions (e.g., rainfall seasonality) would provide valuable insights on N dynamics.

3.4.2 Variables Affecting Nitrogen Concentration in Páramo Soils

The correlation analysis evidenced a positive and strong association between TN and SOC for soils within the study HU. Although positive SOC–TN relationships are commonly assumed in many soil systems, recent evidence indicates that this coupling is inconsistent in Andean Andisols and strongly modulated by land use and management practices (Alavi-Murillo et al., 2022). In this context, the marked SOC–TN coupling observed in the Santurbán–Berlín páramo under green onion cultivation constitutes a relevant finding, suggesting that current agricultural practices may be altering the biogeochemical controls governing nitrogen storage in these high-mountain soils. The coupling of soil carbon and N dynamics has been suggested given the strict proportions of these elements in organisms (Han et al., 2023).

On the other hand, variation of SOC in páramo soils affects their water retention capacity and could promote the spatial and temporal variation of SWC, affecting N transformation and transport (Castellano et al., 2012). This connection arises from the influence of SWC in the redox coupling among oxidized and reduced compounds, which drives N cycling processes such as mineralization, nitrification, and denitrification (Zhu et al., 2018). This relationship aligns with RDA results, indicating that SWC can be considered an explanatory variable for soil N contents. Moreover, BD had a significative negative correlation with TN and with $N\text{-}NH_4^+$. SOM losses lead to an increase in BD and may contribute to soil N depletion. This connection arises from a potential reduction in soil porosity, leading to increased surface runoff during storm events (Patiño et al., 2021). However, in the study area, BD and SWC did not show significant differences between cultivated and non-cultivated plots, making it difficult to determine the specific effect of these properties on N concentrations in the Santurbán-Berlín páramo soils.

Regarding N-NO_3^-, strong positive correlations were evidenced between its concentration and those of other nutrients, including S, K^+, and P. The increased availability of these nutrients in cultivated soils suggests the potential for antagonistic interactions, where an excess of one nutrient negatively affects the plant's uptake of others. That phenomenon may occur due to similar chemical properties of ions, that promote their competition for sites of adsorption, absorption, transport, and function for plant development (Fageria, 2001). Hence, the remarkable increase in ECEC (from 5.43 ± 0.80 to 17.67 ± 8.07 cmol(+)/kg), and particularly in S (from 22.72 ± 2.64 to 107.54 ± 39.20 mg/kg), K^+ (from 0.27 ± 0.04 to 2.34 ± 1.02 cmol(+)/kg), and P (from 19.92 ± 20.55 to 720.90 ± 642.97 mg/kg) contents, resulting from the use of FCM and synthetic fertilizer in cultivated soils, may adversely affect plant N use efficiency (NUE) (Rietra et al., 2017), potentially contributing to Nr losses.

Emphasizing the key role of S in soil N dynamics in páramos, this study revealed significant relationships between S and available N. S uptake by plants exerts a regulatory influence on N assimilation and vice versa (Zhao et al., 1997). As *Allium* crops rely on sulfur for flavor enhancement (Boyhan, 2008), and non-cultivated páramo soils exhibit low S levels, local farmers commonly supplement this nutrient through fertilization practices, particularly using FCM, which may be an important source of S, as shown in the characterization of locally used FCM presented in Chapter II (Table 1). Further, the acidity in the studied soils favors the adsorption of the added sulfate to surfaces of iron and aluminum oxides, hydrous oxides, clay minerals, and organic matter (Schoenau and Malhi, 2008). Therefore, S supplied through fertilization practices could compete with NO_3^- for sites of adsorption, reducing nitrate's availability to plants. Besides impacting NUE, long-term S additions to cultivated soils may have adverse effects on local biogeochemical cycles, such as N cycle (Hermes et al., 2022), yet these

interactions remain poorly understood for many ecosystems (Hinckley et al., 2020; Liu et al., 2024).

According to RDA results, EC was another relevant explanatory variable for soil N contents and had likewise a strong and positive correlation with $N\text{-}NO_3^-$. This relationship was expected considering that EC is a soil quality indicator that can be used as a measure of soluble nutrients (Smith and Doran, 1996), and that other authors have included this edaphic property in soil N predictive models (Chen et al., 2022). Finally, although RDA results showed that pH was not a strong explanatory variable and this variable had weak correlations with the studied N forms (i.e., absolute value of ρ less than 0.3, but statistically significant for TN), this soil property is considered crucial for analyzing N concentrations in páramo soils based on the analysis of PCA results and its influence on N transformation.

3.5 Conclusions

This chapter demonstrated that green onion cropping in a páramo ecosystem significantly alters topsoil N pools, resulting in a decrease in TN from 6.34 ± 0.95 g/kg to 3.83 ± 0.95 g/kg and a noticeable increase in $N\text{-}NO_3^-$ from 8.28 ± 13.20 mg/kg to 75.22 ± 34.01 mg/kg. In contrast, no significant change was observed in $N\text{-}NH_4^+$ concentrations between cultivated and non-cultivated soils. The depletion of TN is linked to local agricultural practices that favor SOM losses, including the use of FCM for fertilization and ploughing for soil preparation. The substantial increase in $N\text{-}NO_3^-$ is associated with agricultural management that promotes the reduction of soil acidity and enhances soil fertility through the incorporation of lime, synthetic fertilizers, and FCM in permanent monocrops. This elevated nitrate content in cultivated soils increases the risk of reactive N losses, with potential environmental impacts and economic inefficiencies.

The high variability observed in $N\text{-}NO_3^-$ and other edaphic properties reflects differences in land management among farmers and soil spatial variability. These findings underscore the importance of continued research on N pools and dynamics in páramos to facilitate decision-making toward sustainable N management in these ecosystems. This study identified soil pH, SOC, EC, K^+, S, P, SWC, and BD as relevant explanatory variables for soil N concentrations in páramo soils. These factors should be included in soil N dynamics models to predict the implications of agricultural management on páramo ecosystem functions.

The influence of green onion cultivation on topsoil N dynamics in the study area differed markedly from findings in other studies assessing the impacts of potato cultivation in other páramo ecosystems, particularly regarding the elevated nitrate concentrations observed in cultivated soils. These contrasting patterns are likely linked to the repeated application of FCM and the general lack of fallow periods, in contrast to potato-based agroecosystems where crop rotation with pasture or fallow land is more common. Furthermore, the geology and mineralogical composition of the soils, characterized by non-allophanic parent material, may explain the higher ammonium concentrations observed here compared to páramos with volcanic soils. Together, these insights emphasize the importance of context-specific studies to better understand the biogeochemical processes shaping nitrogen dynamics in high-altitude Andean ecosystems.

4. Vertical Distribution and Predictive Modelling of Nitrogen Pools: Advancing Understanding in Cultivated Páramo Soils Profiles

4.1 Introduction

Despite the growing recognition of the environmental impacts associated with nitrogen (N) losses, over 50% of applied N is still lost from agricultural soils (Houlton et al., 2019), with much of it reaching surface waters and contributing to eutrophication, harmful algal blooms, and water quality degradation (Wurtsbaugh et al., 2019). Efforts to mitigate these impacts are frequently hampered by the delayed response of water quality to land management interventions (Meals et al., 2010), a delay largely driven by long-term accumulation of soil N (Van Meter et al., 2018). Understanding how N is stored and distributed through the soil profile is thus essential for improving nutrient management in agroecosystems. This aspect is particularly relevant in sensitive high-mountain agroecosystems, such as páramos, where data on soil properties and the factors influencing soil N pools are generally scarce.

Previous research on soil N in páramos has primarily focused on the effects of land-use change, including pine plantations, potato (*Solanum tuberosum*) cropping, cattle grazing, and fallow land (Abreu et al., 2009; Avellaneda-Torres et al., 2018; Chacón et al., 2009; Hofstede et al., 2002; Podwojewski et al., 2002). However, knowledge of soil N dynamics under green onion cropping in páramos remains limited. Moreover, most of the existing literature on these ecosystems has focused solely on surface soils (e.g., 0–20 or 0–30 cm), with limited attention to subsoil N (exceptions include Farley and Kelly, 2004; Quichimbo et al., 2012). Additionally, studies on páramos soil N usually rely on limited temporal resolution, with only a few sampling campaigns,

thus constraining understanding of the vertical distribution patterns of soil N. Moreover, no previous studies on these regions have analyzed temporal changes in soil N pools and their relationship with local agricultural practices. These gaps constrain efforts to promote more resilient and sustainable agricultural practices in Colombian páramos, where green onion cultivation represents the main livelihood for many small-scale farmers (Sarmiento et al., 2017).

Investigating soil N beyond the surface layer is essential for validating functional relationships, such as the effects of edaphic properties, environmental conditions, or management practices on soil N pools, that are often inferred from shallow measurements alone (Smith et al., 2022). Further, the assessment of vertical distribution of soil N can help identifying areas prone to N accumulation, leaching, or runoff, supporting sustainable land use and management decisions. Obtaining such data typically involves field surveys, soil sampling, complex sample preparation and laboratory analysis. Consequently, soil N pools analyses are often hindered by the high costs of time, labor, and the handling of hazardous chemical reagents. To complement these efforts, pedotransfer functions (PTFs) have emerged as empirical tools that predict soil properties based on more easily measurable variables, using data mining techniques (Van Looy et al., 2017). Still, PTFs development must rely on a sound understanding of soil processes to ensure accuracy and relevance.

Studies using multiple linear regressions (MLR) have demonstrated the potential of PTFs to predict soil N across various soil depths using other edaphic properties as covariates (Calazans et al., 2018; Chen et al., 2022; Paltineanu et al., 2024; Y. Wang et al., 2022). However, machine learning (ML) approaches have attracted significant interest due to their improved ability to capture the complex relationships governing soil N dynamics. ML methods enable the construction of non-linear models to predict outcomes through error-minimization techniques, without

requiring predefined assumptions about the relationships between response and predictor variables (Wadoux et al., 2020). Regression and classification tree-based methods (Breiman et al., 1984) are particularly popular ML algorithms, with Random Forest (RF) standing out for its strong predictive performance (Lamichhane et al., 2019). ML-based PTFs have shown promise for estimating soil N content in agroecosystems, incorporating data on edaphic properties, climatic variables, topography, and spectral indexes (Dey and Sarma, 2023; Paltineanu et al., 2024; Sun et al., 2024; Wang et al., 2022).

However, conventional PTFs, often reliant on point measurements, are limited to the pedon-scale, restricting their applicability to broader spatial scales such as plots or watersheds. This limitation has been addressed by integrating remote sensing data, which represent environmental conditions affecting soil properties, as covariates in PTFs, following the digital soil mapping (DSM) framework (Hengl, 2003; McBratney et al., 2003; Scull et al., 2003). Spatial inference systems that combine field data with satellite-based spectral reflectance and topographic indexes derived from digital elevation models have further advanced the ability to predict soil N pools at larger scales (Hengl et al., 2017; Song et al., 2020; Zhang et al., 2019).

Additionally, research on PTFs for páramo soils remains limited, focusing primarily on soil organic carbon (SOC), bulk density (BD), and water retention capacity predictions (Ayala Izurieta et al., 2022, 2021; Beltrán-Dávalos et al., 2025; Carbajal et al., 2024; Díaz et al., 2020; Gebauer et al., 2020). Available literature suggests that no studies have yet developed PTFs for predicting soil N in páramo soils, thereby limiting the applicability of such tools for improving nutrient management in these sensitive landscapes. Consequently, this chapter aimed to (i) analyze the effect of green onion cropping on the vertical distribution patterns of soil total N (TN), ammonium N ($N\text{-}NH_4^+$), and nitrate N ($N\text{-}NO_3^-$) over an extended monitoring period, and (ii) develop PTFs

for predicting these soil N pools. The findings from this study provide a foundation for integrating sustainable agricultural practices with the conservation of páramo ecosystems.

4.2 Methods

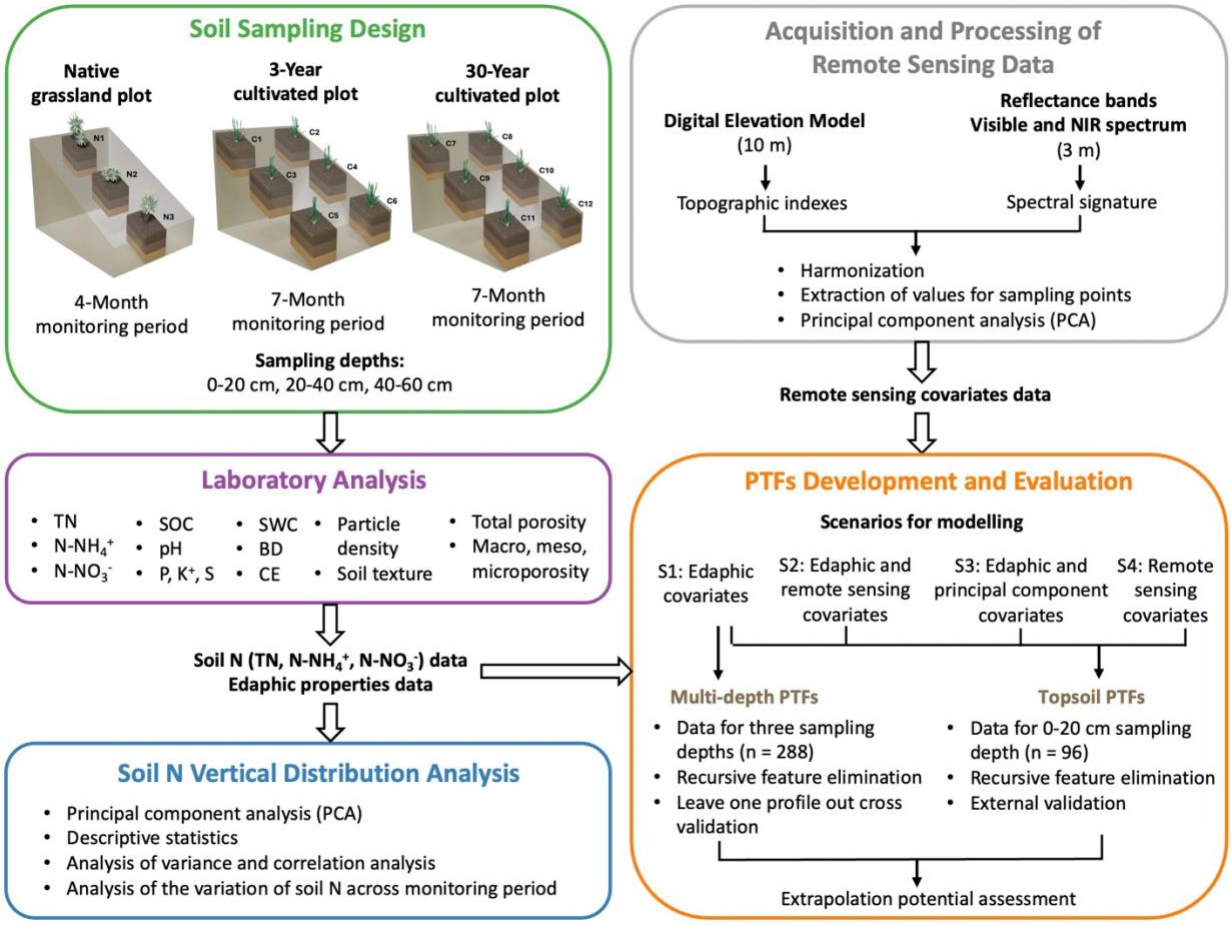
The methodological approach used in this chapter involved the following five phases: i) soil sampling design, ii) laboratory analysis, iii) soil N vertical distribution analysis, iv) acquisition and processing of remote sensing data, and v) PTFs development and evaluation. The workflow is summarized in Figure 8 and described below.

4.2.1 Soil Sampling Design

Three plots of ~250 m^2 with a mean slope of ~3% within the HU were selected for soil sampling (Figure 9). Two of the plots were under green onion cultivation, representing typical management conditions identified in the study area based on the characterization of agricultural practices presented in Chapter II. One plot has been continuously cultivated with green onion for 30 years without crop rotation or fallow, reflecting the long-term monocropping system practiced by most farmers. The second cultivated plot was managed similarly for 15 years (i.e., with green onion crops), then left fallow for a 10-year period following a noticeable decline in soil fertility and has been under green onion cultivation again for the past three years, representing a local practice of temporary abandonment and re-establishment of cropping fields. The third plot is located in native grassland, where the dominant vegetation includes *Calamagrostis effusa, Cortaderia nitida, Diplostephium,* and *Lycopodium clavatum* (Löwer, 2020). According to a 1:25,000-scale soil survey (IGAC, 2015), the first plot is within a soil cartographic unit (SCU) classified as a consociation of Typic Dystrudepts with inclusions of Typic Humudepts. The second plot is within an SCU classified as a consociation of Entic Humudepts with inclusions of Acrudoxic

Melanudand. The third plot is in an SCU consisting of a consociation of Andic Humicryepts with inclusions of Typic Humicryepts, Lithic Melanocryands, and rock outcrops.

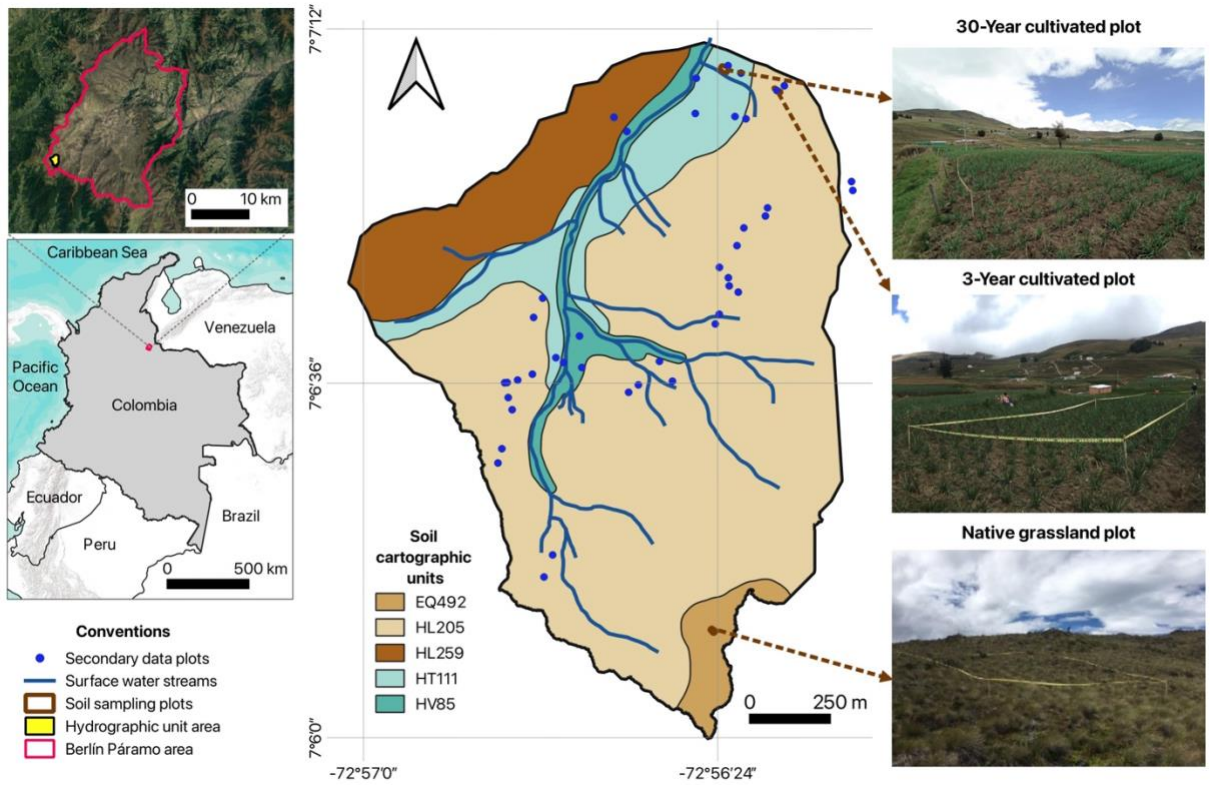
Figure 8. Methodological workflow for the second phase of the doctoral thesis



Since this study aimed to analyze actual green onion cultivation conditions in the páramo, plot selection depended on local farmers' willingness to grant access for soil sampling. As a result, it was not possible to find available plots that strictly matched the SCU. However, despite differences in taxonomic classification, the soils in the selected plots exhibit similar key characteristics to those previously described for the study area in Chapter I. To further support this similarity, the mineralogical composition of the studied soils was analyzed through X-ray

diffraction (Appendix G). This analysis indicate that all three plots share a similar mineralogical composition, dominated by albite, microcline, quartz, gibbsite, muscovite, kaolinite, and vermiculite. Some additional minerals, such as rutile, anatase, and ferruginous muscovite, were detected at deeper layers in the 30-year cultivated plot, suggesting potential pedogenetic differentiation. However, the overall similarity in mineral composition across plots supports the assumption that differences in soil properties are primarily related to land use rather than inherent geological variability.

Figure 9. Location of the sampling plots for the extended soil monitoring period



Note. Soil cartographic units were defined according to IGAC (2015) following the USDA-NRCS classification (Soil Survey Staff, 2022). EQ492: Andic Humicryepts with inclusions of Typic Humicryepts and Lithic Melanocryands; HL205: Entic Humudepts with inclusions of Acrudoxic

Melanudand; HL259: Typic Humudepts with inclusions of Pachic Humudepts and Fluventic Humudepts; HT111: Typic Dystrudepts with inclusions of Typic Humudepts; HV85: Complex of Aquíc Humudepts, Entic Humudepts and Fluventic Humudepts.

Soil sampling was conducted monthly in each plot from August 2023 to February 2024. In the cultivated plots, six sampling points were selected per plot, while in the native grassland plot, three sampling points were considered. At each point, disturbed and undisturbed soil samples were collected at three depths (0–20, 20–40, and 40–60 cm), defined according to the soil depth of the SCUs (IGAC, 2015). The sampling points were distributed across different positions in slope (i.e., upper, middle, and lower) to capture spatial variability due to surface runoff. Disturbed samples were collected with a soil auger and used for chemical and textural analyses, while undisturbed samples were collected using a soil core sampler and 100 cm^3 metallic cylinders for BD and gravimetric soil water content (SWC) assessments.

The cultivated plots were monitored for seven months, yielding 126 samples per plot, whereas the native grassland plot was monitored for four months, resulting in 36 samples. The higher sampling density in the cultivated plots accounted for greater spatial and temporal variability introduced by agricultural management. In contrast, sampling in the native grassland plot was limited due to restricted access, as it is a conservation area. However, given the absence of anthropogenic activity, soil properties were expected to be more stable in the uncultivated plot, making a smaller sample size acceptable for capturing baseline conditions. Irrigation and rainfall occurrences during the monitoring period were identified using secondary data (Naranjo, 2025).

According to reports from the farmers managing the two selected cultivated plots and based on the average nutrient content of the locally used fresh chicken manure (FCM; Table 1), estimated

N inputs per application were approximately 820 kg ha^{-1} of N in the 3-year cultivated plot and 460 kg ha^{-1} of N in the 30-year cultivated plot. These values refer to individual application events, not annual or cumulative inputs. Additionally, these farmers apply up to 688 L ha^{-1} of mixed and diluted agrochemicals, primarily foliar fertilizers and conventional pesticides, every 10 days throughout the crop cycle. Although local farmers commonly dissolve lime (e.g., calcium and magnesium carbonates) in water and spray it onto crops to control pests and diseases and to reduce soil acidity (as discussed in Chapter II), this practice was not observed during the monitoring period. Water is supplied by rainfall and complemented with sprinkler irrigation, typically once a week during dry periods. Manual harvesting occurs every three to four months, leaving at least four pseudo-stems to allow regrowth for the next cycle. One month after each harvest, hilling is commonly performed, consisting of manually mixing of the first 0-20 cm of soil.

4.2.2 Laboratory Analysis

Due to the large number of samples collected during each campaign and the required analysis of edaphic properties, disturbed samples were stored in plastic bags and frozen at -20 °C for up to one week. Prior to their laboratory analyses, samples were defrosted, oven-dried at 60 ˚C for 24 h, and passed through a 2-mm sieve. These sample storage and preparation conditions are acceptable for assessment of soil samples for chemical analysis due to reduced bias introduced by biochemical transformations (Sollen-Norrlin and Rintoul-Hynes, 2024). Undisturbed samples were processed immediately after each sampling campaign to avoid error in SWC measurements. Soil analyses included TN, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, SOC, pH, electrical conductivity (EC), BD, SWC, P, available sulfur (S), and exchangeable potassium (K^+). These properties were measured throughout the monitoring period, since they were identified as key explanatory variables for nitrogen

concentrations in páramo surface soils, as discussed earlier in Chapter III. The methods for these analyses are described in section 3.2.2 (Chapter III).

To further characterize additional factors influencing soil N pools, particle density (PD), soil texture, and soil porosity, were also analyzed. Soil texture was defined by the proportion of clay, silt, and sand fractions, following the USDA classification criteria. Particle-size analysis was conducted using sedimentation procedures (Beretta et al., 2014), with the Robinson pipette method applied to samples with SOC ≥ 2.2% and the Bouyoucos method used for samples with SOC < 2.2%. This approach was adopted to remove SOM before analysis, ensuring effective dispersion of soil aggregates (Jensen et al., 2017). PD was estimated using the pycnometer method (Blake and Hartge, 1986b). Soil porosity types were calculated as follows.

$$\text{Total porosity} = 1 - \frac{\text{BD}}{\text{PD}} \quad (1)$$

$$\text{Macroporosity} = \text{Total porosity} - \theta_{33\text{kPa}} \quad (2)$$

$$\text{Mesoporosity} = \theta_{33\text{kPa}} - \theta_{1500\text{kPa}} \quad (3)$$

$$\text{Microporosity} = \theta_{1500\text{kPa}} \quad (4)$$

Where $\theta_{33\text{kPa}}$ and $\theta_{1500\text{kPa}}$ represent the volumetric soil water content at matric potentials of -33 kPa (field capacity) and -1500 kPa (permanent wilting point), respectively, and were measured using a pressure membrane apparatus (Klute, 1986). Since soil texture, PD, $\theta_{33\text{kPa}}$, and $\theta_{1500\text{kPa}}$ remain relatively stable under short-term soil management, they were measured only once per depth and point during the monitoring period. However, total porosity was monitored throughout the study, as it was calculated using BD values estimated for each sampling point, depth, and campaign.

4.2.3 Soil Nitrogen Vertical Distribution Analysis

Descriptive statistics were applied to edaphic properties data, grouped by sampling plot and depth. Principal component analysis (PCA) was used to explore gradients in edaphic properties potentially associated with land use and/or depth. Since the datasets for the soil properties did not fulfill the normal distribution assumption, as determined by the Anderson-Darling test (Appendix H), Spearman correlation analysis was conducted to identify variables most strongly related to soil N. All statistical analyses were performed in R (R Core Team, 2024), and considered a significance level of 0.05.

To evaluate the effects of land use, soil depth, and sampling month on TN, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ concentrations, linear mixed-effects models (LMMs) were used. For each N form, a core model was fitted including land cover (three levels, i.e., three plots), depth (0–20, 20–40, and 40–60 cm), and month (seven levels) as fixed effects. The interaction between land cover and depth was included to assess whether vertical N patterns differed among land uses. Because repeated measurements were collected at fixed sampling profiles over time, the sampling profile was included as a random intercept to account for the hierarchical and repeated-measures structure. Moreover, for each N form, two supplementary LMMs were additionally fitted for the 3-year and 30-year cultivated plots to evaluate temporal variation within each land use. These within-plot models included depth, month, and their interaction as fixed effects, and sampling profile as a random intercept. All models were fitted by restricted maximum likelihood (REML) using the *lme4* R package (Bates et al., 2015), and Satterthwaite's approximation (via *lmerTest*) (Kuznetsova et al., 2017) was used to obtain degrees of freedom and p-values for fixed effects. Model assumptions were evaluated through visual inspection of residual and random-effects diagnostic plots, and no major deviations from normality or homoscedasticity were detected.

To further assess the implications of agricultural management on soil N vertical distribution, the accumulated N-NH_4^+ and N-NO_3^- stocks within the soil profile were estimated across the three sampling plots. For each sampling point, the mineral N stock at each depth interval was calculated using the measured concentration, BD, and thickness of the soil layer. The accumulated stock per soil profile was then obtained by summing the stocks across the three sampled depths (0–20, 20–40, and 40–60 cm), following the approach outlined below and described by Chen et al. (2022).

$$\text{Accumulated N stock} = \sum C_i \times BD_i \times h_i \div 10 \quad (5)$$

Where accumulated N stock is the total amount of a mineral N form (i.e., N-NH_4^+ or N-NO_3^-) within the 0–60 cm soil profile, expressed in kg ha^{-1} of N; C_i and BD_i represent the concentration of mineral N (mg kg^{-1}) and bulk density (g cm^{-3}), respectively, for a given depth interval *i*; h_i is the thickness of the soil layer (cm); and 10 is the unit conversion factor.

4.2.4 Acquisition and Processing of Remote Sensing Data

Terrain-derived attributes from a digital elevation model (DEM) and spectral reflectance from satellite imagery were used as remote sensing covariates for developing PTFs. The DEM was obtained using the 'elevatr' R package (Hollister et al., 2023). The source of the DEM was the Mapzen Terrain Tiles, which compile data from various sources, including high-resolution datasets, allowing to obtain a spatial resolution of 10 m. Thirteen topographic indexes were computed using the basic terrain analysis module from the System for Automated Geoscientific Analyses (SAGA version 9.2.1) (Conrad et al., 2015). The calculated indexes used as covariates included aspect, channel network base level, channel network distance, convergence index, hillshade, length and steepness factor (LS-factor), plan curvature, profile curvature, relative slope

position, slope, topographic wetness index, total catchment area, and valley depth. A summary of each topographic covariate, including its definition and relevance, is provided in Appendix I.

Additionally, PlanetScope (PS) images (Planet Labs, Inc., USA) were used to derive soil spectral reflectance. PS imagery is among the most widely used remote sensing datasets for environmental studies (Pham-Duc et al., 2025), specially due to its 3-m spatial resolution and near-daily revisit time, offering advantages over traditional optical satellite platforms (Roy et al., 2021). Specifically, the SuperDoves PSB.SD sensor products were used. These products capture images in eight spectral bands: coastal blue (431-452 nm), blue (465-515 nm), green (513-549 nm), green II (547-583 nm), yellow (600-620 nm), red (650-680 nm), red edge (697-713 nm) and near infrared (NIR, 845-885 nm) (Frazier and Hemingway, 2021). PS images were downloaded from Planet Labs website (https://www.planet.com). The spectral covariates used comprised the average reflectance data from those eight raw spectral bands available over the 2021-2023 period.

The ‘terra’ R package (Hijmans, 2025) was used to resample the topographic indexes’ rasters and to extract the remote sensing covariates values for the sampling points. Finally, to reduce predictor dimensionality, we applied PCA separately to each group of remote sensing covariates. The first two principal components (PC1 and PC2) explained 78% of the variance in the topographic dataset, while PC1 alone accounted for 95% of the variance in the spectral dataset. These three principal components were included as additional covariates in the predictive model evaluation. Further details on PCA results are presented in Appendix J.

4.2.5 Pedotransfer Functions (PTFs) Development and Evaluation

Four experimental scenarios were defined to evaluate the relevance of including edaphic properties and remote sensing covariates as predictors in PTFs development. Scenario S1 included

only the edaphic properties measured throughout the whole monitoring period. Scenario S2 expanded on S1 by incorporating the topographic indexes and spectral reflectance data. Scenario S3 combined edaphic properties with principal components (PC1 and PC2 from topographic predictors and PC1 from spectral dataset). Scenario S4 included only topographic indexes and spectral reflectance data.

Two groups of PTFs were developed based on sampling depth coverage: (i) multi-depth PTFs, using data from three depths (0–20, 20–40, and 40–60 cm; n = 288), and (ii) topsoil PTFs, using data only from 0–20 cm (n = 96). Multi-depth PTFs were developed using scenario S1, while topsoil PTFs were developed under scenarios S1 to S4. As all covariates in scenarios S1–S3 were based on field-measured edaphic properties (available only for sampling points), all these models represent pedon-scale PTFs in the sense of using point-based predictors. In contrast, scenario S4 models incorporate only spatial covariates and thus follow a DSM approach.

Covariate selection and model development and evaluation were conducted as two distinct stages. For each target variable (i.e., TN, N-NH_4^+, and N-NO_3^-) and modelling scenario, recursive feature elimination (RFE) was used to optimize predictor selection. RFE is a backward selection algorithm (Guyon et al., 2002), widely used in PTFs development (Wadoux et al., 2020), for reducing model complexity, improving accuracy, avoiding multicollinearity, and preventing overfitting. RFE was implemented following a previous study (Xiao et al., 2022), involving iterative model fitting with RF, ranking variable importance, and sequentially eliminating the least important predictor until the optimal subset was identified. The ‘rfe’ function from the ‘caret’ R package (Kuhn, 2008) was used, performing 10 iterations to ensure stability: covariates were retained if selected in more than 5 iterations.

After completing the RFE procedure and identifying the predictor subset, all subsequent model training and validation steps were performed using only these selected variables. PTFs were modeled using RF and MLR. RF, an ensemble ML technique that builds multiple uncorrelated regression trees on bootstrapped training samples (Breiman, 2001), was implemented with the 'randomForest' R package (Liaw and Wiener, 2002), with 500 trees and mtry set to one-third of the predictor count. MLR was fitted using ordinary least squares with the 'lm' function from the 'stats' R package (R Core Team, 2024). Model performance was evaluated using the square of the Pearson correlation coefficient between observed and modeled values (R^2), the root mean square error (RMSE), and the mean absolute error (MAE).

The leave-one-profile-out cross-validation was used for multi-depth PTFs. This approach provides a robust model evaluation that avoid over-optimistic results by leaving out one soil profile at a time for validation while using the remaining profiles for training (Jaconi et al., 2019). As a result, it was ensured no overlap between training and validation data, preventing biased results due to spatial dependency within profiles. The process was repeated 15 times, ensuring each soil profile (i.e., sampling point) was used at least once for validation. Hence, the mean and standard deviation of the performance metrics were computed to compare models and scenarios.

Moreover, external validation was performed for the topsoil PTFs. Following this approach, the dataset was randomly split into a training set (80%), and a test set (20%), ensuring that both sets maintained the original data distribution. Finally, to assess the extrapolation potential of the PTFs, an additional external validation was performed using data from the initial soil assessment (Figure 9) on topsoil (0–20 cm) properties measured in Chapter III. Appendix K present the comparison of the probability density functions of the primary dataset from this study and the secondary dataset, providing insight into the distributional similarity between both datasets.

4.3 Results

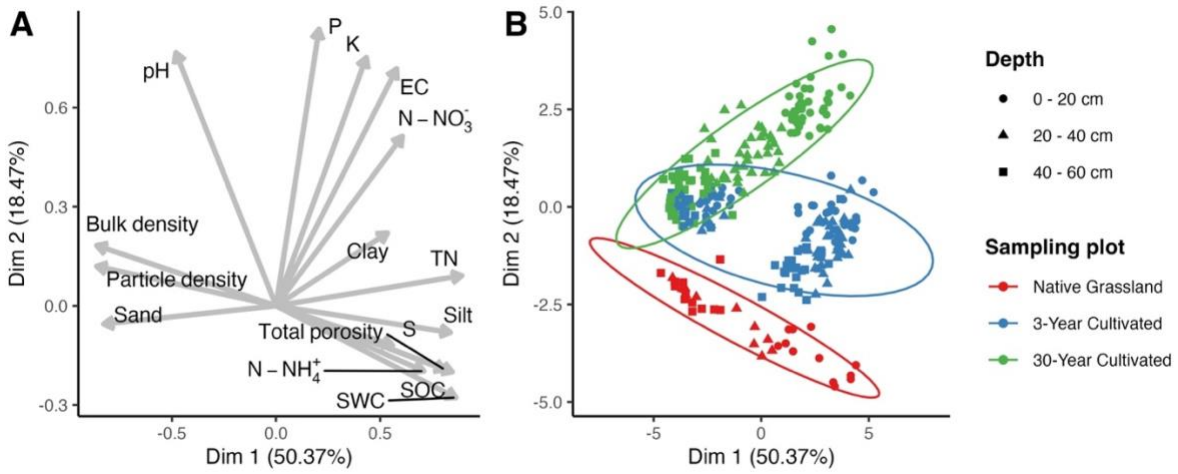
This section begins with an exploratory analysis of soil properties, including PCA, descriptive statistics, and correlations with N forms. It then presents the results of statistical analyses evaluating the effects of depth and sampling month on soil N concentrations, followed by the estimation of accumulated mineral N stocks over time. Subsequently, the selection of relevant covariates for predictive modeling is described. Finally, the performance of the developed PTFs is reported.

4.3.1 Soil Nitrogen and Edaphic Properties across Sampling Plots and Depths

The exploratory PCA conducted on 16 edaphic properties measured across 288 samples revealed that the first two PCs explained 68.84% of the total variance in the data. PC1 accounted for 50.37% of the variance and was strongly positively correlated with TN, $N\text{-}NH_4^+$, SOC, gravimetric SWC, total porosity, and the silt fraction, and strongly negatively correlated with BD, PD, and the sand fraction. PC2 explained 18.47% of the variance and showed strong positive correlations with P, K^+, pH, and EC. Both PC1 and PC2 were moderately positively correlated with $N\text{-}NO_3^-$. These relationships are illustrated in the PCA variables factor map shown in Figure 10A and detailed in Appendix L. The PCA scores biplot presented in Figure 10B shows a clear separation along PC2 between observations from the native grassland plot and those from the 30-year cultivated plot, with the latter exhibiting higher PC2 scores. Observations from the 3-year cultivated plot were distributed between the two groups: some fell within the 95% confidence ellipse of the 30-year cultivated plot, while others occupied an intermediate position along PC2 relative to the other two ellipses. The biplot also suggests a gradient along PC1 related to soil depth, with higher scores for topsoil samples and lower scores for deeper layers. This depth-related

gradient was more evident in the native grassland and 30-year cultivated plots but was less distinct in the 3-year cultivated plot.

Figure 10. Visualization of PCA results for the extended monitoring soil monitoring period



Note. (A) Variables factor map displaying the contribution of original variables to the first two principal components. (B) Biplot of the PCA scores for observations. The ellipses represent 95% confidence intervals for each sampling plot category.

The trends observed in the PCA align with the characterization of edaphic properties across plots and soil depths (Table 9). The descriptive analysis showed that soil TN concentrations were similar among the three plots but exhibited clear stratification with depth. The native grassland and the 30-year cultivated plots displayed similar vertical TN patterns, with higher concentrations in the topsoil and a progressive decline toward deeper layers. In contrast, the 3-year cultivated plot showed a noticeably more homogeneous profile, with comparatively elevated TN concentrations at 20–40 cm and 40–60 cm relative to the other plots. Patterns in N-NH4+ concentrations were broadly similar among plots, although the 30-year cultivated plot consistently exhibited the lowest topsoil ammonium levels. Across depths, the two cultivated plots differed markedly: the long-term

cultivated plot showed lower N-NH4+ concentrations throughout the profile, whereas the 3-year plot retained higher values, particularly in deeper layers.

Vertical patterns in $N\text{-}NO_3^-$ differed substantially among land uses. The native grassland plot maintained uniformly low nitrate concentrations across depths, while the 3-year cultivated plot displayed substantially higher $N\text{-}NO_3^-$ concentrations that remained relatively uniform throughout the profile. In the 30-year cultivated plot, nitrate was strongly concentrated in the topsoil and declined sharply with depth, indicating pronounced stratification and enhanced surface accumulation. Appendix M presents more details of the N pools distribution across the three plots, grouped by depths and position on slope.

Beyond soil N pools, several edaphic properties showed distinct patterns among the three plots (Table 9). Soil acidity decreased with cultivation age, with higher pH values observed in the long-term cultivated plot. Correspondingly, fertilization and soil management practices appeared to increase the concentrations of P and K^+, which were highest in the 30-year cultivated plot. EC also tended to be greater in cultivated soils, particularly in the long-term cultivated plot, reflecting higher ionic content associated with prolonged fertilization. The vertical distribution of S differed from that of other ions, with higher concentrations occurring mainly in the middle and lower depths of the 3-year cultivated plot.

Table 9. Characterization of edaphic properties (mean ± standard deviation) across the three sampling plots and depths over the monitoring period.

Edaphic property	Unit	Native Grassland Plot			3-Year Cultivated Plot			30-Year Cultivated Plot		
		0 - 20 cm	20 - 40 cm	40 - 60 cm	0 - 20 cm	20 - 40 cm	40 - 60 cm	0 - 20 cm	20 - 40 cm	40 - 60 cm
TN	g kg^{-1}	3.77 ± 1.23	1.74 ± 1.27	0.57 ± 0.37	2.90 ± 1.08	2.34 ± 1.10	1.46 ± 0.88	3.49 ± 0.32	1.56 ± 0.67	0.54 ± 0.41
$N\text{-}NH_4^+$	mg kg^{-1}	35.94 ± 11.23	12.02 ± 5.97	6.60 ± 2.80	26.45 ± 8.94	18.19 ± 7.34	12.59 ± 6.86	17.83 ± 6.88	11.20 ± 4.22	7.29 ± 4.19
$N\text{-}NO_3^-$	mg kg^{-1}	0.89 ± 0.48	0.68 ± 0.55	0.73 ± 0.57	30.72 ± 18.02	32.36 ± 20.22	31.10 ± 23.55	50.85 ± 22.07	33.29 ± 11.87	19.45 ± 7.5
SOC	%	8.37 ± 2.41	3.82 ± 3.14	1.29 ± 1.14	4.54 ± 1.82	4.03 ± 1.90	2.63 ± 1.61	4.54 ± 0.40	2.44 ± 1.14	0.81 ± 0.83
P	mg kg^{-1}	7.18 ± 3.44	7.56 ± 6.16	6.52 ± 2.84	288.21 ± 89.88	110.16 ± 64.84	47.81 ± 49.91	2048.90 ± 160.98	1273.81 ± 373.85	489.54 ± 240.93
K^+	cmol(+) kg^{-1}	0.28 ± 0.26	0.11 ± 0.17	0.30 ± 0.91	2.66 ± 0.86	1.94 ± 0.64	1.30 ± 0.49	3.66 ± 1.03	2.27 ± 0.71	1.57 ± 0.60
S	mg kg^{-1}	36.80 ± 15.61	31.70 ± 14.95	36.68 ± 18.71	53.05 ± 20.77	63.33 ± 26.54	78.56 ± 25.30	53.13 ± 16.72	38.76 ± 12.99	31.40 ± 23.39
pH	-	4.75 ± 0.38	4.95 ± 0.45	5.15 ± 0.44	6.01 ± 0.54	5.95 ± 0.76	5.55 ± 0.81	6.77 ± 0.18	7.04 ± 0.21	7.15 ± 0.30
SWC	g g^{-1}	0.72 ± 0.18	0.41 ± 0.26	0.26 ± 0.16	0.46 ± 0.17	0.47 ± 0.17	0.40 ± 0.14	0.47 ± 0.03	0.31 ± 0.10	0.18 ± 0.05
BD	g cm^{-3}	0.66 ± 0.10	0.92 ± 0.18	0.98 ± 0.14	0.90 ± 0.20	0.92 ± 0.25	1.00 ± 0.29	0.80 ± 0.07	1.01 ± 0.14	1.17 ± 0.08
Particle density	g cm^{-3}	2.25 ± 0.03	2.53 ± 0.04	2.60 ± 0.03	2.39 ± 0.11	2.39 ± 0.11	2.41 ± 0.12	2.39 ± 0.00	2.43 ± 0.01	2.50 ± 0.03
EC	µS cm^{-1}	46.92 ± 37.31	13.24 ± 3.89	9.72 ± 5.22	279.10 ± 98.24	187.00 ± 74.91	143.98 ± 59.61	481.42 ± 127.64	216.58 ± 68.32	115.58 ± 40.92
Total porosity	cm^3 cm^{-3}	0.71 ± 0.05	0.64 ± 0.07	0.62 ± 0.05	0.62 ± 0.07	0.62 ± 0.09	0.59 ± 0.10	0.66 ± 0.03	0.58 ± 0.06	0.53 ± 0.03
Macroporosity	cm^3 cm^{-3}	0.27 ± 0.03	0.35 ± 0.05	0.43 ± 0.05	0.18 ± 0.04	0.23 ± 0.06	0.19 ± 0.06	0.25 ± 0.06	0.24 ± 0.02	0.31 ± 0.01
Mesoporosity	cm^3 cm^{-3}	0.04 ± 0.00	0.05 ± 0.01	0.07 ± 0.03	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.01
Microporosity	cm^3 cm^{-3}	0.39 ± 0.02	0.16 ± 0.02	0.07 ± 0.02	0.40 ± 0.11	0.31 ± 0.06	0.35 ± 0.03	0.37 ± 0.04	0.35 ± 0.02	0.21 ± 0.03
Sand	%	51.63 ± 9.10	69.23 ± 3.25	70.09 ± 2.69	32.07 ± 19.38	29.85 ± 19.88	32.56 ± 13.97	37.09 ± 11.39	45.36 ± 14.66	69.14 ± 4.64
Silt	%	29.67 ± 1.31	12.98 ± 2.13	12.14 ± 1.63	35.71 ± 18.32	32.95 ± 16.56	29.09 ± 12.60	28.12 ± 2.07	24.08 ± 8.20	11.66 ± 2.52
Clay	%	18.70 ± 7.81	17.79 ± 1.23	17.77 ± 1.23	32.22 ± 4.37	37.20 ± 10.13	38.35 ± 6.74	34.79 ± 9.50	30.56 ± 6.67	19.20 ± 2.34

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, K^+: exchangeable potassium, P: available phosphorus, S: available sulfur, EC: electrical conductivity.

Soil porosity remained relatively consistent across plots and depths, although the deepest layer of the 30-year cultivated plot showed a slight decline in total porosity. Other physical properties, such as SWC and BD, displayed modest variation across plots; the deepest layer of the 30-year cultivated plot exhibited the highest BD and lowest SWC. SOC was consistently greater in native grassland across all depths, while both cultivated plots showed reduced SOC in surface and subsurface layers. The vertical patterns of SOC in the cultivated plots closely resembled those observed for TN. Textural patterns also differed among plots: the 3-year cultivated plot had a finer texture, with higher proportions of clay and silt across depths, whereas the 30-year cultivated plot showed decreasing clay content with depth, and the native grassland plot was dominated by sand throughout the profile.

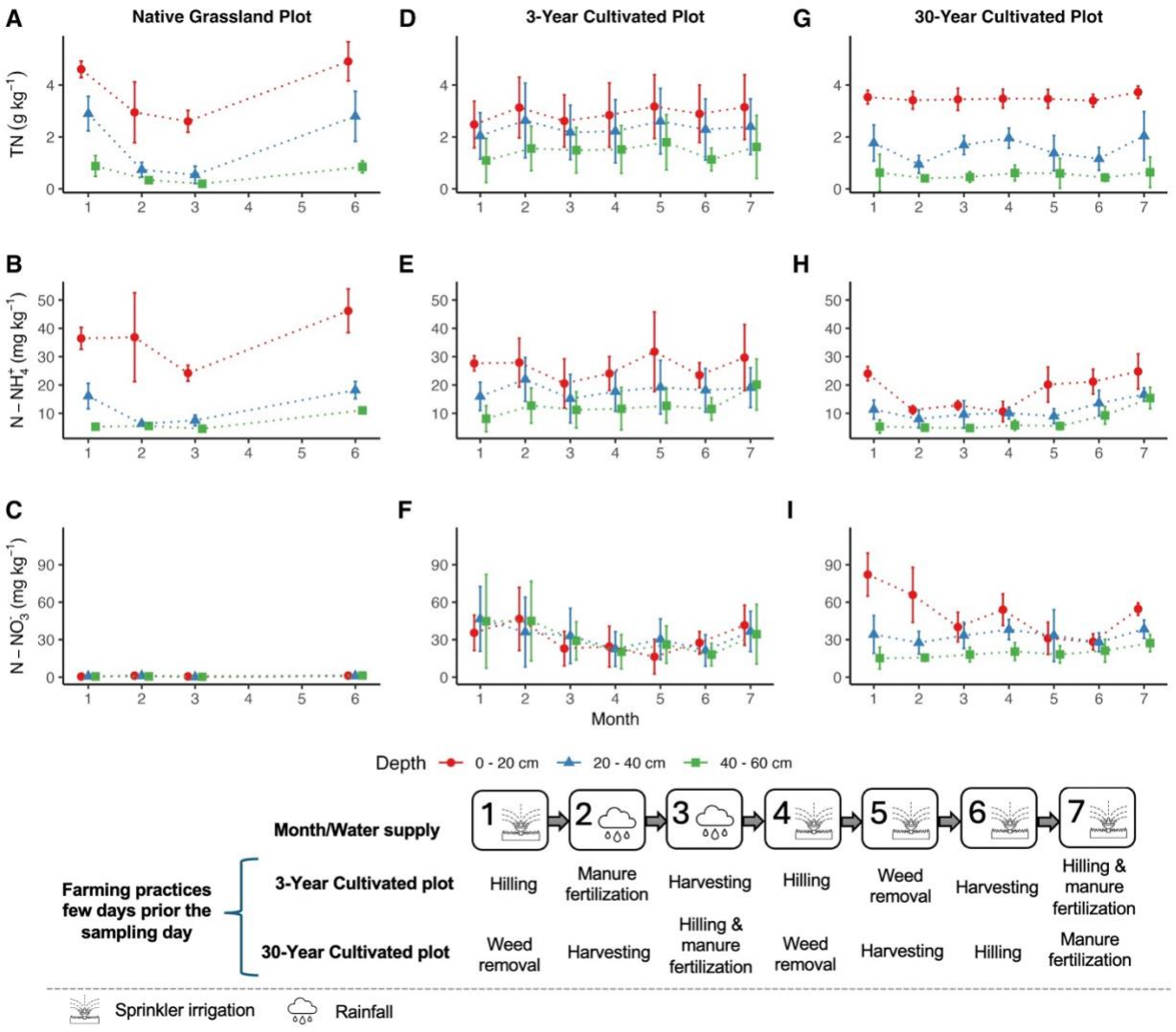
Correlation analysis revealed strong relationships between soil N and several edaphic properties (Appendix N). TN was positively correlated with SOC ($\rho = 0.916$), microporosity ($\rho = 0.794$), N-NH_4^+ ($\rho = 0.783$), SWC ($\rho = 0.759$), TP ($\rho = 0.691$), EC ($\rho = 0.667$), and silt ($\rho = 0.660$), while negatively correlated with BD ($\rho = -0.724$) and sand ($\rho = -0.628$). N-NH_4^+ evidenced positive correlations with SOC ($\rho = 0.802$), microporosity ($\rho = 0.699$), and silt ($\rho = 0.611$); and negative correlation with BD ($\rho = -0.624$). N-NO_3^- displayed weaker correlations with edaphic properties but was positively associated with EC ($\rho = 0.785$), K^+ ($\rho = 0.590$), and P ($\rho = 0.520$); and negatively correlated with sand ($\rho = -0.592$).

4.3.2 Variation of Soil Nitrogen Vertical Distribution Patterns over the Monitoring Period

The variation in soil N concentrations over the monitoring period and soil depths across the three sampling plots is presented in Figure 11, and the LMMs results are summarized in Table

10. The full set of LMM results, including fixed-effect estimates with p-values and confidence intervals and the variances of the random effects, is provided in Appendix O.

Figure 11. Temporal variation of soil N concentrations across the monitoring period and the three sampling plots



Note. Native grassland plot (A–C), 3-Year cultivated plot (D–F), and 30-Year cultivated plot (G–I). Points represent mean concentrations, with error bars indicating standard deviations. The bottom diagram indicates farming practices conducted in plots few days prior the sampling day and the crops water supply source.

Table 10. Fixed-effect estimates from linear mixed-effects models (LMM) for TN, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ across land uses, depths, and sampling months.

	TN LMM			**$N\text{-}NH_4^+$ LMM**			**$N\text{-}NO_3^-$ LMM**		
Fixed effects	**Core model**	**3-year c. plot model**	**30-year c. plot model**	**Core model**	**3-year c. plot model**	**30-year c. plot model**	**Core model**	**3-year c. plot model**	**30-year c. plot model**
Intercept[a]	3.9***	2.48	3.53***	36.79***	27.65***	24.02***	8.11	35.52***	82.05***
Plot (land-use)									
3-year cultivated	-0.97			-10.46**			30.88**		
30-year cultivated	-0.37			-19.08***			51.01***		
Depth									
20 - 40 cm	-2.02***	-0.43	-1.76***	-23.91***	-11.67***	-12.65***	-0.21	11.09	-47.82***
40 - 60 cm	-3.2***	-1.38***	-2.91***	-29.33***	-19.53***	-18.7***	-0.16	9.25	-66.79***
Month									
2nd	-0.22	0.65*	-0.12	-1.36	0.29	-12.8***	-2.83	11.09	-16.18*
3rd	-0.29*	0.14	-0.09	-3.87***	-7.09*	-11.19***	-10.97***	-12.62	-41.92***
4th	0.02	0.36	-0.06	-2.15	-3.59	-13.41***	-11.23***	-11.03	-28.06***
5th	0.09	0.69**	-0.06	0.92	4.12	-3.87*	-15.35***	-19.14*	-51.0***
6th	-0.02	0.41	-0.13	1.81	-4.17	-2.84	-15.07***	-7.98	-53.75***
7th	0.18	0.67*	0.19	5.49***	2.04	0.77	-2.44	6.05	-27.5***
Plot (land-use) x Depth									
3-year cultivated x 20 - 40 cm	1.46***			15.65***			1.85		
30-year cultivated x 20 - 40 cm	0.09			17.28***			-17.35**		
3-year cultivated x 40 - 60 cm	1.76***			15.47***			0.54		
30-year cultivated x 40 - 60 cm	0.24			18.8***			-31.25***		
Depth x Month									
20 - 40 cm x 2nd month		-0.06	-0.71*		5.72	9.45***		-21.62*	9.56

	TN LMM			$N\text{-}NH_4^+$ LMM			$N\text{-}NO_3^-$ LMM		
Fixed effects	**Core model**	**3-year c. plot model**	**30-year c. plot model**	**Core model**	**3-year c. plot model**	**30-year c. plot model**	**Core model**	**3-year c. plot model**	**30-year c. plot model**
40 - 60 cm x 2nd month		-0.19	-0.1		4.33	12.44***		-10.91	16.52
20 - 40 cm x 3rd month		-0.01	0		6.27	9.44***		-0.98	41.09***
40 - 60 cm x 3rd month		0.26	-0.08		10.21*	10.67***		-3.07	44.76***
20 - 40 cm x 4th month		-0.19	0.24		5.29	12.14***		-13.06	31.96***
40 - 60 cm x 4th month		0.06	0.05		7.11	13.89***		-13.37	33.3***
20 - 40 cm x 5th month		-0.13	-0.33		-0.87	1.5		2.86	50.03***
40 - 60 cm x 5th month		0.01	0.04		0.43	4.06		0.42	53.94***
20 - 40 cm x 6th month		-0.17	-0.47		6.34	5.06		-17.23	47.45***
40 - 60 cm x 6th month		-0.37	-0.06		7.6	6.78*		-18.68	59.74***
20 - 40 cm x 7th month		-0.32	0.08		1.05	4.5		-16.11	31.72***
40 - 60 cm x 7th month		-0.15	-0.18		10.0*	9.29***		-16.44	39.46***

Note. TN is expressed in g kg^{-1}; $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ are expressed in mg kg^{-1}. [a]Intercepts correspond to the reference levels: 0-20 cm depth and the first sampling month. For the core models, the native grassland is the reference plot (land-use) level. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

In the core model, TN concentrations did not differ significantly among land-use types at 0–20 cm ($p > 0.05$), whereas depth had a strong effect across all plots ($p > 0.05$). As expected, TN declined with depth; however, the interaction between land use and depth indicated that this decline was less pronounced in the 3-year cultivated plot. In contrast, the vertical TN gradient in the 30-year cultivated plot closely matched that of the native grassland. The supplementary model for the 3-year cultivated plot supports this pattern, showing a relatively homogeneous vertical TN distribution, with only the deepest layer (40–60 cm) differing significantly from the surface. This supplementary model also revealed moderate temporal variability at 0–20 cm, with TN increasing by approximately 0.6–0.7 g kg^{-1} in months 2, 5, and 7 relative to the first sampling month. The absence of significant interactions between month and depth indicates that the shape of the TN vertical profile in this plot remained stable over time. In contrast, the supplementary model for the 30-year cultivated plot showed no meaningful temporal variation in topsoil TN. The depth gradient remained stable throughout the monitoring period, except for a marginal reduction in TN at 20–40 cm during month 2 relative to month 1.

The LMMs for $N\text{-}NH_4^+$ showed that concentrations were strongly influenced by land use and depth (Fig. 11; Table 10). In the core model, both cultivated plots had lower surface $N\text{-}NH_4^+$ concentrations than the native grassland, with the reduction in the 30-year cultivated plot being nearly twice that of the 3-year plot. Although $N\text{-}NH_4^+$ declined with depth in all plots, the decrease was substantially steeper in the native grassland, and significant interactions between land-use and depth indicated that cultivation reduced the vertical gradient, consistent with a redistribution of ammonium throughout the profile. In the 3-year cultivated plot, the supplementary model showed limited temporal variation at the surface, with only a moderate decline in the third month, compared to the first month. Significant interactions between month and depth indicated a

temporary flattening of the depth gradient, with $N\text{-}NH_4^+$ concentrations at 40–60 cm approximately 10 mg kg^{-1} higher than expected from additive effects alone. In the 30-year cultivated plot, surface concentrations decreased by ~12 mg kg^{-1} in months 2–4, followed by a smaller decline (~4 mg kg^{-1}) in month 5. Positive interaction effects between month and depth revealed that subsoil dynamics differed from those at the surface in the 30-year cultivated plot: while the topsoil decreased early in the monitoring period, the middle and deeper layers tended to maintain or increase $N\text{-}NH_4^+$ concentrations, particularly in months 2–4, 6, and 7.

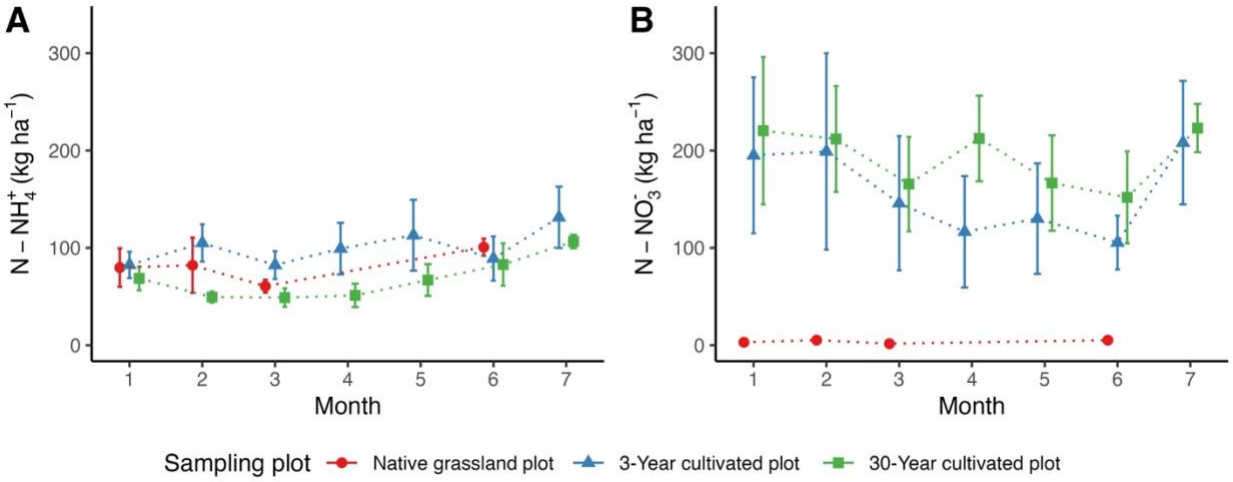
The vertical patterns of soil $N\text{-}NO_3^-$ differed markedly among land uses. The native grassland plot maintained uniformly low nitrate concentrations across depths, with values close to the analytical detection limit (i.e., 0.03 mg kg^{-1}) but consistently above it. The core LMM showed a strong effect of cultivation, with both cultivated plots exhibiting substantially higher nitrate levels. In the 3-year cultivated plot, nitrate concentrations were consistently high and showed no significant depth effect. This vertical uniformity is supported by both the core and supplementary models, which confirmed the absence of depth-related variation despite concentrations being much higher than in the native grassland. Seasonal changes occurred similarly across depths in the 3-year cultivated plot, with most interactions between depth and month being nonsignificant. The only exception was a significant interaction in the second month at 20–40 cm (estimate = -21.6 mg kg^{-1}; $p = 0.040$), indicating a localized early-season depletion at this depth that was not observed at the surface. In contrast, in the 30-year cultivated plot, depth exerted a strong influence on nitrate concentrations. $N\text{-}NO_3^-$ exceeded 80 mg kg^{-1} at 0–20 cm in the first month and decreased sharply with depth (by approximately 48 and 67 mg kg^{-1} at 20–40 cm and 40–60 cm, respectively). The supplementary model yielded a singular fit, with the random intercept variance estimated as zero (Table O3), indicating negligible between-profile differences within this plot. This model also

revealed marked temporal changes in the vertical nitrate profile: from months 3 to 7, several significant interactions between month and depth indicated that the initial steep gradient progressively flattened, reflecting relative increases at deeper layers or reductions at the surface.

4.3.3 Accumulation of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ within the Soil Profile

The accumulated stocks of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ across sampling plots over the monitoring period are presented in Figure 12. $N\text{-}NH_4^+$ accumulation ranged from 38.6 to 163.6 kg ha^{-1} of N and showed similar magnitudes among the cultivated and native grassland plots, with slight temporal fluctuations. In contrast, $N\text{-}NO_3^-$ accumulation ranged from 0.2 to 331.7 kg ha^{-1} of N and was consistently higher in the cultivated plots than in the native grassland, displaying pronounced temporal and spatial variation.

Figure 12. Accumulation of mineral N stocks within the 0-60 cm soil profile under different land use over the monitoring period



Note. Points represent mean concentrations, with error bars indicating standard deviations.

4.3.4 Key Predictors Selected by Recursive Feature Elimination (RFE) for the modelling Scenarios

Detailed results of the RFE for predicting TN, N-NH_4^+, and N-NO_3^- using the multi-depth and topsoil PTFs are presented in Appendix P. For the multi-depth models (S1 scenario), the selected covariates for TN were depth, SOC, P, SWC, EC, K^+, BD, S, and total porosity. For N-NH_4^+, the most relevant predictors included depth, SOC, K^+, pH, SWC, P, and EC. For N-NO_3^-, the selected predictors were SOC, P, K^+, S, pH, SWC, EC, BD, and total porosity.

When only topsoil data (0–20 cm) were considered, RFE identified SOC as the sole relevant predictor for TN across scenarios S1, S2, and S3, while slope was the only predictor selected for scenario S4. This indicates that, when edaphic covariates, particularly SOC, are available, remote sensing variables do not enhance model performance and are excluded. Consequently, scenarios S2 and S3 were excluded for developing topsoil PTFs for TN. For topsoil

$N\text{-}NH_4^+$ prediction, SOC and pH consistently emerged as key predictors across scenarios S1 to S3, with other relevant variables such as SWC, EC, and BD contributing to improved accuracy in some cases. In scenario S3, principal components derived from terrain indexes (PC2) and spectral reflectance (PC1) were also included, providing a dimensionality-reduction approach that helped minimizing model complexity. For scenario S4, a broader range of terrain and spectral predictors were selected, including convergence index, plan and profile curvature, red edge and yellow reflectance, among others. For topsoil $N\text{-}NO_3^-$, common predictors across scenarios S1 to S3 included EC, S, pH, and SOC. In scenario S4, key variables included valley depth, slope, and NIR, among others.

4.3.5 Performance of pedotransfer functions (PTFs)

Tables 10 and 11 summarize the performance of the multi-depth and topsoil PTFs, respectively. Overall, the model performance of multi-depth PTFs ranked highest for TN, followed by $N\text{-}NH_4^+$, and lowest for $N\text{-}NO_3^-$. Both RF and MLR performed similarly for multi-depth predictions of TN and $N\text{-}NH_4^+$, while RF outperformed MLR for $N\text{-}NO_3^-$. Figure 13 presents the results of the external validation, in which multi-depth PTFs were extrapolated using independent data from additional sampling plots within the same HU (i.e., data from the initial soil assessment presented in Chapter III). Among all models, MLR for TN prediction showed the best extrapolation performance (R^2 = 0.80, RMSE = 0.43 g kg^{-1}, MAE = 0.36 g kg^{-1}), with points closely aligned with the 1:1 line. Although MLR also achieved a relatively good performance for $N\text{-}NO_3^-$ extrapolation (R^2 = 0.71), the predictions yielded high error values (RMSE = 25.86 mg kg^{-1}, MAE = 20.36 mg kg^{-1}) and the points were located below the 1:1 line, indicating underestimation of this N pool.

Table 11. Validation metrics (mean ± standard deviation) of multi-depth PTFs using edaphic covariates as predictors (scenario S1) and the leave-one-profile-out cross-validation approach.

Target variable	Random Forest (RF)			Multiple linear regression (MLR)		
	R^2	RMSE	MAE	R^2	RMSE	MAE
TN	0.88 ± 0.11	0.36 ± 0.15	0.28 ± 0.11	0.87 ± 0.14	0.35 ± 0.12	0.28 ± 0.09
$N\text{-}NH_4^+$	0.57 ± 0.23	5.64 ± 1.65	4.31 ± 0.97	0.54 ± 0.22	5.89 ± 0.94	4.42 ± 0.57
$N\text{-}NO_3^-$	0.42 ± 0.25	10.76 ± 5.02	8.17 ± 3.69	0.35 ± 0.24	12.76 ± 4.59	9.72 ± 3.24

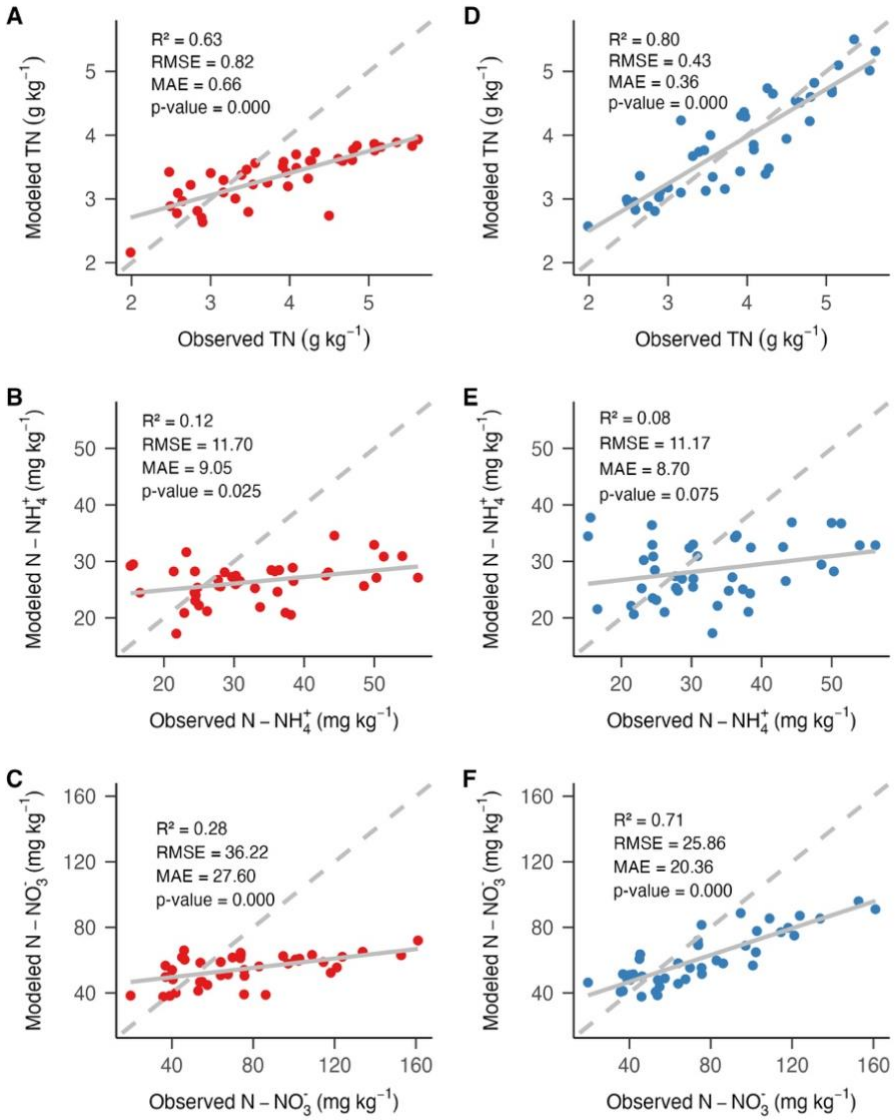
Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen. The units for RMSE and MAE are g kg^{-1} for TN, and mg kg^{-1} for $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$.

Topsoil PTFs validation (Table 11) showed that adding remote sensing covariates (scenarios S2 and S3) did not improve performance compared to models using only edaphic properties (scenario S1), for either $N\text{-}NH_4^+$ or $N\text{-}NO_3^-$. When comparing RF and MLR models across scenarios S1 and S4 using data from the three monitored plots, RF consistently outperformed MLR for the three target variables. Models based solely on remote sensing data (scenario S4) performed worse than those based exclusively on edaphic properties (scenario S1). A comparison of multi-depth PTFs (Table 11) and topsoil PTFs (Table 12) revealed that, while both followed a similar R^2 trend across target variables, the models that included data for the three depths achieved lower RMSE and MAE values, indicating better predictive accuracy across all scenarios and model types.

Regarding extrapolation of the topsoil PTFs using independent plots within the HU (Figure 14), MLR in scenario S1 yielded better results than RF for both TN (R^2 = 0.63, RMSE = 0.62 g kg^{-1}, MAE = 0.51 g kg^{-1}) and $N\text{-}NO_3^-$ (R^2 = 0.64, RMSE = 20.54 mg kg^{-1}, MAE = 17.13 mg kg^{-1}). In both cases, the slope of the regression line between observed and predicted values was close to

1, indicating a proper model fit. However, N-NH_4^+ predictions with RF and MLR under scenario S1 showed poor performance with secondary data. Moreover, none of the topsoil PTFs developed under scenario S4 successfully extrapolated any soil N pools predictions to other plots in the HU.

Figure 13. Extrapolation results for the multi-depth PTFs developed with Random Forest (plots A-C, red) and multiple linear regression (plots D-F, blue) under modelling scenario S1



Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen. The units for RMSE and MAE are g kg^{-1} for TN, and mg kg^{-1} for $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. p-values indicate the statistical significance of the linear relationship between modeled and observed values.

Table 12. Validation metrics of topsoil PTFs for the modelling scenarios using the external validation approach

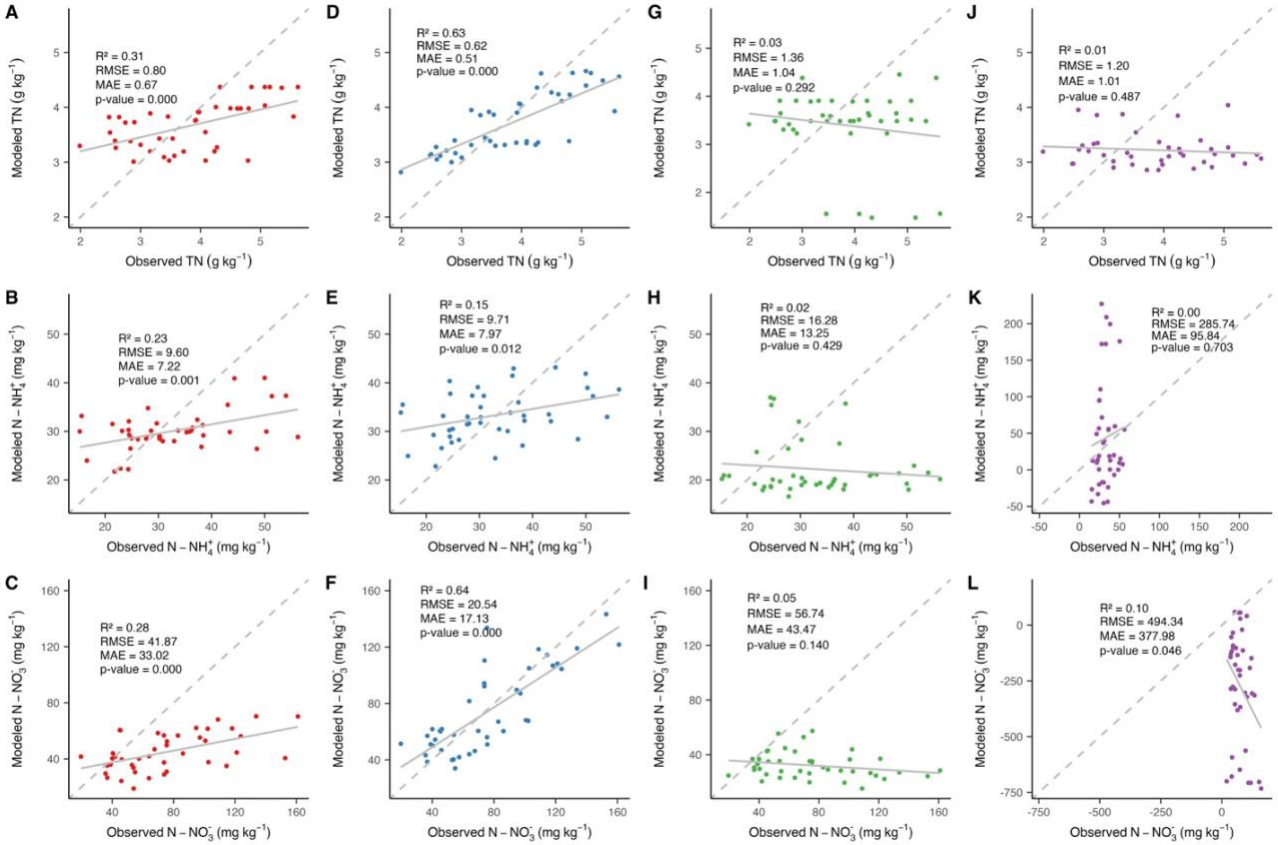
Target variable	Modelling scenario	Random Forest (RF)			Multiple linear regression (MLR)		
		R^2	RMSE	MAE	R^2	RMSE	MAE
TN	S1	0.73	0.50	0.38	0.56	0.67	0.55
	S4	0.53	0.68	0.45	0.22	0.89	0.67
$N\text{-}NH_4^+$	S1	0.70	8.54	5.87	0.45	9.97	7.61
	S2	0.47	9.83	7.32	0.56	9.45	7.02
	S3	0.50	9.58	6.82	0.51	9.64	7.37
	S4	0.31	10.87	8.65	0.29	11.15	8.77
$N\text{-}NO_3^-$	S1	0.59	14.73	12.02	0.43	18.71	15.13
	S2	0.54	15.67	12.50	0.41	18.70	15.19
	S3	0.56	14.82	12.07	0.44	18.32	14.78
	S4	0.37	18.41	13.96	0.35	18.63	14.48

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen. The units for RMSE and MAE are g kg^{-1} for TN, and mg kg^{-1} for $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$.

4.4 Discussion

This section discusses the main findings of the chapter in relation to its two aims: (i) assessing the effects of green onion cropping on the vertical distribution of soil N pools in a páramo ecosystem, and (ii) developing PTFs for estimating N concentrations using edaphic and remote sensing covariates. The implications of these findings for N management in cultivated páramo soils are also addressed.

Figure 14. Extrapolation results of topsoil PTFs using Random Forest (RF) and multiple linear regression (MLR) under two modelling scenarios



Note. TN: total nitrogen, N-NH_4^+: ammonium-nitrogen, N-NO_3^-: nitrate-nitrogen. Plots A–F correspond to scenario S1, while plots G–L correspond to scenario S4. Red and green plots (A–C, G–I) show results from RF, and blue and purple plots (D–F, J–L) show results from MLR. p-values indicate the statistical significance of the linear relationship between modeled and observed values. The units for RMSE and MAE are g kg^{-1} for TN, and mg kg^{-1} for N-NH_4^+ and N-NO_3^-.

4.4.1 Soil Nitrogen Vertical Distribution under Green Onion Cropping

The stratification of TN with depth across sampling plots aligns with the surface accumulation of organic matter and the strong positive correlation between TN and SOC. In contrast to the initial soil assessment in Chapter III, where comparisons across multiple native grassland and cultivated plots consistently showed reduced surface TN under green onion cultivation, the core LMM in the present chapter did not detect a significant land-use effect on TN at 0–20 cm. This difference likely reflects the high spatial heterogeneity of páramo grasslands: the native plot monitored here exhibited comparatively low surface TN values relative to the broader set of reference sites included in the earlier study, thereby reducing the detectable contrast with cultivated plots. Nonetheless, the direction and magnitude of the estimated (but non-significant) reduction in the 3-year cultivated plot (~1 g kg^{-1}) remain consistent with patterns previously attributed to surface application of FCM and the associated priming of native organic matter.

The more uniform TN distribution in the 3-year cultivated plot may be linked to the recent conventional plowing for green onion establishment, which likely homogenized the soil profile. Moreover, as SOC followed a similar vertical trend (Table 9 and Appendix Q), the accumulation of TN at middle and lower depths could also be explained by the downward movement of dissolved organic N (DON), generated through the depolymerization of recalcitrant N-containing organic matter (Wang and Li, 2024). The higher clay content in the 3-year cultivated plot and the observed correlations between TN, particle size fractions, and microporosity suggest the occurrence of physical protection mechanisms, where organic compounds become adsorbed to mineral surfaces or physically occluded within aggregates, limiting further mineralization of DON and reducing their mobility to deeper soil layers (Lehmann and Kleber, 2015).

The LMMs indicated significant temporal variation only in the 3-year cultivated plot. TN increased rapidly following the application of FCM and weed removal, consistent with the addition of fresh organic matter and therefore organic N to the surface soil. In contrast, TN remained stable across all months in the long-term cultivated plot, suggesting a reduced priming response to fresh organic inputs. This subdued temporal variability may reflect the depletion of labile SOM after decades of continuous cultivation, limiting the capacity of recent organic inputs to stimulate additional mineralization.

Lower N-NH_4^+ concentrations in the surface soils of cultivated plots compared to the native grassland (Fig. 4) are consistent with reduced soil acidity associated with agricultural practices, which are known to favor nitrification. This effect was particularly evident in the 30-year cultivated plot, where the highest pH values were recorded. Under such conditions, additional N losses through ammonia (NH_3) volatilization are also plausible, as higher pH levels promote this pathway (Sommer and Ersbøll, 1996), indicating a substantial alteration in N dynamics due to long-term green onion cultivation. The repeated application of FCM is likely to contribute to soil alkalinization through microbial decarboxylation of calcium-organic matter complexes, releasing Ca^{+2} that may promote pH neutralization (Ano and Ubochi, 2007). Additionally, although local farmers occasionally apply lime, its contribution to soil neutralization appears secondary relative to FCM inputs, which is applied at high rates every four to five months.

N-NH_4^+ losses through nitrification and volatilization in long-term cultivated soils may therefore extend beyond the surface layer, as suggested by the lower ammonium concentrations and the pronounced increase in pH observed at all depths in the 30-year cultivated plot (Table 9, Fig. 11 and Appendix Q). Despite surface application of lime and FCM, the observed alkalinization below 20 cm is consistent with mechanisms previously described by de Vargas et al. (2019): (i)

vertical movement of alkaline corrective particles via water flow through macropores, and (ii) transport of Ca^{+2} and Mg^{+2} as soluble organic salts formed by adsorption/desorption with organic anions, enabling their migration and cation exchange with Al^{+3} deeper in the profile. Additionally, long-term FCM inputs may enhance soil pH buffering capacity through the accumulation of dissociable organic functional groups (e.g., –COOH, –OH groups) (Shi et al., 2019), which could help maintain near-neutral pH despite ongoing proton production from nitrification. Taken together, these observations suggest that sustained green onion cultivation is associated with marked shifts in soil acidity and N transformation pathways throughout the soil profile, although the relative contribution of individual mechanisms cannot be isolated within the observational framework of this study.

Temporal variability in ammonium further highlights the contrasting N dynamics between the cultivated soils. In the recently cultivated plot, ammonium responses were mild and transient, consistent with an early-stage system where microbial communities and transformation pathways may still be adjusting to disturbance and fertilization inputs. By contrast, the long-term cultivated plot displayed pronounced fluctuations in surface ammonium over the monitoring period, accompanied by divergent responses at deeper layers. Such vertical decoupling may result from percolation of N during high-rainfall periods, and the slower mineralization of the organic matter added to the soil by fertilization with FCM and weeding.

The markedly higher concentrations of $N\text{-}NO_3^-$ in the cultivated plots compared to the native grassland indicate intense nitrification activity under green onion cropping (Figure 11). In the 30-year cultivated plot, nitrate peaks in the surface soil clearly aligned with FCM applications. In contrast, although the sampling month had a significant effect in the 3-year cultivated plot, concentrations of $N\text{-}NO_3^-$ did not increased following FCM addition. In both cultivated plots, high

$N\text{-}NO_3^-$ concentrations (up to 114 and 70.2 mg kg^{-1} in the 3-year and 30-year cultivated plots, respectively) were found below 20 cm, where root density and nutrient uptake are limited, making these pools particularly vulnerable to leaching losses. Textural differences played a role in shaping the vertical distribution of nitrate across the plots. In the 3-year cultivated plot, the relatively uniform clay content throughout the profile may have contributed to the more even nitrate distribution, possibly due to adsorption onto organo-mineral complexes. In contrast, the 30-year cultivated plot showed a clear increase in sand content with depth, which likely enhanced nitrate mobility, resulting in greater stratification and lower $N\text{-}NO_3^-$ concentrations at 40–60 cm.

The elevated nitrate levels in the middle and lower soil depths of cultivated plots may be primarily due to leaching and vertical transport from the surface, rather than *in situ* nitrification. This is supported by the consistently high macroporosity values across all plots and depths (Table 9), which, in combination with high rainfall and excessive sprinkler irrigation reported for the area (Naranjo, 2025), likely facilitated downward movement of nitrate and other solutes via gravitational water flow (Weil and Brady, 2017). Moreover, subsurface soils in páramos are less favorable for active nitrification due to several factors. First, although SOM is abundant even at 20–40 cm and 40–60 cm depths (see SOC concentrations in Table 9), it is more decomposed and less biologically active than surface inputs such as FCM. Besides, subsurface soil layers typically exhibit lower microbial biomass and activity, limiting mineralization and nitrification rates (Cotrufo et al., 2021; Jones et al., 2018). Additionally, heavier N isotopes, which are less favored by microbial processes such as nitrification, denitrification, and ammonium volatilization, tend to accumulate at depth, further reducing biochemical transformation rates (Högberg, 1997; Rumpel and Kögel-Knabner, 2011).

However, to confirm these hypotheses, future studies should investigate isotopic signatures and microbial activity related to the N cycle across different soil depths, in both cultivated and undisturbed soils. In addition, future research should explicitly quantify vertical profiles of exchangeable Ca^{+2} and Mg^{+2}, lime distribution, and soil buffering capacity to directly test the mechanisms of soil acidity neutralization inferred here and to disentangle the relative roles of FCM inputs, liming practices, and hydrological transport processes. Finally, although the mixed-effects framework appropriately accounts for repeated sampling and unequal replication, the smaller number of sampling points and shorter monitoring period in the native grassland plot may reduce statistical power for detecting some land-use contrasts. This does not compromise the within-plot depth or temporal analyses, but it should be considered when interpreting cross-plot comparisons and motivates additional monitoring efforts in native grasslands.

Finally, as mentioned in the methodology (section 4.2.1), sampling points within each plot were distributed across distinct slope positions (i.e., upper, middle, and lower) to capture potential spatial variability in N concentrations due to runoff. As shown in Appendix M, no differences in TN, $N\text{-}NH_4^+$, or $N\text{-}NO_3^-$ concentrations were observed by slope position in the 30-year cultivated and native grassland plots. However, in the 3-year cultivated plot, consistently lower concentrations of all three N forms were found at the upper slope position across all sampling depths. This pattern may be related to the low uniformity of sprinkler irrigation reported by Naranjo (2025) for this plot. Indeed, previous studies have shown that low and intermittent irrigation uniformity can reduce the spatial homogeneity of soil N distribution within a single plot under surface fertilizer application (Chen et al., 2025). Nevertheless, in the present analysis, N concentrations were interpreted irrespective of slope position, given that soil depth and monitoring period were found to be more influential factors; and that the area, average slope, and distance

between positions in each plot are relatively small (approximately 250 m^2, 3%, and 10 m, respectively). Even so, this finding highlights the relevance of microtopographic variation, suggesting that slope position may warrant more detailed investigation in future studies of nutrient distribution in agricultural soils.

4.4.2 Predicting Nitrogen Pools in Páramo Soils

Although previous studies have reported that random forest (RF) generally outperforms other ML algorithms and linear models for predicting soil N (Dey and Sarma, 2023; Sun et al., 2024; Y. Wang et al., 2022), the results from this chapter indicate that multiple linear regression (MLR) provided superior predictive accuracy for TN when using edaphic properties as covariates. This outstanding performance was evidenced both for the three monitored plots and for secondary plots used in the extrapolation analysis. These findings align with Guevara et al. (2018), who emphasized that predictive model's performance is context-dependent and that algorithms should not be viewed as competing methods but rather as complementary tools, each capturing distinct portions of variability due to differences in underlying assumptions and data characteristics. The multi-depth and topsoil linear PTFs for TN prediction developed in this study achieved high R^2 values and low RMSE and MAE, outperforming results from previous studies applying MLR and RF to predict TN in forest and agricultural soils in Brazil (Calazans et al., 2018), Romania (Paltineanu et al., 2024), and across the EU (Dey and Sarma, 2023).

In this study, the explicit equations produced by MLR (Table 13) not only facilitated accurate predictions but also allowed for clear interpretation of how each covariate influenced TN concentrations. For instance, as shown in Table 13, SOC had a strong positive effect on TN, consistent with previous correlation analysis (see section 4.3.1 and Appendix N). EC and SWC

also had positive effects on TN concentration, while BD showed a consistent negative influence. Other variables that exerted a positive effect on TN but did not show significant correlations in the previous analysis were P, K^+, and S. These influences possibly reflect shifts in nutrient stoichiometry that affect microbial decomposition of SOM (Coonan et al., 2020), thus favoring or limiting N immobilization. Notably, P and K^+ concentrations differed markedly between cultivated plots: at all three depths, P concentrations in the 30-year cultivated plot were up to an order of magnitude higher than in the 3-year cultivated plot, while K^+ levels in the topsoil increased by nearly 40% due to long-term cultivation (see Table 9 and Appendix Q). These contrasting nutrient levels may contribute to the predictive relevance of P and K^+ in the models. On the other hand, the negative coefficient for total porosity, apparently contradictory given the positive correlation with TN, may function as a mathematical correction due to the dominant influence of SOC (Calazans et al., 2018). This strong positive effect of SOC on soil TN pool is also evident in the RFE results for the topsoil PTF, which present this covariate as the only relevant predictor (Table 13).

The MLR approach in this study also allowed assessing and reducing statistical redundancy among predictors, which is particularly relevant when analytical resources are limited. Among the covariates in the multi-depth TN model, only depth, SOC, EC, and K^+ were statistically significant ($p < 0.05$; Appendix R). Further, EC and K^+ were highly correlated (Appendix R), violating a key assumption of linear regression that predictors should be mutually independent or orthogonal. Therefore, simplifying the linear PTFs generated with fewer covariates could be considered (Table 13). To avoid collinearity between predictors, EC was retained given its higher correlation with TN, compared to K^+ (Appendix R). Using simplified functions did not substantially reduce the extrapolation capacity of the TN models for surface soils in the HU, compared to the full model including all covariates selected by RFE. Specifically, the model with depth, SOC, EC, and K^+

achieved an R^2, RMSE, and MAE of 0.79, 0.74 g kg^{-1}, and 0.62 g kg^{-1}, respectively; while the model with depth, SOC, and EC yielded 0.76, 0.91 g kg^{-1}, and 0.79 g kg^{-1}, respectively. However, when applying both simplified models to the full dataset generated in this study, increased prediction errors were found, leading to negative TN estimates for observed TN values ≤ 0.306 g kg^{-1}. This limitation hinders the models' applicability in subsoil layers (20–60 cm), where such low TN concentrations were observed in both cultivated and non-cultivated soils.

Table 13. Pedotransfer functions (PTFs) for predicting nitrogen pools in páramo soils under green onion cropping

Equation	Soil depth	coverage	Relative accuracy
$\mathrm{TN} = \mathrm{a} + 0.3921 \times \mathrm{SOC} + 1.447 \times 10^{-3} \times \mathrm{EC} + 8.273 \times 10^{-5} \times \mathrm{P} + 0.4635 \times \mathrm{SWC} - 1.318 \times \mathrm{BD} + 7.795 \times 10^{-2} \times \mathrm{K^+} + 1.941 \times 10^{-3} \times \mathrm{S} - 3.547 \times \mathrm{Total\_porosity}$ $\mathrm{a} = \begin{cases} 3.5760 \text{ for depth: } 0-20 \text{ cm} \\ 3.3117 \text{ for depth: } 20-40 \text{ cm} \\ 3.2012 \text{ for depth: } 40-60 \text{ cm} \end{cases}$	cm	0–60	++++
$\mathrm{TN} = 0.3921 \times \mathrm{SOC} + 1.447 \times 10^{-3} \times \mathrm{EC} + 7.795 \times 10^{-2} \times \mathrm{K^+}$	cm	0–20	+++
$\mathrm{TN} = 0.3921 \times \mathrm{SOC} + 1.447 \times 10^{-3} \times \mathrm{EC}$	cm	0–20	++
$\mathrm{TN} = 1.607 + 0.3321 \times \mathrm{SOC}$	cm	0–20	+
$\mathrm{N-NO_3^-} = -6.7323 + 5.51 \times 10^{-2} \times \mathrm{EC} + 0.6637 \times \mathrm{S} - 0.7583 \times \mathrm{pH} + 7.2 \times 10^{-3} \times \mathrm{P} - 1.5592 \times \mathrm{SOC} - 1.4714 \times \mathrm{K^+}$	cm	0–20	+

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, K^+: exchangeable potassium, P: available phosphorus, S: available sulfur, EC: electrical conductivity. The units for the variables are those indicated in Table 9.

For mineral N pools, predictive performance was lower. Although RF and MLR provided acceptable predictions for $N\text{-}NH_4^+$ within the three main plots using edaphic properties as predictors, none of the models successfully extrapolated to external plots, limiting their broader applicability. In contrast, topsoil models for $N\text{-}NO_3^-$ showed greater generalizability. This difference may reflect stronger spatial and management-driven variability in $N\text{-}NO_3^-$ patterns compared to the more uniform vertical distribution of $N\text{-}NH_4^+$. The available literature indicates that most PTF studies on N pools have focused on TN, while knowledge on models for mineral N is still incipient. However, a previous study reported high predictive performance for soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ ($R^2 \geq 0.93$, RMSE ≤ 3.14 mg kg^{-1}) using vegetation indexes and climate variables as covariates (Sun et al., 2024).

The linear topsoil PTF developed to predict $N\text{-}NO_3^-$ (Table 13) includes EC as one of the predictors, which aligns with the positive significant correlation identified in the earlier analysis. Other relevant covariates selected were the concentrations of P, S, and K^+. The positive or negative effects of these variables may reflect antagonistic or synergistic ionic interactions, which can respectively hinder or enhance $N\text{-}NO_3^-$ uptake by plants due to increased concentrations of competing or complementary nutrients (Rietra et al., 2017). The linear equation also highlights a negative relationship between $N\text{-}NO_3^-$ and both pH and SOC, likely associated with enhanced mineralization of SOM and increased nitrification following the neutralization of soil acidity. However, the statistical analysis of the MLR model indicated that only EC and S were significant predictors (Appendix R), and these two covariates were not correlated with each other (Appendix R). Nonetheless, the validation metrics obtained for the simplified model using only EC and S revealed poor predictive performance ($R^2 = 0.58$; RMSE = 28.38 mg kg^{-1}; MAE = 22.21 mg kg^{-1}). Considering that relatively weak correlations between $N\text{-}NO_3^-$ and the edaphic properties were

observed in this study, future research could explore the improvement of PTFs for estimating this N pool by incorporating variables related to soil water movement.

Incorporating remote sensing covariates did not improve the accuracy of pedon-scale models or enhance the performance of DSM-based models (scenario S4). This contrasts with studies where PS imagery successfully predicted soil TN (Komariah et al., 2021; Pereira et al., 2022) and with evidence that NIR reflectance is relevant for soil N prediction (Huai et al., 2025). However, in mountainous terrain those in the study area, steep slopes and shading effects can reduce the reliability of surface reflectance data (Wang et al., 2025). Moreover, other studies have shown that vegetation indexes derived from soil spectral signatures outperform individual reflectance values and terrain attributes in predicting soil properties (Fathololoumi et al., 2020). Sun et al. (2024), for instance, demonstrated the utility of the Normalized Difference Vegetation Index (NVDI) combined with climatic variables for predicting soil mineral N. Finally, limited spatial variability in the input data used in this study may have constrained model performance, especially for remote sensing covariates. Future studies should consider expanding the sampling area to increase environmental heterogeneity and test additional predictors, such as vegetation indexes and climatic variables (e.g., temperature, precipitation, solar radiation), which exhibit strong spatial gradients in the Santurbán-Berlín páramo region.

4.4.3 Nitrogen Management Implications

Beyond land cover and land-use effects, this chapter's findings underscore the importance of soil heterogeneity and local agronomic practices in shaping the vertical and temporal distribution of soil N pools. One relevant implication concerns the vertical distribution of soil texture, which differed markedly between the two cultivated plots. The relatively uniform clay

content in the 3-year cultivated plot is consistent with more limited vertical redistribution of N, potentially through enhanced physical protection and retention mechanisms. In contrast, the higher sand content in deeper layers of the 30-year cultivated plot coincided with greater mineral N accumulation at depth, suggesting higher mobility and leaching potential. These observations indicate that assessing soil texture across multiple depths is important for informing fertilization and irrigation practices, particularly in intensively managed páramo soils.

Differences between the continuously cultivated plot and the plot that experienced a decade-long fallow prior to three years of green onion cultivation further highlight the role of land-use history in modulating soil N dynamics. In the long-term cultivated plot, shifts in soil acidity and buffering capacity were evident throughout the profile and were consistent with the cumulative influence of repeated FCM applications over time. In contrast, these effects appeared less pronounced in the plot that underwent a fallow period, suggesting that temporary cessation of cultivation may partially mitigate long-term alterations in soil chemical properties. In páramo ecosystems, fallow periods have been associated with rapid nutrient uptake by early successional vegetation, promoting nutrient storage in plant biomass rather than in soil pools (Llambí and Samiento, 1998). While this dynamic may explain lower available P and K^+, its influence on mineral N appears more complex, as previous studies indicate that subsoil N accumulation can persist for decades following fertilization (Chen et al., 2022).

A particularly relevant aspect of current management practices is their influence on mineral N accumulation and its temporal variability. For instance, the average stocks of $N\text{-}NO_3^-$ fluctuated between 1.6 to 5.2 kg ha^{-1} of N in the native grassland, while in the cultivated plots these average stocks were between 105.4 to 223.1 kg ha^{-1} of N The analysis conducted in this chapter also revealed notable monthly variation in $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ stocks (Figure 12). In the 3-year

cultivated plot, between the FCM application and the days following harvest (months 2 to 3), average stocks decreased by 22.7 kg $N\text{-}NH_4^+$ ha^{-1} and 53.1 kg $N\text{-}NO_3^-$ ha^{-1}. Between the second harvest and the subsequent FCM application (months 6 to 7), stocks increased by 42.3 kg $N\text{-}NH_4^+$ ha^{-1} and 102.7 kg $N\text{-}NO_3$ ha^{-1}. In the 30-year cultivated plot, a loss of 0.5 kg $N\text{-}NH_4^+$ ha^{-1} and 46.3 kg $N\text{-}NO_3^-$ ha^{-1} was recorded between the first harvest and the subsequent FCM application (months 2 and 3). Between months 6 and 7, following the last hilling and shortly after the final FCM application during the monitoring period, the stock increased by 23.7 kg $N\text{-}NH_4^+$ ha^{-1} and 71.2 kg $N\text{-}NO_3^-$ ha^{-1}. In contrast, the largest month-to-month decline in the native grassland plot was just 25.1 kg N ha^{-1}, reinforcing that these large fluctuations are primarily driven by agricultural management.

The magnitudes of the negative shifts in mineral N average stocks in cultivated plots suggest substantial N losses, especially considering that, N removal through green onion harvest is estimated at 34–55 kg N ha^{-1} (see Table 2 in Chapter II). The strong temporal fluctuations in accumulated mineral N also reflect the complexity of N dynamics in cultivated páramo soils and highlight the challenges in optimizing fertilization management for green onion. Future research should assess how N stocks respond to different fertilization strategies to support improved decision-making and promote sustainable soil management in páramo ecosystems.

Finally, to support improved N management, the use of the linear PTFs developed in this study (Table 13) is proposed for estimating TN and $N\text{-}NO_3^-$ concentrations in soils under green onion cropping in the study area. These functions may be applicable to other sites within the Santurbán-Berlín páramo, provided that the soils share similar taxonomic characteristics to those of the SCU units present in the study HU (Figure 9), and that the distributions of predictor variables data align with those used in model development (Appendix K). Model relative accuracy levels

and depth coverage reported in Table 13 should be considered when applying these functions for decision-making, both within and beyond the study area.

4.5 Conclusions

Green onion cultivation alters the vertical distribution of soil N pools in páramo soils through interacting effects of management and soil physical properties. Total N concentrations declined with depth across all plots; however, recently cultivated soils, despite their long agricultural legacy prior to a decade of fallow, exhibited a more homogenized TN profile. This pattern is consistent with the combined effects of recent plowing and contrasts in soil texture, particularly the higher clay content in the 3-year cultivated plot and the sandier subsoil in the 30-year cultivated plot, which appear to modulate N retention and mobility.

Inorganic N pools showed significant changes driven by cultivation. Ammonium displayed a shallower vertical gradient in cultivated soils, while nitrate exhibited substantial enrichment and distinct depth-dependent patterns relative to the native grassland. Subsurface $N\text{-}NO_3^-$ concentrations reached up to 114 mg kg^{-1}, compared to only 1.76 mg kg^{-1} in native grassland. Month-to-month declines in average mineral N stocks were also greater in cultivated soils, reaching up to 75.8 kg N ha^{-1}, nearly three times higher than the maximum decline observed in uncultivated soil (25.1 kg N ha^{-1}), indicating substantial N losses. These patterns co-occurred with shifts in soil acidity and buffering capacity observed under sustained fertilization with fresh chicken manure, and were particularly evident below the typical rooting zone. Together, these findings suggest that current management practices are associated with increased subsurface mineral N accumulation, potentially limiting plant uptake efficiency and increasing vulnerability to leaching losses.

Among the developed pedotransfer functions (PTFs), multiple linear regression (MLR) models based on edaphic properties achieved the best performance and extrapolation capacity for TN (R^2 = 0.80, RMSE = 0.46 g kg^{-1}, MAE = 0.36 g kg^{-1}) and N-NO_3^- (R^2 = 0.64, RMSE = 20.54 mg kg^{-1}, MAE = 17.12 mg kg^{-1}). No model reliably predicted N-NH_4^+, and the inclusion of remote sensing covariates did not improve performance. These findings highlight the importance of analyzing vertical N patterns over extended monitoring periods to understand how agricultural practices reshape soil N dynamics and to support predictive modelling for improved N management in páramo ecosystems. The ability to predict TN and N-NO_3^- with relatively high accuracy is particularly valuable, as these pools serve as indicators of soil fertility and potential N losses. The developed models can be applied to other green onion–cultivated soils in the Santurbán-Berlín páramo, as long as they share similar taxonomy and management conditions with the monitored plots.

5. Taxonomic and Functional Dynamics of the Nitrogen-Cycling Microbiota in Páramo Soils under Green Onion Cropping

5.1 Introduction

Soil biodiversity encompasses the variety of life forms that inhabit the soil, ranging from genes and species to the communities they form and the ecological complexes to which they belong (FAO et al., 2020). In terms of taxonomy, function, and adaptive strategies, the diversity of soil organisms is remarkable (Orgiazzi et al., 2016). Soil organisms play essential roles in food production through the ecological functions they perform, particularly those related to nutrient cycling (Bender et al., 2016). For instance, nitrogen (N) availability in soil depends on several transformation processes mediated by complex networks of metabolically versatile microorganisms (Takai, 2019). These transformations constitute the biogeochemical N cycle, which includes major processes such as ammonification, molecular nitrogen (N_2) fixation, assimilation, nitrification, anaerobic ammonium oxidation (anammox), and denitrification (Stein and Klotz, 2016).

Based on the biochemical pathways involved, N cycling in soil can be subdivided into three main mechanisms: i) decomposition, ii) assimilatory processes, and iii) dissimilatory processes. As described by other authors (Flores and Herrero, 1994; Levy-Booth et al., 2014), decomposition involves the breakdown of soil organic matter (SOM) and plant litter, releasing high molecular weight soil organic N (SON), which is subsequently transformed into low molecular weight dissolved organic N (DON). Assimilatory processes refer to the incorporation of N compounds into microbial biomass, while dissimilatory processes involve the use of N compounds as electron

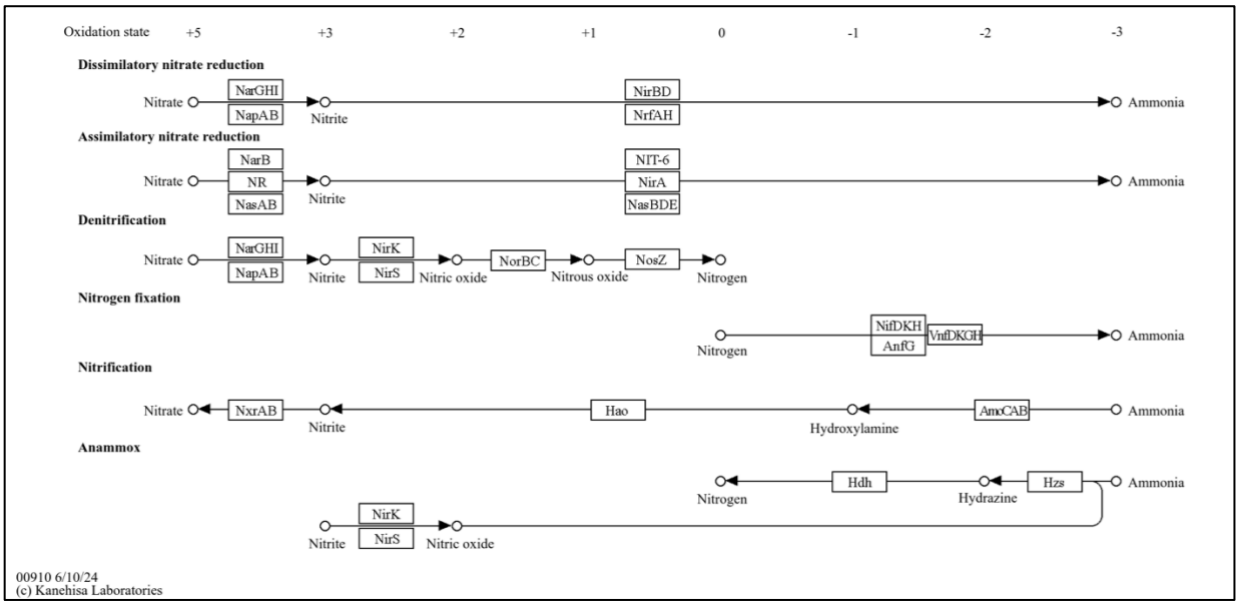
acceptors or donors to gain energy. Figure 15 presents the N metabolism pathways and their associated functional genes, as defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG). In line with this, recent studies have emphasized that N cycling is not driven by individual microbes in isolation, but rather by the soil microbial community as a collective entity (Han et al., 2025).

Changes in the composition and functionality of soil microbial communities are key indicators of the effects of environmental and anthropogenic factors on N dynamics, given the rapid response of these organisms to external disturbances (Rincon-Florez et al., 2013). To better capture these microbial responses, the characterization of microbial communities using molecular tools based on DNA and/or RNA has increased, offering an alternative to traditional cultivation methods, which are limited by the low proportion of culturable microbial strains in terrestrial ecosystems (~25%) (Martiny, 2019). Among these tools, targeted DNA metabarcoding is the most widely used approach for studying microbial biodiversity. This technique involves the amplification of taxonomically informative genetic markers, such as 16S and 18S rRNA genes, via polymerase chain reaction (PCR), followed by high-throughput sequencing of the resulting amplicons (Francioli et al., 2021). The sequencing data are then processed through bioinformatics pipelines and compared with reference databases to identify and quantify microbial taxa (Hernández et al., 2020) and to infer their functional roles within ecosystems (Djemiel et al., 2022).

Microorganisms involved in N transformation processes are traditionally classified based on their functional roles (e.g., N_2 fixers, nitrifiers, denitrifiers). However, genome sequencing studies over the past decade have challenged this categorization, revealing that many of these organisms possess highly versatile and overlapping metabolic capabilities (Kuypers et al., 2018). Consequently, beyond taxonomic profiling and functional inference, it is essential to directly

assess functional gene markers, such as those involved in specific N transformations, using quantitative PCR (qPCR) (Aguilera et al., 2014). This approach offers more accurate insights into the functional potential of microbial communities and helps elucidate the effects of agriculture on soil N dynamics (Ouyang et al., 2018).

Figure 15. Reference nitrogen metabolism pathways and associated functional genes based on KEGG map 00910



Source: (Kanehisa et al., 2025)

Although molecular tools have been widely used to assess the impact of agricultural activities on N-cycling microorganisms, findings from studies in other contexts cannot be readily generalized to high-mountain ecosystems such as páramos, which are characterized by extreme environmental conditions. Microorganisms inhabiting these environments have evolved distinct strategies to cope with challenges such as low temperatures, high solar radiation, and limited nutrient availability. These adaptations include the synthesis of cold-shock proteins, cryoprotectors, and antifreeze molecules, as well as seasonal dormancy and modifications to

enzyme kinetics (Pandey and Yarzábal, 2019), which enable them to sustain key biogeochemical processes, such as nutrient mobilization and the mineralization of SOM, at low temperatures (Pandey et al., 2019). In addition, these microorganisms possess highly efficient and precise DNA repair systems that protect them from ionizing and ultraviolet radiation (Zhu et al., 2024).

To 2026, research on soil microorganisms in páramos has focused primarily on conserved ecosystems (i.e., areas unaffected by human intervention) and on evaluating the effects of potato (*Solanum tuberosum*) cultivation and fallow land on the composition of microbial communities or specific functional groups involved in biogeochemical cycles. Although páramo ecosystems extend across several Andean countries, most peer-reviewed studies on soil microbial communities, particularly those examining agricultural impacts, have been conducted in Colombian páramos. Early studies used culture-dependent techniques to identify cellulolytic microorganisms in undisturbed páramo soils (Avellaneda-Torres et al., 2014; Bernal Pinilla et al., 2006; Lizarazo-Medina and Gómez-Vásquez, 2015). Other culture-based studies reported a high abundance of proteolytic microorganisms, which may provide substantial inputs of organic N to the soil and tend to predominate over other N-cycling groups, regardless of land use (Cañón-Cortázar et al., 2012; Flórez-Zapata et al., 2011).

More recent studies in different Colombian páramos reported contrasting results regarding the influence of potato cultivation and fallow periods on the structure of culturable functional groups involved in N_2 fixation, phosphate solubilization, and cellulose degradation (Avellaneda-Torres et al., 2020; Farfán et al., 2020), suggesting that local agricultural practices and edaphic variability may play a central role in shaping microbial community dynamics. While most previous studies in páramo soils have relied on cultivation techniques to characterize microbial communities, more recent research has incorporated metabarcoding (Reyes-Ardila et al., 2024;

Vélez-Martínez et al., 2024) and shotgun metagenomic sequencing (Rangel Ibáñez, 2025) to explore microbial diversity in conserved páramo soils, offering new insights into these highly diverse and environmentally extreme ecosystems.

Despite these pioneering efforts, the understanding of how agricultural activities affect the microorganisms involved in N dynamics in páramo soils remains limited. Available literature suggest that no studies in these ecosystems have comprehensively assessed the functional potential of soil microbial communities involved in multiple key N transformation pathways, limiting the understanding of their ecological roles under changing land-use conditions. Moreover, the effects of green onion (*Allium fistulosum*) cultivation have not been previously addressed, despite its prominence as a key economic activity among smallholder farmers in certain Colombian páramo regions (Sarmiento et al., 2017). Therefore, this chapter aims to evaluate shifts in the soil bacterial community composition and the dynamics of the N-cycling microbiota in response to green onion cropping in páramo soils. This study represents one of the first efforts to integrate taxonomic and functional molecular approaches to investigate N-cycling microbial dynamics in a high-Andean páramo agroecosystem. The findings offer valuable insights for assessing the sustainability of current farming practices and may inform biodiversity-based strategies to improve N use efficiency and reduce dependence on external inputs.

5.2 Methods

This section outlines the methodology used to evaluate shifts in the soil bacterial community composition and the dynamics of N-cycling microbiota in response to green onion cropping. It begins with a description of the soil sampling design and DNA extraction protocols. Details of the high-throughput sequencing of the 16S rRNA gene are then provided, followed by

the bioinformatics analyses conducted for taxonomic profiling. Subsequent subsection describe the quantification of functional genes using qPCR and functional inference. Finally, the statistical and analytical approaches employed for data analysis are described.

5.2.1 Soil Sampling Design and DNA Extraction

In parallel with the soil monitoring activities described in Chapter IV, soil samples were collected for microbial community analysis using molecular techniques. Sampling was conducted in the same three plots described previously: (i) a plot cultivated with green onion for three years following a fallow period, (ii) a plot under continuous green onion cultivation for 30 years without fallow or rotation, and (iii) a plot with native grassland that had never been cultivated. The location of these plots is shown in Figure 9 (Chapter IV). Three sampling points were established within each plot, and soil samples were taken from the 0–20 cm layer. In cultivated plots, samples were collected from the rhizosphere, the zone with the most complex and dynamic plant-microbiota interactions (Backer et al., 2018). Sampling was performed monthly between August and October 2023, yielding a total of 27 samples (3 months × 3 plots × 3 sampling points). Samples were collected in sterile 50 mL capped plastic tubes and stored at –20 °C for up to two months, following established protocols to preserve sample integrity for molecular analysis (Rubin et al., 2013). During the sampling period, agricultural practices in the study plots were monitored through field observations and farmer interviews.

For DNA extraction, 250 mg of soil (fresh weight) per sample was processed using the DNeasy PowerSoil Pro Kit (QIAGEN, USA), following the manufacturer's protocol. For each soil sample, two replicate DNA extracts were obtained: one of them was used for quantification of functional genes via qPCR, and the other for 16S rRNA gene amplicon sequencing. Although

different extracts were used for each application, both were replicates derived from the same soil sample, and differences between them were limited to DNA concentration and quality. The DNA concentration and purity were assessed using an IMPLEN NP80 nanophotometer (IMPLEN, Germany). According to the criteria reported in the literature (Alejos Velázquez et al., 2014), DNA quality was considered adequate when: (i) the concentration was ≥5 ng μL^{-1}; (ii) the 260 nm to 280 nm absorbance ratio (A260/A280) was ≥1.8, indicating minimal protein contamination; and (iii) the 260 nm to 230 nm absorbance ratio (A260/A230) ranged between 2.0 and 2.2, with values outside this range suggesting possible contamination by carbohydrates or phenolic compounds. The concentration and absorbance values for all samples are provided in Appendix S. Most A260/A230 ratios were below the recommended lower limit, likely due to the high organic matter content of the soils. However, DNA quality was deemed acceptable for downstream applications, as PCR amplification is more strongly affected by the A260/A280 ratio than the A260/A230 ratio (Krsek and Wellington, 1999). DNA integrity was further confirmed by 1% agarose gel electrophoresis. All DNA extracts were stored in 1.5 mL plastic tubes at –20 °C for up to 10 months.

5.2.2 Taxonomic profiling and diversity estimation

Soil bacterial communities were characterized via high-throughput sequencing. The extracted DNA was used to amplify the V3–V4 region of the prokaryotic 16S rRNA gene using universal primers targeting this region (Klindworth et al., 2013), generating a ~460 bp amplicon. The full-length sequences of primers used were as follows:

- Forward: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′

- Reverse: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTA TCTAATCC-3′

Library preparation was conducted using the Nextera XT DNA Library Preparation Kit, following the manufacturer's protocol (Illumina, 2022). Sequencing was carried out at the Kyushu Institute of Technology (Japan) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) with paired-end 300 bp reads. The samples from the first monitoring month were sequenced in September 2023, while those from the second and third months were sequenced in January 2024.

The raw FASTQ files generated from sequencing were processed using the QIIME 2 platform (version 2024.2) (Bolyen et al., 2019). The pipeline included removal of Illumina adapters and primers, quality filtering of sequences, and grouping them according to error profiles within samples to identify amplicon sequence variants (ASVs). Sequence trimming and denoising were performed using the DADA2 plugin in QIIME 2 (Callahan et al., 2016).

To determine trimming parameters and assess sequence quality, the FASTQC tool (https://www.bioinformatics.babraham .ac.uk/projects/fastqc/) was used to assess Phred scores. These scores range from 4 to 60, with higher values indicating greater confidence in base calls (Ambardar et al., 2016). A Phred score of 30, corresponding to 99.9% base call accuracy, was used as the quality threshold. Appendix T shows the read positions where the Phred score dropped below this threshold for each sequencing run. Based on the FASTQC results, several filtering parameter sets were tested, and those retaining the highest proportion of high-quality sequences across samples were selected. The final trimming and filtering parameters are provided in Appendix U. The outputs from the two DADA2 runs (i.e., samples sequenced on September 2023 and January 2024) were merged for subsequent taxonomic assignment using the SILVA database (Pruesse et al., 2007), with a naïve Bayes classifier (silva-138-99-nb-classifier.qza).

Microbial community composition and diversity were analyzed using the Phyloseq R package (McMurdie and Holmes, 2013). Taxon abundances were calculated at the phylum, family, and genus levels, and alpha diversity was estimated using the Shannon and Simpson indexes (Shannon, 1948; Simpson, 1949). Beta diversity was assessed using Bray-Curtis dissimilarities (Beals, 1984) and visualized through Principal Coordinate Analysis (PCoA).

5.2.3 Quantification of N-cycling genes and functional inference

The absolute abundance of key N-cycling genes was quantified using qPCR. The target genes included: *nifH* (encoding nitrogenase reductase for biological N_2 fixation), *amoA* from archaea (AOA) and bacteria (AOB) (encoding ammonium monooxygenase for the oxidation of NH_3 to NH_2OH), *nirS* (encoding nitrite reductase for the reduction of NO_2^- to NO during denitrification), and *nosZ* (encoding nitrous oxide reductase for the final reduction step of N_2O to N_2 in denitrification). These genes are widely used as functional markers to evaluate the effects of agricultural practices on N dynamics in soils, as their abundances are often significantly correlated with corresponding process rates (Ouyang et al., 2018; Zhong et al., 2023). The qPCR analyses were performed in the Biochemistry and Microbiology Research Group (GIBIM) Laboratory at the Universidad Industrial de Santander (UIS, Colombia).

To verify amplification specificity and optimize the conditions for each target gene, end-point PCRs were initially conducted using soil genomic DNA and primers selected from previous studies. Initial reaction conditions (e.g., annealing temperature and cycle number) were adjusted according to the literature. The specificity of the PCR products was verified via 1% agarose gel electrophoresis, confirming the presence of a single fragment of the expected size. If multiple bands, no product, or unexpected fragment sizes were observed, the reaction conditions (i.e.,

thermal cycling parameters and reagent concentrations) were adjusted until a clear, specific amplification product of adequate intensity was obtained. The concentration of each PCR product was measured using a microvolume spectrophotometer (IMPLEN, Germany). Once specificity was confirmed, standard curves were generated from tenfold serial dilutions for subsequent qPCR analysis.

The qPCR reactions were performed in a final volume of 20 µL, comprising: 10 µL of Luna® Universal qPCR Master Mix (New England Biolabs, USA), forward and reverse primers at an initial concentration of 10 µM (0.8 µL each for *nirS*; 0.4 µL each for the other genes), 1 µL of template DNA, and nuclease-free water to complete the volume. The reactions were run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). The thermal profile consisted of an initial denaturation at 95 °C for 3 minutes, followed by n amplification cycles (32 cycles for *nirS*, 35 cycles for the other genes) of 95 °C for 10 seconds and gene-specific annealing temperatures for 45 seconds. A melting curve analysis was conducted at the end of each reaction to confirm amplification specificity. All the qPCR assays included negative controls (without template DNA) to rule out contamination. The primer sequences, amplicon lengths, annealing temperatures, and additional reaction conditions for each gene are provided in Appendix V.

To infer the functional potential of microbial communities related to N cycling, the PICRUSt2 pipeline was applied (Douglas et al., 2020) following approaches described in previous studies (Shen et al., 2021; K. Wang et al., 2022). Representative ASV sequences were placed into a reference phylogenetic tree, and the functional potential of each ASV was predicted based on its phylogenetic proximity to reference genomes with known gene content. These predictions were then used to estimate the abundance of functional genes in each sample. The functional predictions focused on N metabolism pathways by selecting orthologs from the KEGG database

(https://www.genome.jp/kegg/). The metabolic pathways, genes, and KEGG Orthologs (KOs) included in the analysis are listed in Table 14.

Table 14. Nitrogen metabolism pathways and corresponding KEGG Orthologs (KOs) included in the functional inference

Pathway	Gene	KO
Ammonia (NH_3) oxidation	*hao*	K10535
	amoA	K10944
	amoB	K10945
	amoC	K10946
Ammonification (i.e., organic N to NH_4^+)	*ureA*	K01430
	ureB	K01429
	ureC	K01428
Assimilatory nitrate (NO_3^-) to nitrite (NO_2^-) reduction	*nasB*	K00360
	narB	K00367
Assimilatory nitrite (NO_2^-) to ammonium (NH_4^+) reduction	*nirA*	K00366
	nasE	K26138
	nasD	K26139
Dissimilatory nitrate (NO_3^-) reduction or nitrite (NO_2^-) oxidation	*narG/nxrA*	K00370
	narH/nxrB	K00371
Dissimilatory nitrate (NO_3^-) to nitrite (NO_2^-) reduction	*narI*	K00374
	napA	K02567
	napB	K02568
Dissimilatory nitrite (NO_2^-) to ammonium (NH_4^+) reduction	*nirB*	K00362
	nirD	K00363
	nrfA	K03385
	nrfH	K15876
Nitric oxide (NO) to nitrous oxide (N_2O) reduction	*norC*	K02305
	norB	K04561
Nitrite (NO_2^-) to nitric oxide (NO) reduction	*nirK*	K00368
	nirS	K15864
Nitrogen (N_2) fixation	*anfG*	K00531
	nifD	K02586
	nifH	K02588
	nifK	K02591
Nitrous oxide (N_2O) to nitrogen (N_2) reduction	*nosZ*	K00376

Notably, KEGG assigns a single KO identifier to each enzymatic function based on catalytic activity, without necessarily distinguishing the ecological role or directionality of the process. This may introduce ambiguity when interpreting functional potential. In this study, such ambiguity was observed for K00370 (*narG* and n*xrA*) and K00371 (*narH* and *nxrB*), which may encode either nitrate reductase or nitrite oxidoreductase subunits. Consequently, in interpreting the results, these KOs were grouped into a combined category representing both the dissimilatory reduction of nitrate to nitrite and the oxidation of nitrite to nitrate.

Finally, to identify the main taxa contributing to each N metabolic pathway, the predicted KO abundance table generated by PICRUSt2 was merged with the ASV taxonomy assignments. For each KO, the relative contribution of each taxon was calculated by summing the abundances of ASVs annotated to that taxon and associated with the KO. Then, the contributions of individual KOs were aggregated according to the N metabolic pathway in which they are involved. Taxonomic contributions were summarized at the genus level to facilitate interpretation.

5.2.4 Statistical Analyses

The effect of the sampling plot on alpha diversity was evaluated with the Kruskal-Wallis test, followed by Dunn's *post-hoc* test for pairwise comparisons. The influence of the sampling plot on community structure was tested via permutational multivariate analysis of variance (PERMANOVA). To explore the relative abundances of the dominant taxa and key N-cycling genera across sampling plots and months, stacked bar plots were generated. Group comparisons were performed using STAMP software (Parks et al., 2014), applying Welch's t-test with Bonferroni correction to evaluate differences between plots (i.e., native grassland vs. cultivated plots, and between cultivated plots).

The influence of green onion cropping on the N-cycling functional potential was evaluated using the absolute abundance of functional genes (quantified via qPCR) through a two-way repeated-measures analysis of variance (ANOVA). Log10 transformation was applied to improve normality and homogeneity of variances prior to ANOVA-based analyses. *Post hoc* comparisons were conducted using Tukey's honestly significant difference (HSD) test. For genes showing no significant interaction between plot and month, pairwise comparisons were performed separately for the main effects of land use and sampling month.

Changes in the prokaryotic functional potential related to N-cycling were further explored using gene abundance predictions generated by PICRUSt2. For each sample, the abundances of KOs associated with the 11 nitrogen-related pathways identified (Table 14) were summed, and the relative contribution of each pathway was expressed as a proportion of the total predicted N-function abundance. Differences in pathway-level inferred abundances between land uses were tested using t-tests or Mann–Whitney U tests, depending on data distribution. The contribution of individual genera to each pathway was inferred from the stratified PICRUSt2 output, and genera contributing ≥5% to a given pathway were considered dominant contributors. To validate the functional predictions, Spearman's rank correlation was used to assess the relationship between PICRUSt2-predicted gene abundances and absolute gene copy numbers obtained by qPCR.

Finally, to evaluate the influence of soil physicochemical properties (from Chapter IV) on microbial composition, redundancy analysis (RDA) was performed using the vegan package in R (Oksanen et al., 2023). As described in Chapter III, RDA is a constrained ordination method that quantifies the proportion of variance in community composition explained by environmental variables (Ramette, 2007). For this analysis, genus-level ASV abundances were Hellinger-transformed, and the following soil variables were included: total nitrogen (TN), ammonium (N-

NH_4^+), nitrate ($N\text{-}NO_3^-$), soil organic carbon (SOC), available phosphorus (P), exchangeable potassium (K), available sulfur (S), pH, gravimetric soil water content (SWC), bulk density (BD), total porosity, electrical conductivity (EC), and percentages of sand, silt, and clay. The significance of the RDA model was tested using permutation tests (Paliy and Shankar, 2016). In addition, Spearman's correlation was applied to explore associations between these soil properties and both the abundance of key N-cycling genera and the absolute abundance of functional genes measured by qPCR.

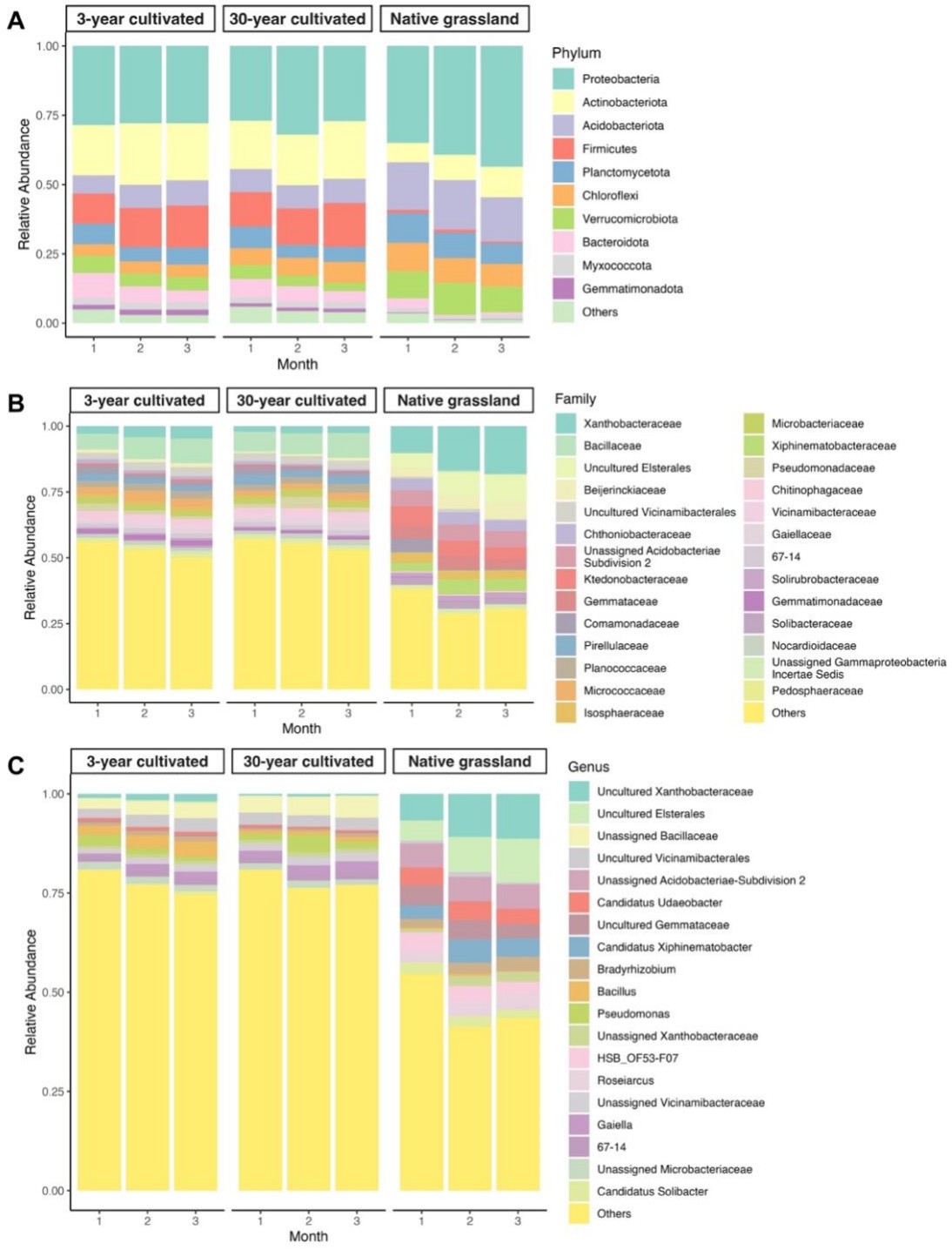
5.3 Results

5.3.1 General Description of Bacterial Communities

The relative abundances of the dominant bacterial taxa at the phylum, family, and genus level are presented in Figure 16 and results of the statistical comparisons are summarized in Appendixes W-AB. Significant differences were observed in the abundances of the ten dominant phyla between native grassland and cultivated plots ($p < 0.01$; Figure 16A).

Compared to native grassland, green onion cropping significantly reduced the relative abundance of Proteobacteria (Pseudomonadota), Acidobacteria (Acidobacteriota), Verrucomicrobiota, Chloroflexi (Chloroflexota), and Planctomycetota, whereas the opposite trend was detected for Firmicutes (Bacillota), Actinobacteriota (Actinomycetota), Bacteroidota, Gemmatimonadota, and Myxococcota (Appendix W). Additionally, Gemmatimonadota and Chloroflexi differed significantly between the two cultivated plots, with the former being more abundant and the latter less abundant in the 3-year cultivated plot ($p < 0.05$; Appendix X).

Figure 16. Relative abundance of dominant taxa across sampling plots and months at the phylum (A), family (B), and genus (C) levels. Taxa with mean relative abundances <1% were grouped under the "Others" category.



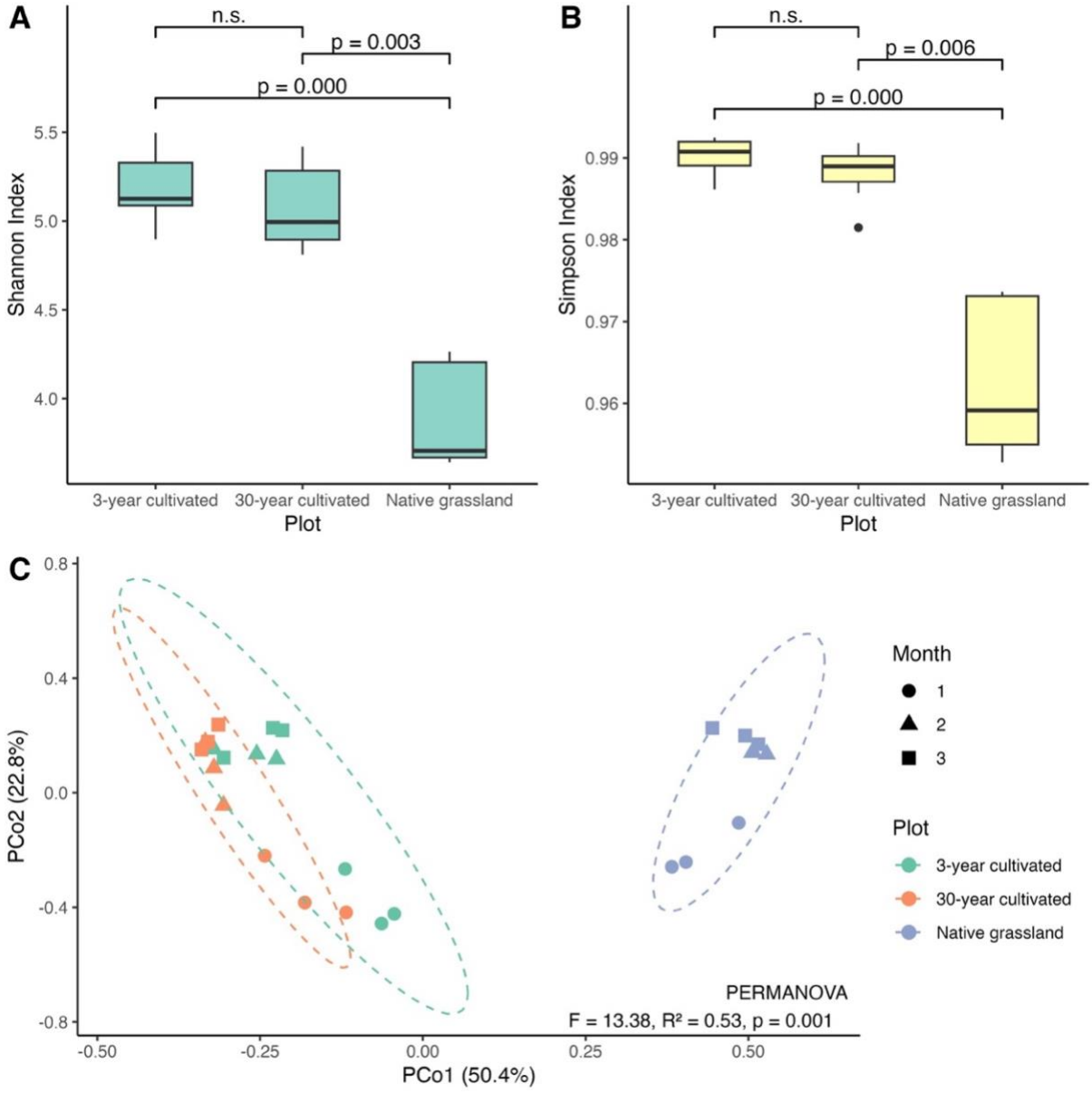
Twenty-seven dominant families were identified, including uncultured taxa from the Elsterales and Vicinamibacterales orders (Figure 16B), most of which presented significant differences in abundance between the native grassland and the cultivated plots ($p < 0.01$; Appendix Y). Notable examples include higher abundances of Xanthobacteraceae and an uncultured family from Elsterales in the native grassland, and increased abundance of Bacillaceae in cultivated plots. Gemmatimonadaceae was the only family that differed significantly between cultivated plots and was more abundant in the 3-year cultivated plot ($p < 0.001$; Appendix Z).

Nineteen dominant genera were detected across the samples (Figure 16C), including uncultured genera from Xanthobacteraceae and Gemmataceae, and from the Elsterales and Vicinamibacterales orders. Several unassigned (i.e., unclassified) genera from Bacillaceae, Xanthobacteraceae, Vicinamibacteraceae, Microbacteriaceae and the Subdivision 2 of Acidobacteria were also prevalent. All dominant genera except *Pseudomonas, Bacillus,* and an unassigned genus from Xanthobacteraceae differed significantly between the native grassland and cultivated soils ($p < 0.05$; Appendix AA). The most notable reductions due to cultivation were observed for uncultured genera from Xanthobacteraceae and Elsterales, while the unassigned genus from Bacillaceae increased significantly in cultivated plots. No significant differences in dominant genera were observed between the two cultivated plots ($p > 0.05$; Appendix AB).

Alpha and beta diversity metrics at the genus level are shown in Figure 16. The Shannon index ranged from 3.64 to 5.59 (Figure 17A) and the Simpson index from 0.953 to 0.993 (Figure 17B). Both metrics were significantly higher in cultivated plots compared to native grassland ($p < 0.01$), with no significant differences between the two cultivated plots. The Bray–Curtis-based PCoA (Figure 17C) explained 73.2% of the total variance across axes 1 and 2. PERMANOVA confirmed a significant effect of sampling plot on microbial community structure ($F = 13.38$, $R^2 =$

0.53, p = 0.001) with significant differences between cultivated plots and native grassland (p = 0.001), but not between the cultivated plots (p > 0.05).

Figure 17. Alpha and beta diversity metrics at the genus level



Note. A: Shannon index; B: Simpson index; C: PCoA based on Bray–Curtis dissimilarities. PERMANOVA indicated a significant effect of the sampling plot on community structure.

5.3.2 Nitrogen-Cycling Microbiota Diversity and Functional Potential

Figure 18 presents the absolute abundances of functional genes quantified by qPCR, grouped by sampling plot and month. The two-way repeated-measures ANOVA showed that land use (i.e., sampling plot) had a significant effect on *nifH*, *nosZ*, *nirS*, and *amoA* (AOB) abundances. Significant differences were observed between the cultivated plots and the native grassland ($p < 0.05$), whereas no significant differences were detected between the two cultivated plots ($p > 0.05$). Detailed statistical results are provided in Appendix AE. The abundances of *nifH* and *nosZ* were significantly reduced (by up to one order of magnitude) in cultivated plots compared to the native grassland. In contrast, green onion cropping was associated with increased *nirS* abundance, although values remained within the same order of magnitude ($\approx 10^7$ copies g^{-1}). A more pronounced increase was observed for *amoA* (AOB), whose abundance ranged from 0 to 3.0×10^8 copies g^{-1}, with the highest values recorded in cultivated plots. The effect of sampling month was significant only for *nifH* and *amoA* (AOA) abundances ($p < 0.05$). A significant interaction between sampling month and plot was detected exclusively for *amoA* (AOB), with temporal differences observed only in the native grassland plot ($p < 0.05$).

The merging of PICRUSt2 functional predictions with genus-level taxonomic assignments revealed that 428 taxa contributed to dissimilatory NO_2^- to NH_4^+ reduction, 281 to ammonification (i.e., organic N to NH_4^+), 160 to dissimilatory NO_3^- to NO_2^- reduction, 138 to dissimilatory NO_3^- reduction or NO_2^- oxidation, 119 to NO to N_2O reduction, 118 to NO_2^- to NO reduction, 95 to assimilatory NO_3^- to NO_2^- reduction, 87 to N_2O oxide to N_2 reduction, 82 to N_2 fixation, 72 to assimilatory NO_2^- to NH_4^+ reduction, and 14 to NH_3 oxidation. The 30 dominant taxa (i.e., those contributing more than 5% to any one pathway) are detailed in Table 15, with their abundances shown in the heatmap in Figure 19. Most of the key genera contributed to multiple pathways.

Figure 20 shows the mean proportional abundance of the 11 N cycle pathways, while Appendix AD provides the scaled abundances for each functional gene across the sampling plots and months. Statistically significant differences ($p < 0.01$) between cultivated and native grassland soils were observed for all pathways except for NO to N_2O reduction and dissimilatory NO_3^- to NO_2^- reduction (Appendix AE). Functional predictions suggest that green onion cropping reduced the potential for ammonia oxidation, ammonification, assimilatory NO_2^- to NH_4^+ reduction, and N_2 fixation, but increased the potential for assimilatory NO_3^- to NO_2^- reduction, dissimilatory NO_2^- to NH_4^+ reduction, NO_2^- to NO reduction, N_2O to N_2 reduction, and dissimilatory NO_3^- reduction or NO_2^- oxidation.

Gene abundance quantification results for *nifH* and *nirS* were consistent with the functional predictions, reflecting reduced potential for N_2 fixation and increased potential for NO_2^- to NO reduction in cultivated plots. However, discrepancies were observed for *nosZ* and *amoA*. The quantification using qPCR indicated increased *amoA* abundance and decreased *nosZ* abundance in cultivated plots, despite functional predictions suggesting the opposite trends. These contrasting results were supported by the correlation analysis (Appendix AF), which revealed significant positive correlations only for *nifH*.

Figure 18. Absolute abundances of nitrogen cycle genes across sampling plots and months (log10 scale)

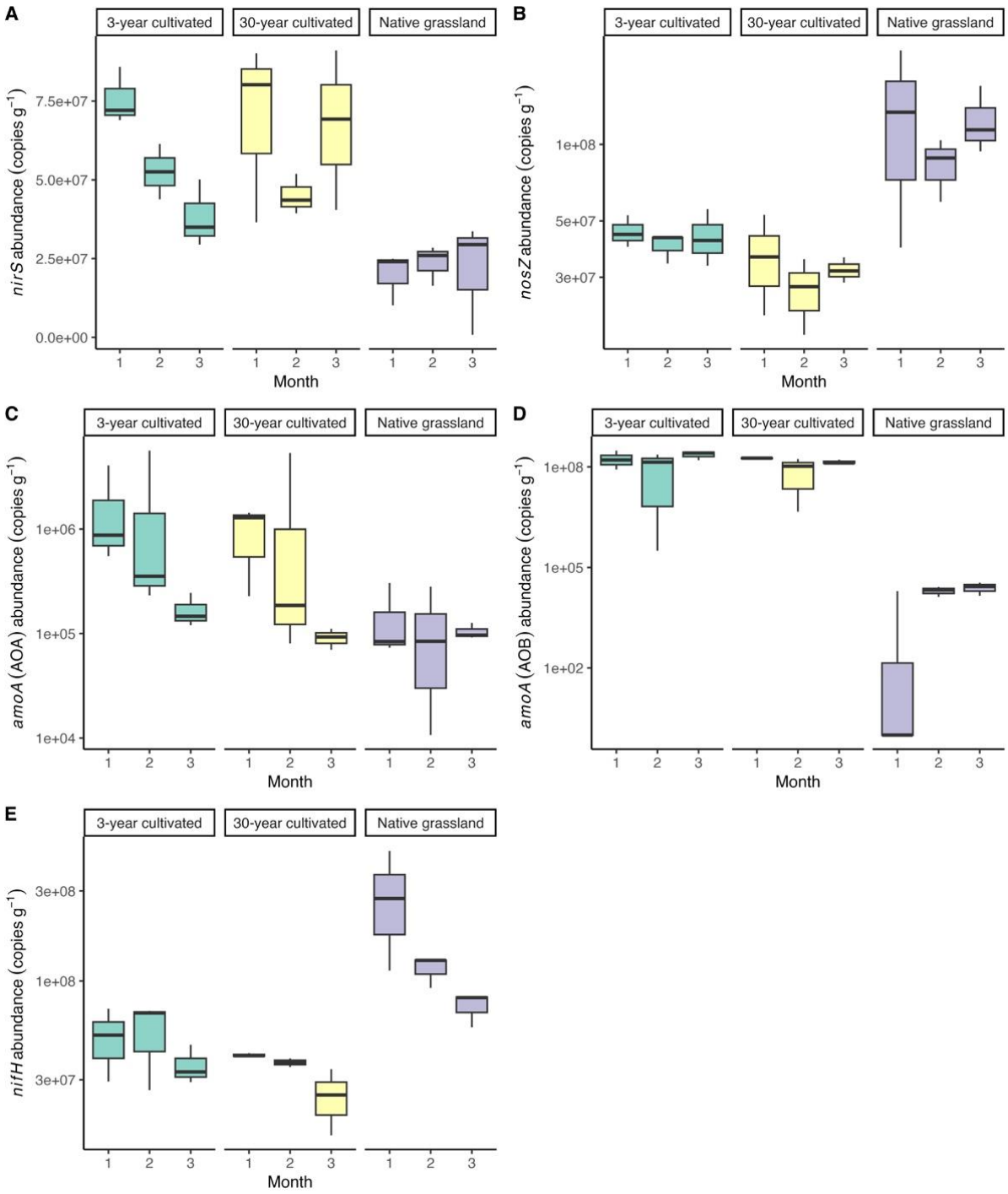
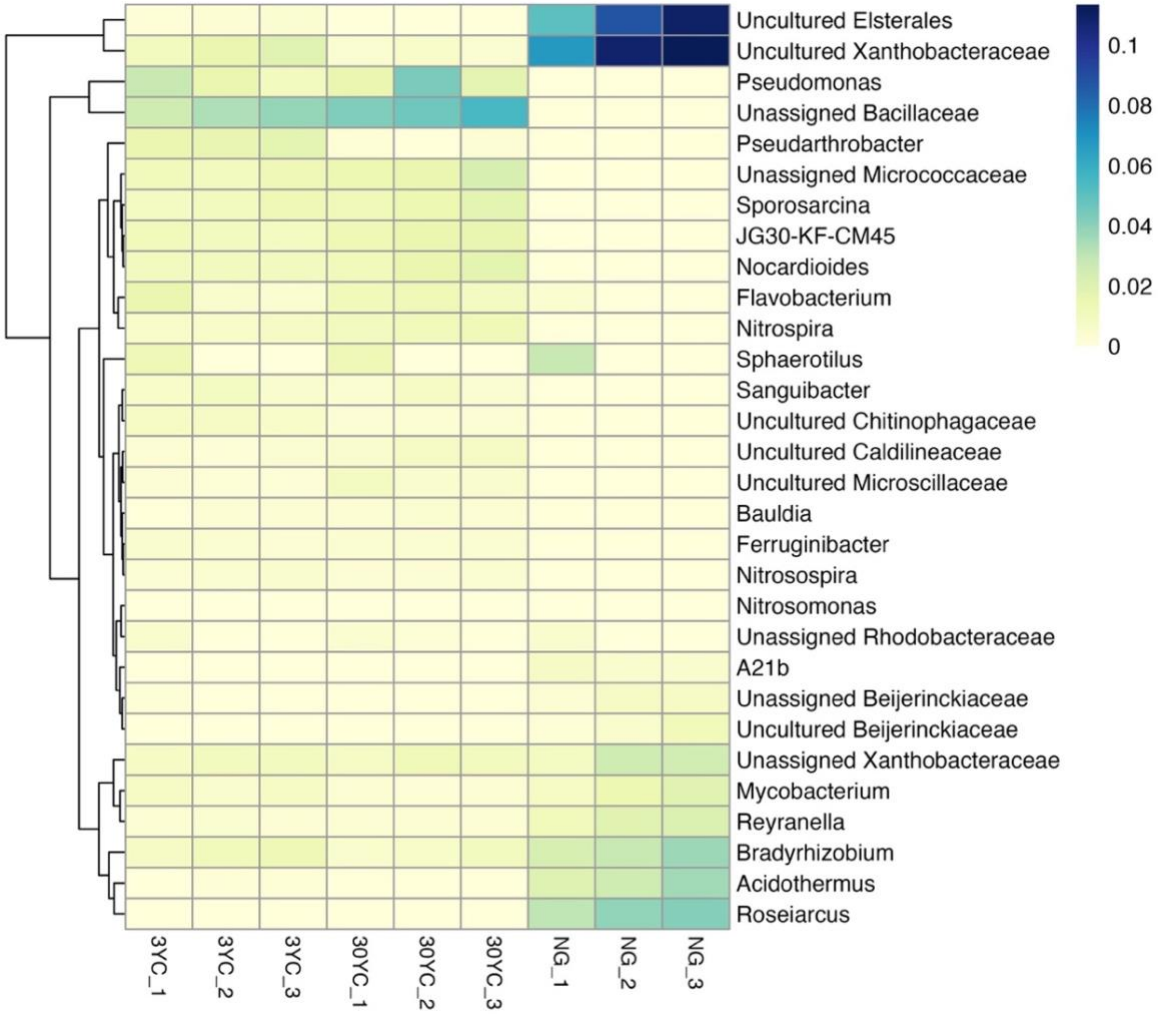


Table 15. Relative contribution of dominant genera to soil nitrogen-cycling pathways

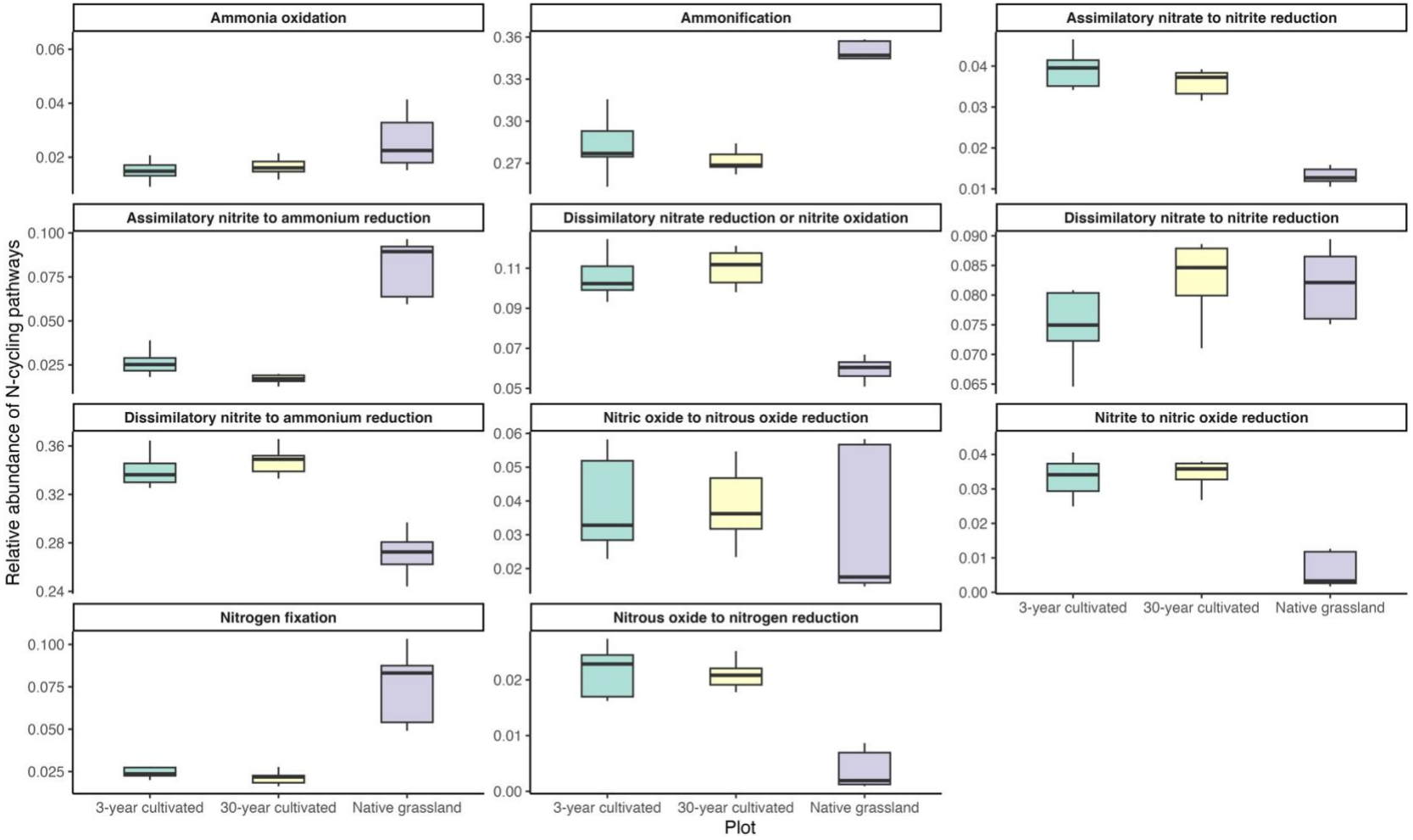
Genus	Ammonia oxidation	Ammonification	Assimilatory nitrate to nitrite reduction	Assimilatory nitrite to ammonium reduction	Dissimilatory nitrate reduction or nitrite oxidation	Dissimilatory nitrate to nitrite reduction	Nitric oxide to nitrous oxide reduction	Nitrite to nitric oxide reduction	Nitrogen fixation	Nitrous oxide to nitrogen reduction
A21b (Burkholderiales)	0.108									
Acidothermus			0.131		0.080					
Bauldia	0.057									
Bradyrhizobium		0.055		0.117		0.114			0.073	
Ferruginibacter										0.063
Flavobacterium			0.061				0.064	0.063		
JG30-KF-CM45 (Thermomicrobiales)								0.057		
Mycobacterium					0.075					
Nitrosomonas	0.063									
Nitrosospira	0.313									
Nitrospira	0.056				0.071			0.074		
Nocardioides			0.085							
Pseudarthrobacter			0.078							
Pseudomonas						0.064	0.065	0.058		0.088
Reyranella					0.065					
Roseiarcus	0.151			0.120						
Sanguibacter								0.052		
Sphaerotilus		0.050				0.117	0.209			
Sporosarcina					0.060					
Unassigned Bacillaceae					0.074					
Unassigned Beijerinckiaceae	0.055								0.059	
Unassigned Micrococcaceae			0.113							
Unassigned Rhodobacteraceae							0.052			
Unassigned Xanthobacteraceae				0.098					0.211	
Uncultured Beijerinckiaceae	0.170									
Uncultured Caldilineaceae										0.051
Uncultured Chitinophagaceae										0.076
Uncultured Elsterales		0.077				0.063	0.056		0.193	
Uncultured Microscillaceae										0.065
Uncultured Xanthobacteraceae				0.359						

Figure 19. Heatmap representing the mean relative abundances of the key nitrogen-cycling genera across sampling plots and months.



Note. 3YC: 3-year cultivated plot; 30YC: 30-year cultivated plot; NG: Native grassland plot. The numbers next to the plot labels indicate the sampling months.

Figure 20. Mean proportional abundance of predicted nitrogen cycle pathways, relative to the total predicted abundance of all 11 pathways per sampling plot.

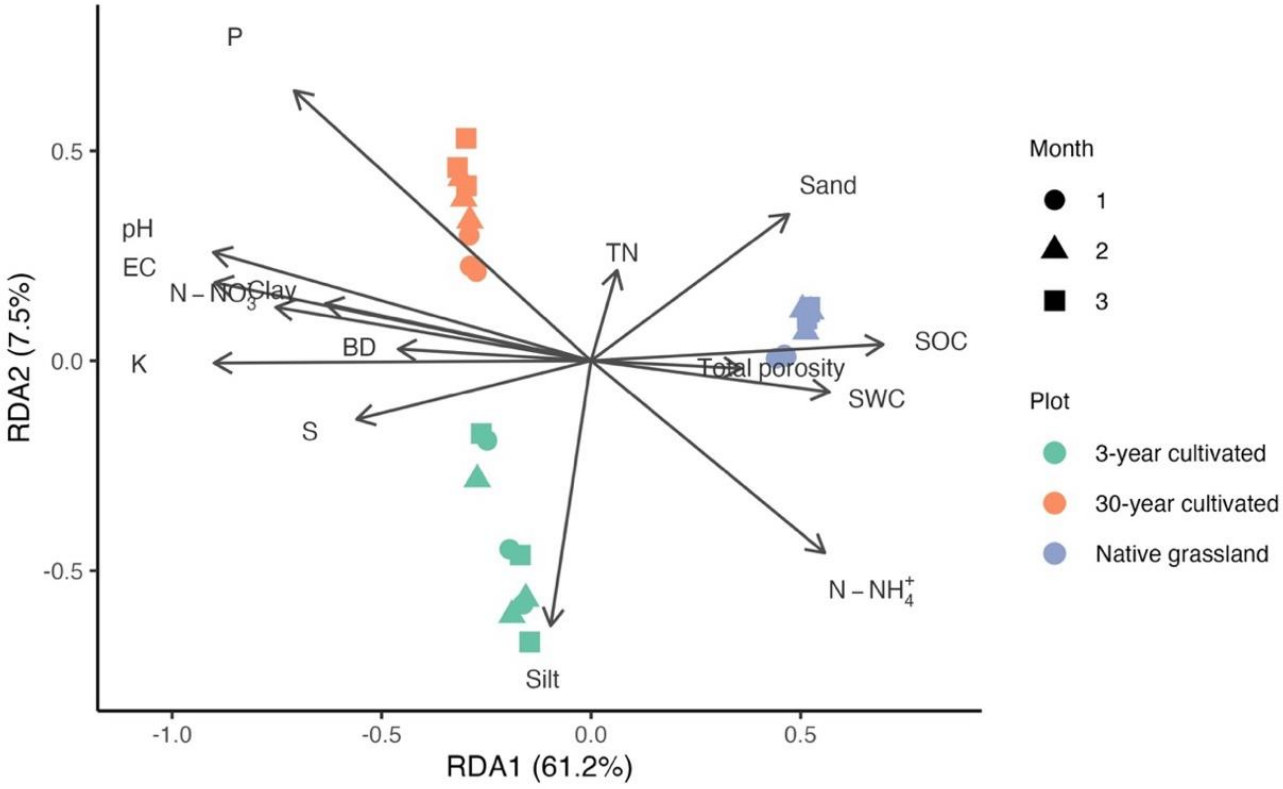


5.3.3 Relationships among Soil Properties, Microbial Communities and N-Cycling Genera and Genes

Descriptive statistics for the soil properties measured across the sampling plots during the monitoring period (three months) are summarized in Table 16. The RDA model showed that the soil physicochemical properties explained 87.3% of the variation in the microbial community composition. The first axis (RDA1) captured 61.2% of the variance, clearly separating cultivated plots from native grassland samples (Figure 21). A subtle gradient between the two cultivated plots was observed on RDA2 (7.5% variance explained). The model was statistically significant ($F = 5.036$, $p = 0.001$). Among the explanatory variables, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, SOC, and K had the strongest influence on community structure ($p < 0.01$). P, S, pH, and EC had marginal effects (i.e., p value ranging from ~0.06 to 0.09), while TN, SWC, BD, total porosity, and soil texture were not significant.

Figure 22 presents the results of the Spearman correlation analysis among the three groups of variables considered: i) 15 physicochemical soil properties, ii) the quantified abundance of five N-cycle functional genes; and iii) the relative abundances (Hellinger-transformed) of 30 key N-cycling genera. Robust (i.e., $|\rho| > 0.65$) and statistically significant ($p < 0.05$) correlations were found between certain soil properties and microbial variables. For instance, *nifH* was positively correlated with $N\text{-}NH_4^+$ and SOC, and negatively correlated with P, K, pH, and EC. *nirS* was strongly positively correlated with $N\text{-}NO_3^-$, P, and K, whereas *nosZ* was positively correlated with SOC and negatively correlated with P, pH, and EC. Among the *amoA* genes, only *amoA* (AOB) showed a strong positive correlation with $N\text{-}NO_3^-$, whereas no robust correlations were detected between *amoA* (AOA) and any soil physicochemical property.

Figure 21. Redundancy analysis (RDA) biplot showing relationships between the genus-level microbial community composition (Hellinger-transformed) and the soil physicochemical properties across sampling plots and months.



Note. TN: total nitrogen, N-NH_4^+: ammonium-nitrogen, N-NO_3^-: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, K^+: exchangeable potassium, P: available phosphorus, S: available sulfur, EC: electrical conductivity.

The concentrations of N-NO_3^-, P, and K, pH, and EC (properties whose gradients reflect the land-use transition from native grasslands to green onion cropping, as discussed in Chapters III and IV) showed consistent strong ($|\rho| > 0.65$, $p < 0.05$) correlations with the relative abundances of several key N-cycling genera. Positive correlations with these properties were observed for

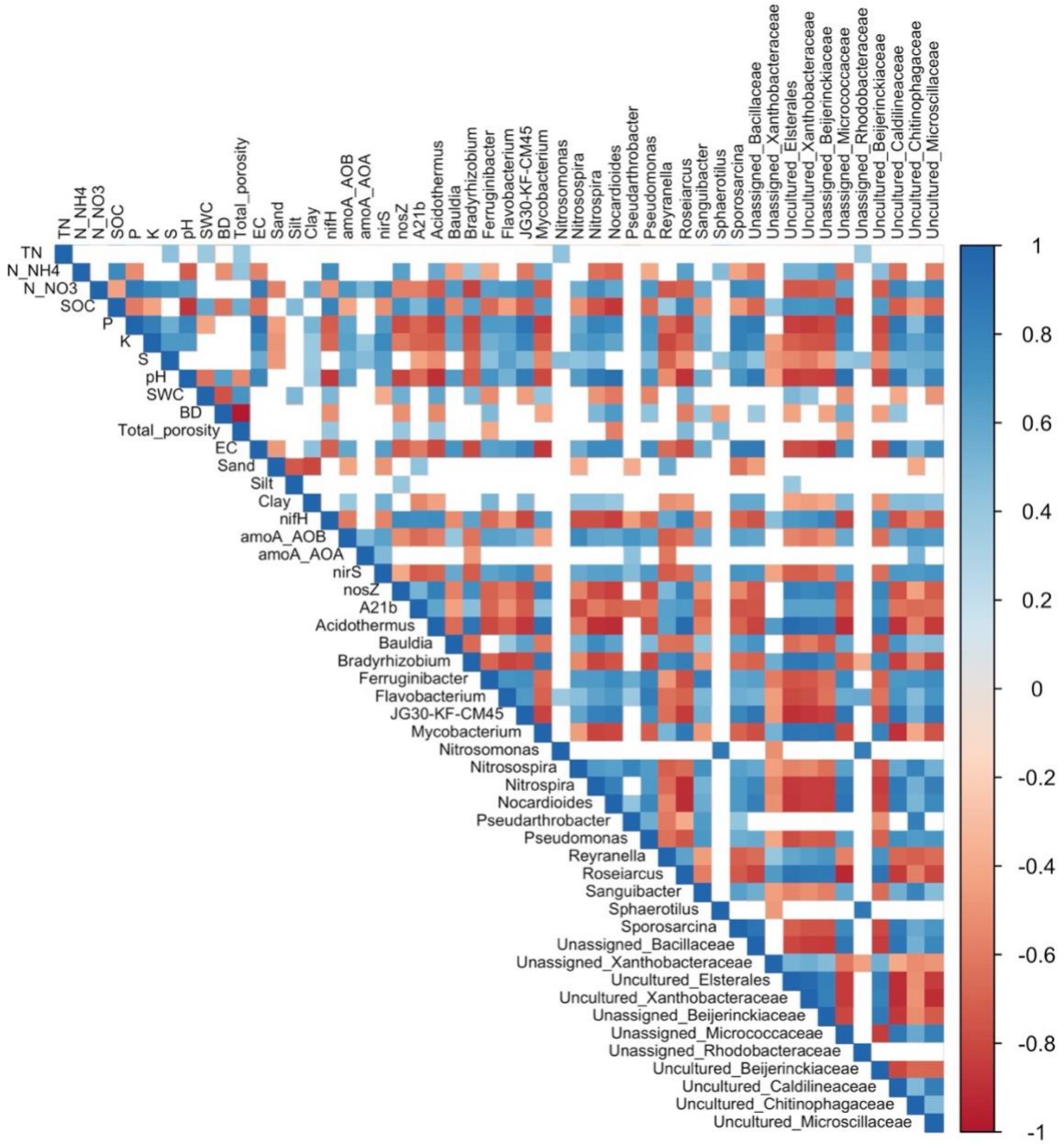
JG30-KF-CM45, *Nitrospira*, *Pseudomonas*, *Sporosarcina*, an unassigned genus from Bacillaceae, and uncultured genera from Caldilineaceae and Microscillaceae.

Table 16. Descriptive statistics of soil properties at 0-20 cm depth measured during the three months of sampling

Soil property	3-Year cultivated plot (n = 9)			30-Year cultivated plot (n = 9)			Native grassland plot (n = 9)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
TN (g kg^{-1})	2.7 ± 1.0	1.3	4.2	3.7 ± 0.3	2.9	4.0	3.4 ± 1.1	1.9	4.9
$N\text{-}NH_4^+$ (mg kg^{-1})	26.7 ± 7.7	13.6	35.8	16.9 ± 7.1	9.3	27.5	32.5 ± 10.3	20.8	52.1
$N\text{-}NO_3^-$ (mg kg^{-1})	33.5 ± 19.2	13.7	66.4	60.9 ± 25.7	29.1	99.5	0.8 ± 0.5	0.0	1.4
SOC (%)	4.3 ± 1.5	2.2	5.4	4.7 ± 0.3	3.9	5.0	7.7 ± 2.4	5.0	11.3
P (mg kg^{-1})	337 ± 63	229	430	2123 ± 189	1931	2511	7.8 ± 3.8	4.5	16.8
K^+ (cmol(+) kg^{-1})	2.8 ± 0.7	2.1	3.7	3.4 ± 0.9	2.7	5.8	0.3 ± 0.3	0.1	1.1
S (mg kg^{-1})	56.8 ± 18.2	25.6	82.6	63.5 ± 18.8	39.6	93.8	35.3 ± 17.6	19.3	77.6
pH	6.0 ± 0.5	5.3	7.0	6.6 ± 0.2	6.4	6.8	4.7 ± 0.4	4.0	5.1
SWC (g g^{-1})	0.5 ± 0.2	0.2	0.7	0.5 ± 0.0	0.5	0.5	0.7 ± 0.2	0.4	0.9
BD (g cm^{-3})	0.8 ± 0.2	0.7	1.2	0.8 ± 0.1	0.6	0.9	0.7 ± 0.1	0.5	0.8
EC (µS cm^{-1})	358 ± 94	230	523	549 ± 77	450	660	45 ± 41	22	146
Total porosity (cm^3 cm^{-3})	0.6 ± 0.1	0.5	0.7	0.7 ± 0.0	0.6	0.7	0.7 ± 0.0	0.6	0.8
Sand (%)	29.8 ± 20.5	15.6	57.1	33.9 ± 15.4	13.5	46.3	51.6 ± 9.2	39.5	59.8
Silt (%)	40.7 ± 19.9	14.2	55.5	29.2 ± 2.4	27.3	32.4	29.7 ± 1.3	28.7	31.4
Clay (%)	29.5 ± 2.0	27.7	32.1	37.0 ± 13.0	25.9	54.1	18.7 ± 7.9	11.5	29.0

Note. TN: total nitrogen, $N\text{-}NH_4^+$: ammonium-nitrogen, $N\text{-}NO_3^-$: nitrate-nitrogen, SWC: soil water content, BD: bulk density, SOC: soil organic carbon, K^+: exchangeable potassium, P: available phosphorus, S: available sulfur, EC: electrical conductivity. SD: Standard deviation; n: number of samples.

Figure 22. Spearman correlation matrix between soil physicochemical properties, N-cycle functional gene abundances (qPCR), and the relative abundances (Hellinger-transformed) of 30 key nitrogen-cycling genera.



Note. Only significant correlations ($p < 0.05$) are shown.

In contrast, negative correlations were found for those soil properties and the abundances of *Acidothermus*, *Bradyrhizobium*, *Mycobacterium*, *Reyranella*, *Roseiarcus*, an unassigned genus from Beijerinckiaceae, and uncultured genera from Elsterales, Xanthobacteraceae, and Beijerinckiaceae. For most of these genera, SOC also showed strong and significant correlations, but in the opposite direction to those observed for $N\text{-}NO_3^-$, P, K, pH, and EC.

Fewer robust correlations were identified between $N\text{-}NH_4^+$ and the abundance of N-cycling genera. Notable examples include a positive correlation with an unassigned genus from Beijerinckiaceae and negative correlations with *Nocardioides* and an unassigned genus from Micrococcaceae. No strong correlations were found between TN, BD, SWC, S, total porosity, or soil texture and the abundance of either functional genes or key genera involved in the N cycle.

5.4 Discussion

This discussion is structured in two main subsections to comprehensively explain the effects of green onion cultivation on the soil N-cycling microbiota. The first subsection examines overall shifts in bacterial community composition and diversity, highlighting changes in the relative abundance of dominant taxa, trends in alpha and beta diversity, and the key soil physicochemical properties driving community structure. The second subsection focuses on the functional dynamics of the N-cycling microbiota, addressing each pathway individually to evaluate how agricultural practices influence the microbial potentials for key transformations within the N cycle.

5.4.1 Shifts in Soil Bacterial Community Composition and Diversity

Green onion cultivation induced notable shifts in the structure and abundance of soil bacterial communities, with significant differences in taxon abundances between cultivated plots

and native grassland, observed not only at deep taxonomic levels (e.g., family and genus) but also at broader levels such as the phylum level (Figure 16). These changes corresponded with the results of the PCoA and PERMANOVA, which indicated evident changes in the composition of the communities between cultivated plots and the native grassland plot. The Proteobacteria phylum was significantly affected by land-use change, with higher relative abundance in the native plot (up to 43.6% in month 3) and lower levels in the cultivated plots (27–32%). This group includes many plant-associated and N_2-fixing taxa, such as members of the Rhizobiales order (Jones, 2015), and their greater prevalence in native grasslands may reflect the presence of more stable symbiotic associations. Moreover, the observed reduction in Proteobacteria and Acidobacteriota under cultivation could limit atmospheric N_2 fixation, as previous studies have identified common microbial networks dominated by Acidobacteriota, Alphaproteobacteria, and Bacteroidota as key contributors to this function (Han et al., 2025).

The abundance of Actinobacteriota exhibited a marked increase in cultivated plots (18–22%) compared to native grasslands (7–11%). Similarly, the abundance of Firmicutes increased approximately tenfold under cultivation. Notably, the enrichment of Firmicutes has been linked to chicken manure inputs, as this phylum is among the most abundant in the avian gastrointestinal tract (Lopes et al., 2025). The increase in Actinobacteriota may also reflect the presence of antibiotic-resistant taxa, potentially linked to the use of antimicrobials in poultry production (Parente et al., 2021). Moreover, both phyla have been associated with a positive priming effect, i.e., the process by which the addition of fresh organic matter stimulates microbial activity and accelerates the decomposition of native SOM (Bernard et al., 2022; Kuzyakov et al., 2000). Their higher relative abundance in cultivated plots may reflect the increased microbial biomass and activity triggered by repeated inputs of fresh chicken manure for fertilization, and this pattern is

consistent with the lower soil organic carbon (SOC) levels measured in cultivated soils (~4.3–4.7%) relative to native grassland (~7.7%). These changes suggest that the mineralization of both added and native SOM may contribute to increased N losses in inorganic forms. Conversely, Acidobacteriota showed a higher relative abundance in native grasslands (~17%) compared to cultivated plots (~8%), as did Verrucomicrobiota, which declined by half under cultivation. Similar patterns have been reported in response to elevated inputs of reactive N, favoring microbial communities less capable of decomposing recalcitrant SOM (Ramirez et al., 2012). In the study area, such reactive N enrichment likely stems from the continuous application of fresh chicken manure and synthetic foliar fertilizers containing mineral N.

The taxonomic shifts observed across land-use types were consistent with the multivariate patterns captured by the RDA, which demonstrated a strong association between bacterial community composition and soil nutrient status. Specifically, the increased relative abundances of copiotrophic phyla such as Actinobacteriota and Firmicutes in cultivated plots aligned with elevated mean concentrations of $N\text{-}NO_3^-$ (33.5–60.9 mg kg^{-1}) and K^+ (2.8–3.4 cmol(+) kg^{-1}), and lower levels of SOC (4.3–4.7 %), compared to the native grassland plot ($N\text{-}NO_3^-$: 0.8 mg/kg, K^+: 0.3 cmol(+)/kg, SOC: ~7.7%). These variables were among the main drivers of the community structure in the ordination space. In addition, the higher concentration of $N\text{-}NH_4^+$ in the native grassland plot (~32.5 mg kg^{-1} vs. 16.9–26.7 mg kg^{-1}) may be linked to a greater abundance of taxa associated with N_2 fixation (e.g., Xanthobacteraceae, *Bradyrhizobium*) and limited nitrification due to lower pH (~4.7 vs. ~6.0–6.6), further helping to distinguish this group in the RDA (Figure 21). While diversity indexes and pairwise comparisons of dominant taxa abundances did not reveal significant differences in overall community composition between the two cultivated plots, the subtle separation observed in the RDA suggests that variation in the abundance of a few sensitive

taxa may be influenced by the notable differences in P availability between these plots (i.e., mean concentrations of 337 mg kg^{-1} vs. 2511 mg kg^{-1}).

The Shannon and Simpson diversity indexes calculated for the study plots were higher than those reported by Rangel (2025) for undisturbed soils dominated by *Espeletia spp*. in the Santurbán páramo (Santander, Colombia), but lower than those recorded in other undisturbed páramos across the country (Reyes-Ardila et al., 2024; Vélez-Martínez et al., 2024). Although all plots presented high bacterial diversity, statistical comparisons revealed significantly greater diversity in the cultivated plots (Figure 17A–B). This trend is consistent with the genus-level relative abundance profiles (Figure 16C), which show an increase in the proportion of non-dominant taxa (i.e., those with relative abundances <1%) from approximately 50% in the native grassland to over 75% in cultivated plots.

Similar increases in soil bacterial diversity have been reported in other agricultural systems where FCM is applied, compared with unfertilized soils (Lopes et al., 2025; Parente et al., 2021). A plausible explanation is that prolonged manure application may introduce a wide array of gut-associated, antimicrobial-resistant bacteria, which are abundant in poultry feces (Kyakuwaire et al., 2019). The repeated use of fresh chicken manure may also contribute to the accumulation of veterinary antibiotics in soils (Gržinić et al., 2023), creating selective pressures that favor the establishment and proliferation of these resistant taxa. These bacteria could exhibit greater tolerance to stressors associated with cultivation, such as fluctuations in nutrient availability or the proliferation of pathogenic fungi, enabling them to colonize and persist in cultivated soils. This microbial influx may contribute not only to higher diversity but also to the displacement of native taxa less adapted to the altered conditions created by agricultural management. In addition to the observed increase in microbial diversity and potential influx of manure-associated taxa, notable

shifts were also detected in the composition and relative abundance of the dominant genera. Many of these genera are functionally relevant to N cycling and may underlie the differences in N transformation processes between cultivated and native grassland plots, as discussed in the following subsection.

5.4.2 Functional Dynamics of the Nitrogen-Cycling Microbiota

The significant reduction in *nifH* gene abundance in cultivated soils suggests a decline in the potential for biological N_2 fixation. This pattern is consistent with the strong correlation between *nifH* gene abundance and soil N-NH_4^+ concentration, suggesting that biological N_2 fixation may contribute to maintaining higher ammonium availability in native grassland soils. Biological N_2 fixation is downregulated under conditions of high N availability and oxygen, as these increase the energetic cost of nitrogenase activity relative to low-N, low-oxygen environments (Smercina et al., 2019). Accordingly, the reduced fixation potential under green onion cultivation may result from repeated N inputs and enhanced soil aeration due to frequent hilling by local farmers.

Among the taxa potentially contributing to this pathway, *Bradyrhizobium* showed a marked decline in relative abundance, decreasing from ~2.9% in the native grassland to ~0.9% in cultivated plots (Figure 19; Table 15). In this study, *Bradyrhizobium* emerged as one of the most abundant genera in páramo soils with native vegetation (Fig. 16C), in contrast with previous metabarcoding studies in páramo ecosystems where this genus was not identified as dominant (Reyes-Ardila et al., 2024; Vélez-Martínez et al., 2024). Nevertheless, in other high-mountain ecosystems *Bradyrhizobium* has been recognized as a key taxon within diazotrophic communities (Li et al., 2026). Another relevant contributor to this pathway in the study area may be an unassigned genus

from the Beijerinckiaceae family (Table 15), a group widely recognized for its N_2-fixing potential (Marín and Arahal, 2014), whose abundance was strongly and positively correlated with soil N-NH_4^+ concentration. Recent work has isolated several culturable diazotrophic bacteria from undisturbed páramo soils, highlighting the potential importance of biological N_2 fixation in these ecosystems (Carreño León et al., 2026).

Nitrification potential also showed a marked change associated with cultivation. Soil chemical patterns indicated enhanced nitrification in cultivated plots, as reflected by lower N-NH_4^+ concentrations and noticeable higher N-NO_3^- levels compared with native grassland (Table 16). This pattern was supported by qPCR results, which showed significantly higher abundances of *amoA* gene from ammonia-oxidizing bacteria (AOB) in cultivated plots, confirming an increased potential for NH_3 oxidation under green onion cultivation (Figure 18C). The neutralization of soil acidity through local agricultural practices (Rey-Romero et al., 2025) may indirectly influence nitrifying communities, as reduced soil acidity leads to lower concentrations of exchangeable aluminum, which is known to inhibit nitrifying populations (Li et al., 2018). Fertilization practices, particularly the combined use of fresh chicken manure and synthetic fertilizers, may also contribute, as N fertilization tends to increase AOB populations (Yang et al., 2024).

Among the predicted main contributors to the nitrification pathway (Table 15), *Nitrosomonas* and *Nitrosospira* are well-established AOB (Hayatsu et al., 2008), whereas *Nitrospira* is notable for its capacity to perform complete oxidation of NH_3 to NO_3^- (comammox) (Daims et al., 2015). The abundances of *Nitrosomonas* and *Nitrosospira* remained relatively stable across land uses. *Nitrosomonas* abundance was not related to *amoA* AOB abundance ($\rho = -0.036$, $p = 0.86$), likely reflecting its limited ecological relevance in páramo soils. This genus typically prefers neutral pH conditions and is often inhibited in acidic environments (Song et al., 2016). In

contrast, *Nitrosospira* showed a strong positive correlation with *amoA* AOB gene abundance ($\rho = 0.76$, $p < 0.01$), indicating it may be among the dominant AOB in native páramo soils. On the other hand, the abundance of *Nitrospira* increased markedly in cultivated plots (Figure 19) and displayed a moderate positive correlation with *amoA* AOB abundance ($\rho = 0.60$, $p < 0.01$), along with a robust positive correlation with N-NO_3^- concentrations (Figure 22). Taken together, these results suggest a shift toward comammox-dominated nitrification under green onion cropping.

Interestingly, taxonomic profiling did not detect *Nitrobacter*, a well-known nitrite-oxidizing bacteria (NOB). This absence may reflect its higher sensitivity to acidic conditions, compared to *Nitrospira* (Wang et al., 2014) or limitations of the metabarcoding approach, as *Nitrobacter* 16S rRNA gene sequences share high similarity with those of other taxa (e.g., *Bradyrhizobium*), complicating discrimination (Li et al., 2018). Furthermore, this study did not assess heterotrophic nitrification, which may contribute to N transformation (Hayatsu et al., 2008), but remains challenging to evaluate due to the lack of universal genetic markers and limited biochemical characterization of its pathways (Li et al., 2018). Since the *amoA* gene is typically absent in heterotrophic nitrifiers (Pulikova et al., 2025), the qPCR approach primarily captures autotrophic nitrification and may underestimate the contribution of heterotrophic processes to soil NO_3^- accumulation.

The response of denitrification potential to green onion cultivation varied among process steps. The qPCR results indicated that agricultural practices increased the potential for NO_2^- reduction to NO mediated by the *nirS* gene. The higher abundance of *nirS* in cultivated soils (Fig. 3A) may be associated with increased populations of *Flavobacterium*, *JG30-KF-CM45*, and *Pseudomonas* (Table 15), which showed positive and significant correlations (Figure 22) and may be stimulated by elevated nutrient availability. This is supported by the strong positive correlations

between *nirS* and N-NO_3^-, P, and K. Previous studies similarly report increases in *nirS* abundance in response to N surpluses from organic and inorganic fertilizers (Ouyang et al., 2018), particularly amendments with low C/N ratios such as fresh chicken manure (Yang et al., 2024).

Cultivation also contributed to a shift in the final step of denitrification (N_2O to N_2), mediated by the *nosZ* gene, with a reduced potential in cultivated soils compared to native grassland (Figure 18B). While some studies have shown that N fertilization can increase *nosZ* abundance by up to 75% (Ouyang et al., 2018), others have reported that stable organic amendments with high C/N ratios (e.g., compost) more strongly promote the abundance of *nosZ* genes (Yang et al., 2024). In this regard, the high levels of well-humified organic matter typical of native grassland soils may help explain both the greater *nosZ* abundance and its positive correlation with SOC. The combined increase in *nirS* and reduction in *nosZ* abundances raises concerns about the environmental consequences of green onion cropping in páramo ecosystems. Since denitrification is a major source of N_2O emissions from soil (Levy-Booth et al., 2014), a potential imbalance between NO_2^- and N_2O reduction steps could enhance N_2O release, with implications for greenhouse gas emissions and climate regulation in these sensitive high-altitude ecosystems.

Complementing these findings, functional inference suggests that cultivation increases the potential for assimilatory NO_3^- reduction to NO_2^- while decreasing the potential for the subsequent reduction of NO_2^- to NH_4^+ (Figure 20). Assimilatory N transformations involve the incorporation of inorganic N into microbial and plant biomass, representing a key pathway for N retention in soils (Silver et al., 2005). The predicted pattern may constrain temporary N immobilization, thereby limiting its capacity to mitigate soil N losses driven by dissimilatory processes (e.g., nitrification, denitrification). The potential decline in this pathway may be associated with a reduced relative contribution of taxa involved in NO_2^- assimilation, including *Bradyrhizobium*,

Roseiarcus, and an unassigned genus within the Xanthobacteraceae family (Table 15 and Figure 19). Since assimilatory processes tend to be suppressed in N-rich environments (Levy-Booth et al., 2014), the plausible shift in these pathways may be associated with the elevated concentrations of $N\text{-}NO_3^-$ in cultivated soils (Table 16).

The reduced concentrations of $N\text{-}NH_4^+$ in cultivated soils (Table 1) may also reflect the predicted decrease in the potential for ammonification (Fig. 5), particularly the ureolytic pathway involving *ureA*, *ureB*, and *ureC* genes, which are responsible for the hydrolysis of urea and the mineralization of organic N into NH_4^+. This pathway is especially relevant in the study context, as it constitutes the final step in the decomposition of uric acid, a compound that accounts for up to 70% of the total N content in fresh chicken manure (Nahm, 2003). A decline in ammonification potential may limit short-term N availability to plants, even under high external N inputs. Although a direct link between fertilization and ureolytic microbial communities has not been established, the observed decline may result from indirect effects of agricultural practices on soil pH, carbon availability, and nutrient levels (Fan et al., 2025).

Moreover, although an increase in the potential of dissimilatory NO_2^- to NH_4^+ reduction was predicted in cultivated soils (Figure 20), the complete dissimilatory NO_3^- reduction to NH_4^+ (DNRA) pathway is generally not dominant in well-aerated, non-flooded soils (Silver et al., 2001). While current irrigation practices may lead to excess water inputs (Naranjo, 2025), the study area's medium to coarse soil textures (IGAC, 2015) and hilling practice by local farmers promote natural drainage, likely limiting DNRA activity. This observation, together with elevated NO_3^- concentrations and increased *amoA* gene abundance, supports the hypothesis that nitrification is enhanced under green onion cultivation.

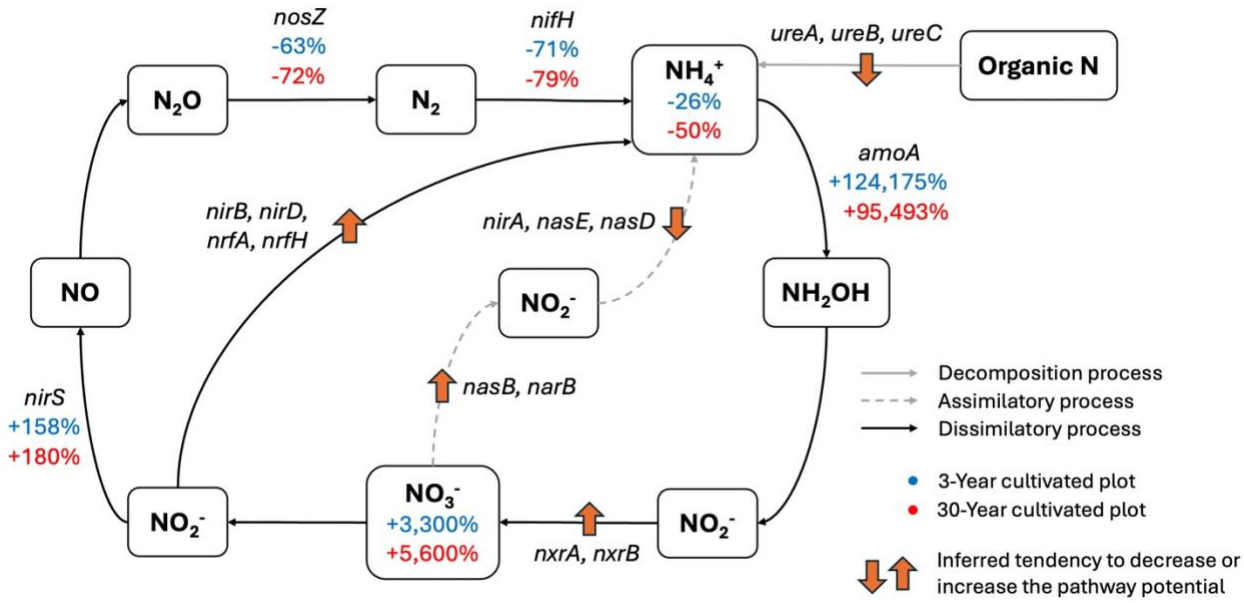
Although functional inference results provide insights into plausible functional changes in microbial communities, they should be interpreted with caution. Ambiguity arose in the interpretation of NO_2^- oxidation, the second step of nitrification, because the genes *nxrA* and *nxrB* that mediate this pathway, share KO identifiers (K00370 and K00371) with *narG* and *narH* which are involved in dissimilatory NO_3^- reduction to NO_2^-. Although PICRUSt2 predicted higher abundances of these KOs in cultivated plots (Figure 20), this overlap renders functional interpretation uncertain. However, taxonomic cross-referencing identified *Nitrospira,* a well-established NOB (Hayatsu et al., 2008), as one of the main contributors to these KOs in cultivated soils. In contrast, other genes associated with dissimilatory NO_3^- to NO_2^- reduction (i.e., *narI*, *napA*, *napB*) showed stable abundances across land uses (Fig. 5), suggesting that this pathway was not substantially affected by green onion cultivation.

Additionally, this study revealed inconsistencies between functional inference and qPCR results for NH_3 oxidation and the final step of denitrification. Indeed, correlation analysis between gene abundances measured by qPCR and those predicted by PICRUSt2 (Appendix AF) revealed only significant positive correlation for *nifH*. A major limitation of prediction tools based on DNA metabarcoding is the incomplete representation of reference genomes, particularly in complex environments such as soils (Djemiel et al., 2022; Wang et al., 2022). The discrepancies observed here may therefore reflect the limited capacity of these tools to accurately capture the functional potential of poorly characterized microbial communities in high-mountain ecosystems, where microbiomes are underrepresented in reference databases and subject to complex taxonomic–functional decoupling.

Figure 23 summarizes the percentage changes in the mean absolute abundance of genes associated with the major N cycle pathways. This visual synthesis reinforces the chapter's main

findings by illustrating how green onion cultivation alters the functional capacity of microbial communities, particularly through increases in nitrification and NO_3^- accumulation, along with reductions in the potential for N_2 fixation, assimilatory processes, and complete denitrification. These trends are consistent with a shift toward N loss pathways in cultivated soils.

Figure 23. Summary of changes in the functional potential of key nitrogen cycle pathways and soil inorganic nitrogen concentrations associated with green onion cultivation.



Note. The numbers next to the arrows indicate the percentage increase (+) or decrease (–) in the mean absolute abundance of functional genes involved in each pathway in cultivated soils relative to native grassland. Orange arrows indicate predicted increase or decrease in pathway abundance according to PICRUSt2 results. The percentages shown below the ammonium (NH4+) and nitrate (NO3-) labels represent the relative changes in mean soil concentrations of these pools in cultivated plots compared to native grassland.

Finally, it is relevant to recognize that the main limitation in the analysis of functional potential presented in this chapter is the discrepancy between results from functional inference and absolute gene abundance quantified by qPCR. Predictions from amplicon-based functional profiling are inherently biased toward existing reference genomes, reducing the likelihood of detecting rare, environment-specific functions (Douglas et al., 2020), a limitation particularly relevant for the unique microbial assemblages of páramo soils. Future research should assess the accuracy of functional predictions by incorporating metrics such as the Nearest Sequenced Taxon Index (NSTI) score and the use complementary approaches such as shotgun metagenomics (Langille et al., 2013). In addition, alternative predictive tools like Tax4Fun2 (Wemheuer et al., 2020) could be employed for cross-validation, thereby improving the robustness of functional profiling in these ecosystems.

5.5 Conclusions

Green onion cultivation significantly altered the composition of soil bacterial communities across multiple taxonomic levels. Agricultural management practices favor the proliferation of copiotrophic organisms associated with soil organic matter decomposition via the priming effect. In contrast, native grassland soils were dominated by oligotrophic taxa with high N_2 fixation potential. These shifts were driven primarily by increased concentrations of $N\text{-}NO_3^-$ and K^+, along with reductions in SOC and $N\text{-}NH_4^+$, as a result of land-use change.

Substantial changes were also observed in the microbial functional potential related to N cycling. Green onion cultivation reduced the potential for N_2 fixation and complete denitrification, while increased the potential for nitrification. Functional inference additionally suggested reduced assimilatory pathways and ureolytic ammonification, although these predictions should be

interpreted cautiously. Overall, cultivated soils exhibited a transition toward increased reliance on external N inputs and N loss pathways, implying higher vulnerability to nitrate leaching and greenhouse gas emissions in páramo ecosystems.

Although one of the cultivated plots had undergone a fallow period, its bacterial community composition and structure did not differ significantly from those of the plot under continuous cultivation. This suggests that prolonged green onion cultivation did not lead to further marked shifts in overall microbial diversity compared to shorter-term cultivation after fallow. However, the increased availability of P appeared to influence specific taxonomic groups within the cultivated soils. Moreover, the minor differences observed in the abundance of functional genes involved in N cycling between the two cultivated plots underscore the need to deepen the assessment of microbial functional shifts associated with the intensification and duration of farming practices.

Together, these findings underscore the need to reconsider nutrient management strategies under green onion cultivation to mitigate environmental impacts and support more sustainable land use in páramo landscapes. Moreover, the high relative abundance of uncultured and unclassified organisms further underscores the need to advance the study of soil microbiota through targeted cultivation efforts and metagenomic sequencing. These approaches are essential to better characterize these poorly known taxa and elucidate their ecological roles, particularly in understudied high-Andean environments.

6. Pathways Toward Sustainable Nitrogen Management in Páramo Agroecosystems

6.1 Introduction

Improving the management of nitrogen (N) in agroecosystems is a major challenge for sustainable development, particularly in developing countries, due to the dual role of this nutrient: it is an essential input for food production but also a major pollutant, with emissions affecting ecosystems at local, regional, and global scales (Kanter et al., 2020). Reducing N losses can thus simultaneously increase farmers' net income by lowering fertilizer requirements and decrease the economic and health costs associated with pollution, while enhancing ecosystem services and contributing to climate change mitigation (Gu et al., 2023).

Recognizing these interlinked benefits, N has gained prominence in environmental policy. At the global level, the United Nations (UN) has made reducing N waste a strategic priority. The Colombo Declaration (2019), emerging from a UN high-level science–policy dialogue in Sri Lanka, launched the UN Global Campaign on Sustainable Nitrogen Management, committing to halve N waste by 2030, a milestone target that placed N firmly on the international environmental agenda. The Berlin Declaration (2021), adopted at the 8th Global Nitrogen Conference organized by the International Nitrogen Initiative (INI), reinforced the urgency of reducing global flows of reactive N and called for integrated policy responses. In 2022, the UN Environment Assembly adopted a resolution (UNEP/EA.5/Res.2) urging member states to accelerate actions to substantially reduce N losses by 2030.

Despite these global commitments, translating policy goals into effective action at the local level remains challenging. Agricultural systems in high-mountain Andean regions, such as the Colombian páramos, face unique environmental, socioeconomic, and cultural constraints that require tailored solutions. In these sensitive ecosystems, N mismanagement has led to severe impacts on water quality, biodiversity, and rural livelihoods, making context-specific strategies essential. Therefore, this chapter analyzes potential strategies to improve N management and reduce losses in green onion agroecosystems in the Santurbán–Berlín páramo, drawing on the findings from each specific objective of this doctoral research.

Strategies to reduce N losses from agricultural systems can generally follow three complementary approaches (Xia et al., 2020). The first focuses on controlling N losses at the source by improving N use efficiency through balanced fertilization, precision application, and management practices that reduce N surplus in the soil. The second aims to intercept and transform N during its transport within the landscape, for example through vegetated buffer strips, cover crops, or ecological drainage features that enhance assimilation, sorption, sedimentation, and denitrification processes (Birgand et al., 2007; Dollinger et al., 2015). The third approach involves final treatment or remediation measures that act as the last barrier before N reaches sensitive environments, such as riparian corridors, constructed wetlands, and infiltration systems (Passeport et al., 2013). Among these, source control measures are particularly relevant for achieving long-term agricultural sustainability in the páramo context, as they simultaneously reduce environmental losses and improve input use efficiency while supporting soil health and productivity. Therefore, this proposal emphasizes source control strategies.

Building on this framweork, the proposed strategies for the Santurbán-Berlín páramo context focus on: (i) improving fertilization and irrigation practices; (ii) reducing reliance on

monocropping through crop diversification, rotation, and fallow systems; (iii) managing soil microbial biodiversity; and (iv) promoting public policies aimed at reducing nitrogen pollution. In particular, they align with selected principles of the United Nations Economic Commission for Europe (UNECE) Guidance Document on Integrated Sustainable Nitrogen Management (Sutton et al., 2022), which emphasize reducing N losses while safeguarding productivity, tailoring interventions to local environmental vulnerability, integrating N with water and soil carbon (C) management, enhancing nutrient use efficiency, and fostering multi-stakeholder responsibility. The following discussion addresses the challenges and opportunities for these strategies, identified from current agronomic management in the study area. These findings can guide decision-making by diverse stakeholders to facilitate the transition toward sustainable agricultural practices in Colombian páramo ecosystems.

6.2 Improving Fertilization and Irrigation Practices

As discussed in previous chapters, the long-term and continued use of fresh chicken manure (FCM) as the primary fertilizer source is a major driver of N losses under green onion (*Allium fistulosum*) cultivation in the Santurbán–Berlín páramo. This practice is deeply ingrained among local farmers: all interviewees in the farming practices characterization (Chapter II) emphasized that both FCM and synthetic fertilizers are essential to meet market standards for onion size and appearance. These market pressures, coupled with a widespread perception of low fertility in undisturbed páramo soils, likely contribute to the overapplication of N. The absence of soil testing, lack of input records, and non-standardized application methods (Chapter II) further indicate a disconnect between perceived and actual nutrient requirements. This was corroborated by the partial N balance carried out on three representative farms (Table 2), which revealed substantial N surpluses (i.e., 136 to 430 kg ha^{-1}). Given the conservative assumptions applied in

this balance, actual surpluses are likely even greater, underscoring the need for improved nutrient monitoring and locally adapted fertilization guidelines, an approach consistent with the UNECE principle of avoiding nutrient surpluses while maintaining productivity.

Improving fertilization practices in this context requires building farmers' capacity to develop fertilization plans based on crop needs and soil nutrient status, while gradually replacing FCM with stabilized organic fertilizers. Short-term improvements could include modifying the method and frequency of FCM application. For instance, incorporating manure below the soil surface can reduce ammonia volatilization and promote conversion to ammonium, thereby improving N availability for crops (Strock, 2008). Similarly, adopting split fertilization (i.e., reducing the dose per application and increasing the frequency) can improve N use efficiency (Khalsa et al., 2022). Both measures align with UNECE recommendations to improve nutrient-use efficiency through timing, placement, and matching of supply to demand.

In the medium term, fertilization should be guided by N budgets that incorporate periodic soil and plant tissue analyses (Khalsa et al., 2022). Where resources limit laboratory analyses, the pedotransfer functions (PTFs) developed in this thesis (Chapter IV) could provide an accessible alternative for estimating total N and nitrate-N concentrations, enabling more informed nutrient planning. This approach aligns with UNECE principles on balancing nutrient inputs and outputs and on tailoring interventions to local conditions. Fertilization plans should also aim to enhance soil organic matter (SOM) as a means to improve soil health and resilience. In this regard, the stoichiometric balance between fresh organic inputs and microbial biomass is critical for promoting microbial productivity and organic matter stabilization (Buchkowski et al., 2019). Findings from Chapter IV showed that long-term green onion cultivation tends to increase exchangeable potassium (K^+) and available phosphorous (P), particularly in plots cultivated

continuously without fallow, possibly due to reduced acidity (Table 9). However, these nutrient accumulations, if not balanced with other elements, may not translate into SOM gains. Consistent with UNECE principles, strategies to improve N use efficiency should also safeguard soil C stocks and quality, recognizing that C sequestration is inherently linked to N sequestration through stable C:N ratios in soils. This calls for a shift in fertilization plans from a crop-only focus to a system-oriented approach that meets the needs of both plants and soil microorganisms (Coonan et al., 2020).

Reducing N losses will also require replacing FCM with stabilized organic fertilizers, such as composts or anaerobically digested products, sourced from local agricultural residues. However, interviews from Chapter II revealed strong farmer skepticism toward composted products, with some believing composting removes nutrients, and others citing an initiative conducted by the regional environmental authority in which the use of composted manure produced onions that failed to meet market size standards. These perceptions highlight the UNECE principle of integrating stakeholder engagement and socio-economic realities into nutrient management. Therefore, while composting and anaerobic digestion remain promising long-term strategies, immediate progress could be made through participatory research aimed at optimizing FCM use. Such work could identify the minimum effective application rates that sustain yields under varying rainfall and irrigation conditions, while also assessing nitrate and particulate N leaching. This evidence-based approach could build trust, inform tailored recommendations, and lay the groundwork for the gradual adoption of alternative organic fertilizers. Complementary pilot projects, such as composting FCM with onion residues or food waste, could also be explored, as demonstrated by previous studies in the Santurbán–Berlín páramo, where these substrates produced bioproducts with high agronomic potential (Oviedo-Ocaña et al., 2022; Ríos, 2025).

Improving irrigation management is equally important for enhancing N use efficiency and minimizing losses. Reliance on empirical scheduling in the study area leads to inefficient water use and greater leaching risk. Transitioning toward more precise irrigation scheduling, supported by basic training on crop water requirements and drainage management, could reduce N losses while maintaining productivity. As evidenced from agricultural practice characterization (Chapter II), farmers themselves linked excess soil moisture to greater disease incidence, suggesting an entry point for change by framing water-saving practices to improve crop health and yield. Establishing local mechanisms for regulating irrigation practices and fostering collective action could also enhance equity in water distribution and promote long-term sustainability (Leroy, 2019). Such measures align directly with UNECE principles on integrating water and nutrient management to minimize losses and optimize resource use.

Furthermore, results from Chapter IV indicated that the vertical distribution of soil texture, together with the use or absence of fallow periods, are major drivers of soil N distribution in the studied agroecosystems. Coarser-textured subsoil layers can facilitate nitrate leaching, particularly under excessive fertilization or inefficient irrigation, while finer-textured layers may promote greater N retention but also increase the risk of denitrification losses under prolonged saturation. Integrating these findings into fertilization and irrigation planning can help tailor N application rates and water management to site-specific soil profiles, aligning with UNECE principles that emphasize adapting nutrient strategies to local biophysical conditions and preventing nutrient losses to water and air.

6.3 Reducing Reliance on Monocropping: Crop Diversification, Rotation, and Fallow Systems

The deep-seated reliance on green onion monocropping as the main economic activity represents a major constraint to sustainable N management in the Santurbán–Berlín páramo. As evidenced in the characterization of the farming practices and local soil knowledge in Chapter II, seed renewal is infrequent due to high costs, prompting farmers to keep fields continuously under cultivation without rotation or fallow, except when fertility declines markedly. This practice reduces opportunities for nutrient replenishment, enhances pest and disease pressure, and increases the risk of N imbalances. Promoting crop rotation and fallow systems offers multiple benefits for N cycling. Rotations with legumes or deep-rooted crops can enhance biological N fixation, improve N uptake from subsoil layers, and redistribute N vertically, an especially relevant finding given that Chapter IV identified that current management leads to the accumulation of elevated stocks of mineral N below the root zone, increasing the risk of N leaching.

Multiple barriers hinder crop diversification in the local context. While some farmers also cultivate potato (*Solanum tuberosum*), it is perceived as risky due to labor demands and frost vulnerability. Attempts to introduce alternative crops have been limited by their poor frost tolerance, while tenant farmers face additional constraints such as the need for landowner approval and reluctance to invest under short, uncertain contracts. Other limiting factors include restricted market access, insufficient technical training, limited land availability, and high investment costs for establishing new production systems. These findings underscore that technical knowledge gaps, land tenure and market conditions can directly influence N management decisions and the feasibility of sustainable practices.

Native species with high agronomic potential, such as *Symplocos theiformis* (Romero-Murcia, 2019), could diversify production while maintaining ecosystem compatibility. Expanding potato cultivation within rotations could be made more resilient by introducing a broader range of varieties, as only two or three are typically grown in páramos (Blake et al., 2023) despite Colombia's rich genetic diversity (Berdugo-Cely et al., 2017). Introducing or reinforcing the cultivation of legumes such as faba beans (*Vicia faba*), already occasionally grown by farmers (as discussed in Chatper II), could enhance soil fertility through nitrogen fixation and reduce the reliance on synthetic inputs. Promoting fallow periods, which historically support nutrient recycling in Andean ecosystems (Pestalozzi, 2000; Sarmiento and Bottner, 2002) could further improve soil fertility. The benefits of fallow for sustainable N management could also be enhanced by incorporating páramos' native legumes (Sarmiento et al., 2012). In this regard, forage-based fallows have shown potential in highland Andean systems to improve biomass production, nutrient retention, and crop health while maintaining soil health and productivity (Vanek et al., 2020).

Future research could also assess the feasibility of cultivating less frost-tolerant crops under greenhouse conditions. Protected agriculture has shown high potential for improving food security and agricultural resilience in high-mountain regions (Dorji et al., 2024), but in the páramo context it faces two significant constraints: the need for substantial initial investment and high operating costs related to energy demand. Moreover, greenhouses must be specifically adapted to local conditions in the Andean highlands, accounting for high solar radiation, extreme and unpredictable climatic fluctuations, and large daily temperature variations (Chiriboga et al., 2021).

For rotation and fallow systems to succeed, agronomic strategies must be paired with enabling measures, including improved market access for alternative crops, participatory research to adapt practices to local conditions, and extension services to strengthen farmer capacity.

Financial incentives and cost-sharing mechanisms from governmental agencies, environmental funds, and private-sector actors could help offset short-term income losses and investment costs, reducing barriers to adoption. By directly addressing these challenges, such measures advance the core aim of sustainable N management outlined in the UNECE principles: to safeguard human health, protect the climate and ecosystems, and ensure food security through efficient and balanced N inputs. Achieving this requires shared responsibility across all actors in the food chain, alongside a fair distribution of the costs and benefits of improved practices.

6.4 Managing Soil Microbial Biodiversity

Given the prominent contribution of soil organisms to key processes related to plant development, managing soil biodiversity in agroecosystems has strong potential to positively influence sustainable N management (Erisman et al., 2016). In recent years, there has been growing interest in plant growth-promoting microorganisms (PGPM) adapted to high-mountain environments because their management can reduce dependence on agrochemicals while maintaining high crop yields (Pandey and Yarzábal, 2019). PGPM can enhance N cycling in agroecosystems through multiple mechanisms, including biological N fixation, solubilization of other nutrients (e.g., phosphorus, potassium, and zinc), production of phytohormones and siderophores, stimulation of root growth, and increased plant resistance to biotic and abiotic stresses, among others (Meena et al., 2017).

To harness their potential benefits in agriculture, various strategies have been developed to optimize the roles of PGPM in nutrient supply and crop protection. Two main approaches are commonly distinguished: (i) the use of microbial inoculants and (ii) the management of endogenous soil microbiota (Barea, 2015). However, the incorporation of microbial inoculants into

a given agroecosystem requires a rigorous process of prior studies to select strains, produce the inoculum, and determine the appropriate application method (Hungria et al., 2005). Moreover, Hu and He (2018) emphasize that agronomic practices can be used to manipulate soil physicochemical properties in ways that favor the development of native microorganisms capable of promoting the sustainable use of N.

It is essential that future research assess the potential of implementing soil microbiota management strategies in the páramo, using as a baseline the findings of this doctoral thesis on native bacterial communities and the influence of current agronomic practices on their composition and function. *Bradyrhizobium* emerges as a particularly relevant taxon for potential bioinoculant applications, given its dominance in native páramo soils and its association with N-cycle functions such as assimilation, ammonification, and biological N_2 fixation (Chapter V). However, identifying key microorganisms for improving N management requires targeted studies employing metagenomic analyses or cultivation-based approaches to elucidate their ecological roles, especially considering that a high proportion of taxa detected in Chapter V were either uncultivable or unidentified by 16S rRNA sequencing. Other promising genera include *Bacillus* and *Pseudomonas*, both dominant in páramo soils (Figure 16) and known for their potential to enhance N use efficiency and suppress pathogens (Pandey and Yarzábal, 2019). Indeed, certain strains of these genera have been reported as effective PGPM in potato-cultivated soils from other high-mountain Andean ecosystems (Yarzábal and Chica, 2021).

Agronomic strategies discussed earlier, such as improving fertilization and irrigation practices, and adopting crop rotation and fallow schemes, can contribute to fostering more resilient soil microbiomes with enhanced N-cycling capacity. This, in turn, could increase plant-available N while reducing losses. In the study area, the results suggest rapid mineralization of the manure

used for fertilization, coupled with high nitrification rates driven by the availability of fresh organic matter and low soil acidity. Substituting FCM with more stabilized organic amendments could slow the hydrolysis of urea- and uric acid-containing materials, thereby reducing ammonia emissions and moderating nitrification rates, an approach consistent with UNECE principles for sustainable N management. In contrast, nitrification inhibitors, although effective against autotrophic nitrifying bacteria are not recommended in this context, as they may be less effective against nitrifying archaea and heterotrophic nitrifiers (Li et al., 2018), and do not address the root causes of N imbalance and pollution.

Despite the potential of soil microbiota management for sustainable N use, its practical adoption in the study area is likely constrained by gaps in local knowledge. The results from the first methodological phase of this doctoral research indicate limited farmer knowledge about weed diversity, soil fauna, and microbiota, and their roles in nutrient cycling and soil fertility. Similarly, technical knowledge of pest and disease management is limited. Farmers identified key onion diseases, such as *peca* (freckle), caused by viruses transmitted by *Thrips tabaci* and *Frankliniella occidentalis*; *ceniza* (ash), referring to downy mildew caused by *Peronospora destructor*; and *amarillo* (yellow), linked to yellow dwarf virus transmitted by aphids. However, local farmers often attributed these to weather conditions rather than biological causes. Promoting agrobiodiversity awareness and soil biota management could help improve nutrient retention, reduce disease pressure, and support more sustainable N management.

6.5 Promoting Public Policies aimed at Reducing Nitrogen Pollution

An essential pathway for the transition toward sustainable N management in páramos is the development and implementation of public policies that explicitly recognize nitrogen's dual

role: as an essential input for agriculture and as a driver of significant environmental harm. Globally, most national and regional policies still prioritize food production over environmental protection. As noted by Kanter et al. (2020), nitrogen-related regulations often focus on incentivizing its use or managing its trade, with emission controls typically targeting a single sink (e.g., water or air). This fragmented approach increases the risk of simply shifting N pollution from one environmental compartment to another.

In Colombia, three legal instruments currently establish guidelines for páramo ecosystem management (Resolution 886 of 2018, Law 1930 of 2018, and Resolution 963 of 2025), yet none explicitly regulate N management in agricultural systems. Instruments that could indirectly guide this transition, such as Resolution 30021 of 2017, which sets requirements for Good Agricultural Practices in primary vegetable production, and Resolution 00150 of 2003, which regulates the sale and responsible use of fertilizers and soil conditioners, require critical review. Their applicability to the biophysical and sociocultural conditions of Colombian páramos is uncertain, and current guidelines do not account for the specific N dynamics of these high-mountain ecosystems.

As disccussed by Kanter et al. (2020), in the long term, integrated N management policies are needed to reduce, recycle, store, and ultimately remove excess N via denitrifcation. Such policies should adopt a cross-sink perspective, preventing trade-offs between different N pollutants and aligning with international guidance on sustainable N management. As an intermediate step, incentives could be established for adopting mitigation measures that target total N pollution, addressing its root causes rather than isolated symptoms, such as nutrient budgeting, cross-sink monitoring systems, and payment schemes for practices that enhance N use efficiency while reducing losses.

7. General Conclusions and Perspectives

7.1 General Conclusions

Overall, this doctoral research advances the understanding of nitrogen (N) dynamics in páramo soils under green onion cultivation by integrating agronomic practices, soil properties, vertical N distribution, and microbial biodiversity. The findings demonstrate that cultivation reshapes both the physicochemical and biological foundations of N cycling, from altering soil properties that regulate nutrient pools to restructuring microbial communities and their functional potential. By coupling detailed monitoring with predictive modeling, the thesis not only identifies mechanisms driving N losses but also provides tools to anticipate them across cultivated landscapes. Together, these insights underscore the dual challenge of sustaining agricultural livelihoods and safeguarding the ecological integrity of páramos, offering a scientific basis to guide more sustainable N management strategies in high-mountain Andean agroecosystems.

Regarding the *first specific objective,* this thesis shows that current fertilization practices, particularly the continued and long-term use of fresh chicken manure with highly variable composition, combined with inadequate agrochemical applications and the absence of fertilization plans, are major drivers of changes in N dynamics in cultivated soils compared with native grasslands. Excessive irrigation further exacerbates these effects. Together, these practices increase the potential for N losses via runoff, leaching, and gaseous emissions, thereby limiting the possibility of aligning productive activities with ecosystem conservation.

At the hydrographic unit scale, the interaction of management practices and environmental conditions resulted in a depletion of total nitrogen (TN) in topsoil (0-20 cm) by up to 40%, while

nitrate-nitrogen ($N\text{-}NO_3^-$) concentrations were nearly eight times higher in green onion plots than in non-cultivated soils. The concentration of ammonium-nitrogen ($N\text{-}NH_4^+$), however, remained relatively stable between cultivated and native grassland surface soils. TN losses were linked to soil organic matter depletion, driven by fresh chicken manure fertilization and mechanized plowing. Neutralization of soil acidity under agricultural management favored nitrification, further enhancing the risk of $N\text{-}NO_3^-$ leaching and runoff. Key soil properties influencing surface soil N pools included pH, soil organic carbon, electrical conductivity, available potassium, available sulfur, available phosphorus, soil water content, and bulk density.

While many of the agricultural practices and aspects of local soil knowledge documented in this thesis are consistent with findings from other páramo ecosystems in Colombia, supporting the transferability of the insights, variations in parent material and mineralogical characteristics across páramo soils introduce important differences in edaphic properties. These differences represent a limitation when extrapolating the results, underscoring the need for site-specific considerations when applying the knowledge generated here to other páramo ecosystems.

Addressing the *second specific objective,* this thesis demonstrated that green onion cropping significantly altered the vertical distribution of soil mineral N in the intensively monitored plots. The sustained application of fresh chicken manure as fertilizer contributed to soil acidity neutralization and enhanced buffering capacity throughout the profile, promoting high concentrations of mineral N, particularly $N\text{-}NO_3^-$ below the typical rooting zone, limiting plant uptake and increasing the risk of leaching losses. Besides land-use change, factors such as the presence of fallow periods and the vertical distribution of soil texture in cultivated plots also influence N dynamics and should be considered in agricultural decision-making.

Regarding the pedotransfer functions (PTFs) for estimating soil N pools, this study demonstrated that including data from all three soil depths improved prediction accuracy across the three intensively monitored plots, compared to models based solely on topsoil data, regardless of the modeling scenario. Among the predictive models tested, multiple linear regression using edaphic properties as covariates performed best for estimating TN concentrations across the profile and $N\text{-}NO_3^-$ in the surface layer. These two pools are key indicators of soil fertility and potential N losses, making them critical for guiding nutrient management and mitigating environmental impacts. These developed models can be applied to other green onion-cultivated soils in the Santurbán-Berlín páramo, provided they share similar taxonomy and management characteristics with the plots monitored in this study.

In line with the *third specific objective,* and based on the intensively monitored plots, this thesis demonstrated that green onion cultivation significantly altered soil bacterial communities at multiple taxonomic levels. Agricultural management favored copiotrophic organisms associated with soil organic matter decomposition through the priming effect, whereas native grassland soils were dominated by oligotrophic taxa with high N_2 fixation potential.

Cultivation also modified the microbial functional potential related to N cycling. The decline in assimilatory pathways, ureolytic activity, and biological N_2 fixation indicates a reduced capacity for N retention in soils, increasing reliance on external N inputs and potentially compromising sustainability. In contrast, greater nitrification potential promoted soil nitrate accumulation, raising the risk of leaching and water pollution. The enhanced incomplete denitrification potential further suggests a higher likelihood of nitrous oxide (N_2O) emissions, with implications for greenhouse gas fluxes in páramo ecosystems.

Moreover, this thesis underscores a set of complementary strategies to improve N management and enhance agricultural sustainability in the Santurbán-Berlín páramo agroecosystems. Following a source-control approach to mitigate N pollution, the strategies focus on: (i) improving fertilization and irrigation practices; (ii) reducing dependence on monocropping through diversification, rotation, and fallow systems; (iii) managing soil microbial biodiversity; and (iv) promoting public policies to reduce N pollution. Together, these strategies aim to minimize N losses while safeguarding productivity, adapt interventions to local environmental vulnerability, integrate nitrogen management with water and soil carbon, improve nutrient use efficiency, and foster shared responsibility among stakeholders.

In summary, improving fertilization and irrigation in the Santurbán–Berlín páramo requires a progressive shift from input-heavy, perception-driven practices toward evidence-based nutrient management that balances crop productivity with environmental protection. Leveraging locally adapted tools such as the PTFs developed in this thesis, engaging farmers in participatory research, and aligning recommendations with market realities are essential to ensure adoption. Integrating water and nutrient management, reducing nutrient surpluses, and improving organic fertilizer use will not only address current inefficiencies but also create the enabling conditions for complementary strategies, such as crop rotation and fallow systems, to deliver greater long-term sustainability. The findings provide a basis for guiding decision-making and supporting the transition toward sustainable agricultural practices in Colombian páramo ecosystems.

7.2 Perspectives for Future Research

To further advance understanding of soil N dynamics in páramo ecosystems, participatory research with local farmers is essential. Evaluating the strategies proposed in this thesis through

experimental plots should be prioritized, ensuring that study designs integrate local perceptions and definitions of soil fertility. Such an approach will facilitate communication among researchers, communities, and institutions while strengthening farmers' capacities, particularly regarding soil biodiversity management, an area where local knowledge remains limited.

Future studies should also expand the analysis of soil N pools beyond those considered here, incorporating dissolved organic N, potentially mineralizable N, and microbial biomass N, which are key indicators of soil health and fertility. Stable isotopic techniques warrant exploration to better understand how agricultural practices influence N transformations and losses. In parallel, the predictive models developed in this thesis could be strengthened by incorporating additional covariates and employing geostatistical or data mining approaches, enabling more accurate spatial predictions to support sustainable N management.

Deeper insight into the soil microbial dynamics of the N cycle is also needed. Future studies should move beyond bacterial taxonomic profiling toward more comprehensive approaches, incorporating additional gene markers (e.g., other 16S rRNA fragments, 18S rRNA, or ITS) and metagenomic shotgun sequencing to refine functional characterization of bacteria, archaea, and fungi. At the same time, it is critical to address the discrepancy observed in this study between functional predictions from amplicon-based inference and absolute gene abundances quantified by qPCR. Because such predictions are inherently biased toward existing reference genomes, they risk overlooking rare or environment-specific functions, a limitation particularly relevant for the unique microbial assemblages of páramo soils. To improve robustness, future research should integrate quality metrics to assess prediction accuracy and employ complementary strategies such as RNA-based molecular tools, enzyme activity assays, and alternative predictive frameworks. Combining these approaches would not only strengthen functional profiling but also generate

process-level insights, enabling the design of more precise microbiota management strategies to support sustainable agriculture in páramo ecosystems.

Another priority is to address the role of fresh chicken manure, a widely used amendment with diverse and largely uncharacterized microbiota. Its microbial composition, along with transformation processes during generation, storage, and application, likely affects soil N cycling. Future research should clarify how these microbial communities interact with soils and crops, influencing N transformations and assimilation.

Finally, it is worth noting that future research should also account for broader drivers and policy implications. Climate change is expected to alter precipitation and temperature regimes in páramo ecosystems, with potential consequences for soil N cycling and hydrological services. Establishing long-term monitoring networks would provide the temporal resolution needed to capture these shifts and assess their impact on N dynamics. Advancing research in these directions will be critical to deepen understanding of soil N dynamics in páramos and to support the transition toward agricultural systems that minimize N losses while maintaining productivity.

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