Soil Quality and Microbial Responses to Tree Species and Land Terracing in Rwanda

Thesis presented in partial fulfilment of the requirements for the degree of Doctor of Sciences (PhD)

Peter Rwibasira

Supervisor: Prof. Monique Carnol
Co-supervisors: Prof. Donat Nsabimana & Prof. Francois Xavier Naramabuye

University of Liège, Faculty of Sciences
Laboratory of Plant and Microbial Ecology
Department Biology, Ecology, Evolution, UR-InBioS
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Supervisory Committee

Prof. Monique Carnol, ULiège (supervisor)

Prof. Donat Nsabimana, UR (co-supervisor)

Prof. Francois Xavier Naramabuye, UR (co-supervisor)

Prof. Bas van Wesemael, UCLouvain (member)

Prof. Kris Verheyen, UGent (member)

Jury

Prof. Monique Carnol, ULiège (supervisor)

Prof. Donat Nsabimana, UR (co-supervisor)

Prof. Francois Xavier Naramabuye, UR (co-supervisor)

Dr Carole Rougeot (president)

Prof. Bas van Wesemael, UCLouvain (secretary)

Prof. Karen Vancampenhout, KULeuven (member)

Prof. Erik Verbruggen, UAntwerpen (member)

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The fieldwork for this research was carried out in farmers' fields and in the Arboretum of Ruhande in Huye district, Southern Rwanda. I would like to acknowledge the support of the Rwanda Forestry Authority (RFA) and owners of selected farms for allowing this research to take place in these sites. Special thanks to Mr. Emmanuel Nsabimana, an experienced caretaker of the arboretum with more than 40 years of experience, for assisting with field work. His knowledge of the site, plots, tree species, and management history was helpful in setting up the experimental plots and soil sampling.

I feel indebted to my family for the continuous encouragement and motivation during my studies. Most importantly, my heartfelt gratitude is expressed to my beloved wife, Mrs. Fridah Ingabire for your love, patience, encouragement, and most importantly enduring all family duties during my absence. I also extend my thanks to my lovely children, your revitalizing evening calls during my stays abroad kept me motivated. Ndabakunda!

Summary

Soil conservation measures, including forest plantations and land terracing, have been implemented worldwide to restore degraded soils and/or counter land degradation processes. However, these anthropogenic actions may have contrasting effects on soil quality and ecosystem functioning, depending on climate and biophysical characteristics. In Rwanda, afforestation and land terracing are the two major land-use forms commonly implemented, not only for restoring the country's severely degraded soils, but also as a means to provide for wood-derived goods and services and to enable the cultivation of its steep terrains. In this thesis, we assessed the responses of soil quality, through the measurement of physical, chemical and microbiological indicators, to commonly planted tree species and agricultural terracing in southern Rwanda.

We investigated the long-term effects on soil quality of 3 eucalyptus, 3 agroforestry, 2 native species and native species mixed in a self-regenerated plot in the Ruhande arboretum, Rwanda. Potential effects were measured in the upper soil layers at 0-5 cm and 5-10 cm depth. Our results indicate that significantly higher values and more pronounced effects of tree species on most soil properties and microbial processes were restrained in the upper 0–5 cm layer, highlighting the importance of this thin layer for soil quality and ecosystem functioning under these forest plantations. Planting native tree species (i.e., *Entandrophragma excelsum* and *Polyscias fulva*) improved soil quality via alleviation of soil acidity, increasing concentrations of exchangeable base cations, and promoting higher microbial biomass and activity. Eucalyptus species acidified the soil, but also significantly increased soil organic matter contents and did not adversely affect microbial biomass and activity. For example, results showed a significant increase in microbial biomass under *Eucalyptus grandis* and increased N mineralization under *Eucalyptus maidenii*, despite reports on detrimental effects of eucalyptus species on growth and activity of soil microorganisms, due to their soil acidifying effects and secretion of allelopathic compounds. This study therefore suggests that we cannot generalize the effects of planting Eucalyptus on soil quality in general and, in particular, on soil microbial biomass and activity.

Labile fractions of soil organic matter, particularly those extracted with hot water, were the main drivers of differences in soil microbial activity between tree species, indicating that they would better indicate tree-induced changes in substrate availability and soil

quality than total soil organic matter. Further, results suggest that combining analysis of these labile C and N fractions with that soil microbial biomass and activity would give an early indication of management-induced changes in soil conditions.

This study also evaluated the effects of planted tree species on the abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB), as well as their contribution to the rates of soil nitrification, which are important indicators of N cycling in terrestrial ecosystems. Abundance of the *amo*A gene (ammonia monooxygenase–subunit A) of AOA and AOB and their activity demonstrated the numerical and functional dominance of AOA over AOB in terms of *amo*A gene copies and potential soil nitrification rates across tree species. These results are consistent with reports indicating higher abundance and activity of AOA under low pH and limited substrate availability. Soil pH and labile nitrogen were found to influence the differences in abundance and activity of nitrifiers between tree species. Generally, *Polyscias fulva*, *Eucalyptus grandis*, *Grevillea robusta*, and *Cedrela serrata* showed highest potential nitrification rates both by AOA and AOB.

The influence of land terracing was investigated in three paired terraced – unterraced agricultural plots. Land terracing did not affect most soil physico-chemical properties, which were mostly influenced by hillslope position both in terraced and unterraced fields. The results from this study contradict our hypothesis about the effects of land terracing on decline of total SOM and associated soil properties. The reduction of SOM was expected following the construction of terraces which disrupts soil structure through excavation, leading to vertical soil redistribution and thus oxidation of SOM once stored in deeper soil layers. The results, however, supported our hypothesis in which soil quality increases in lower hillslope position as a result of long-term erosional movement and sedimentation of fertile topsoil downwards. Despite the increase in labile C and N fractions as well as soil microbial parameters, especially downslope of terraced land, the overall results did not allow us to draw an explicit conclusion on soil quality restoration by land terracing in the studied sites.

Keywords: soil quality, native tree species, Eucalyptus spp., land terracing, microbial processes, Rwanda.

Résumé

Des mesures de conservation des sols, comprenant des plantations forestières et l'aménagement de terrasses agricoles, ont été mises en œuvre dans le monde entier pour restaurer les sols dégradés et/ou contrer les processus de dégradation des terres arables. Cependant, ces actions anthropiques peuvent avoir des effets contrastés sur la qualité des sols et le fonctionnement des écosystèmes, en fonction du climat et des caractéristiques biophysiques des sols. Au Rwanda, l'afforestation et l'aménagement de terrasses agricoles sont les deux principales formes d'utilisation des terres couramment mises en œuvre, non seulement pour restaurer les sols gravement dégradés du pays, mais aussi pour fournir des biens et services dérivés du bois et permettre la culture des terrains en pente. Dans cette thèse, nous avons évalué les réponses sur la qualité des sols, par le biais de la mesure d'indicateurs physiques, chimiques et microbiologiques, aux espèces d'arbres couramment plantées et au terrassement agricole dans le sud du Rwanda.

Nous avons étudié les effets à long terme sur la qualité des sols de 3 essences d'eucalyptus, 3 essences agroforestières, 2 essences d'arbre indigènes et une brousse naturellement régénérée comprenant un mélange d'espèces indigènes dans l'arboretum de Ruhande, au Rwanda. Les effets potentiels ont été mesurés dans les couches supérieures du sol, à 0-5 cm et à 5-10 cm de profondeur. Nos résultats indiquent que des valeurs significativement plus élevées et des effets plus prononcés des espèces d'arbres sur la plupart des propriétés du sol et des processus microbiologiques étaient observés dans la couche supérieure de 0 à 5 cm, soulignant l'importance de cette fine couche pour la qualité des sols et le fonctionnement des écosystèmes sous ces plantations forestières. La plantation d'espèces d'arbres natives (comme *Entandrophragma excelsum* et *Polyscias fulva*) améliorait la qualité des sols en atténuant l'acidité du sol, en augmentant les concentrations de cations basiques échangeables et en favorisant une biomasse et une activité microbienne plus élevée. Les espèces d'eucalyptus acidifiaient le sol, mais augmentaient également de manière significative les teneurs en matière organique du sol et n'affectaient pas négativement la biomasse et l'activité microbienne. Par exemple, les résultats ont montré une augmentation significative de la biomasse microbienne sous *Eucalyptus grandis* et une minéralisation accrue de l'azote sous *Eucalyptus maidenii*, malgré des rapports sur les effets néfastes des espèces d'eucalyptus sur la croissance et l'activité des micro-organismes du sol, en raison de leurs capacités à acidifier les sol et de la sécrétion de composés allélopathiques. Cette étude suggère donc que nous ne pouvons

pas généraliser les effets de la plantation d'eucalyptus sur la qualité des sols en général et, en particulier, sur la biomasse et l'activité microbienne du sol.

Les fractions labiles de la matière organique du sol, notamment celles extraites à l'eau chaude, étaient les principaux facteurs expliquant les différences d'activité microbiologique du sol entre les espèces d'arbres, ce qui montrait qu'elles indiqueraient mieux les changements induits par les arbres dans la disponibilité des substrats et la qualité des sols que la mesure de la matière organique totale du sol. De plus, les résultats suggèrent que la combinaison de l'analyse de ces fractions labiles de carbone et d'azote avec celle de la biomasse et d'activité microbienne du sol donnerait une indication rapide des changements de la qualité du sol causés par les pratiques de gestion du sol.

Cette étude a également évalué les effets des espèces d'arbres plantées sur l'abondance des archées oxydant l'ammoniac (AOA) et des bactéries oxydant l'ammoniac (AOB), ainsi que leur contribution aux taux de nitrification du sol, qui sont des indicateurs importants du cycle de l'azote dans les écosystèmes terrestres. Les résultats sur l'abondance des gènes *amo*A (ammonium monooxygenase) des AOA et des AOB, ainsi que l'analyse de leur activité ont démontré la prédominance numérique et fonctionnelle des AOA par rapport aux AOB en termes de copies des gènes *amo*A et de taux potentiels de nitrification du sol entre les espèces d'arbres. Ces résultats sont cohérents avec d'autres études indiquant une plus grande abondance et activité des AOA dans des conditions acides et de disponibilité limitée de substrat. Le pH du sol et l'azote labile (fractions d'azote extractible à l'eau chaude) étaient fortement associées à des différences d'abondance et d'activité des nitrificateurs entre les espèces d'arbres. Généralement, *Polyscias fulva*, *Eucalyptus grandis*, *Grevillea robusta* et *Cedrela serrata* ont avaient les taux potentiels de nitrification les plus élevés à la fois pour les AOA et les AOB.

L'influence du terrassement agricole a été étudiée dans trois sites agricoles. Les résultats montrent que le terrassement n'a pas changé significativement la plupart des propriétés physico-chimiques du sol, qui étaient principalement influencées par la position dans la pente à la fois dans les champs terrassés et non terrassés. Les résultats de cette étude contredisent notre hypothèse concernant les effets du terrassement sur la diminution de la matière organique totale (SOM) et des propriétés du sol associées. La diminution de la matière organique était attendue après la construction des terrasses qui perturbe la structure du sol par creusement, conduisant à la redistribution verticale des couches du sol et donc à l'oxydation accélérée de la matière organique lorsqu'elle est stockée dans

les couches profondes du sol. Les résultats ont cependant confirmé notre hypothèse selon laquelle la qualité du sol augmente en position basse de la pente à cause du transport érosif des sols fertiles vers le bas et l'accumulation des sédiments sur le long terme. Malgré l'augmentation des fractions labiles de carbone et de l'azote ainsi que des paramètres microbiologiques du sol, en particulier à la position basse de la pente des terres terrassées, les résultats globaux ne nous ont pas permis de tirer une conclusion explicite sur l'amélioration de la qualité du sol par le terrassement dans les sites étudiés.
Mots clés: *qualité du sol, espèces d'arbres indigènes, Eucalyptus, terrassement du sol, processus microbiens, Rwanda*.

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List of acronyms

AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
ATU	Allyl-thiourea
C:N	Carbon to nitrogen ratio
EBC	Exchangeable base cations
FAO	Food and Agriculture Organization
FONERWA	Rwanda's National Fund for Environment
GoR	Government of Rwanda
HWC	Hot water-extractable carbon
HWN	Hot water extractable nitrogen
ICRAF	World Agroforestry Centre
IUCN	International Union for Conservation of Nature
LMM	Linear mixed models
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MBP	Microbial biomass phosphorus
MINAGRI	Rwanda's Ministry of Agriculture and Animal Husbandry
MINILAF	Ministry of Land and Forestry
NISR	National institute of statistics of Rwanda
Nmin	Net nitrogen mineralization
PCA	Principal component analyses
PCR	Polymerase Chain Reaction
PNR	Potential Nitrification Rate
PSTA	Rwanda Strategic Plan for Agriculture Transformation
qCO2	Metabolic quotient
qmic	Microbial quotient
RAB	Rwanda Agriculture Board
REMA	Rwanda Environmental Authority
RFA	Rwanda Water and Forest Authority
SOM	Soil organic matter
TOC	Total soil organic carbon
TEA	Total Exchangeable Acidity
TN	Total soil nitrogen
WAS	Water Aggregate Stability
WHC	Water holding capacity
WSC	Water soluble carbon
WSN	Water soluble nitrogen

Scientific communications from this PhD research

Journal Articles

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol (2021). Long-Term Effects of Forest Plantation Species on Chemical Soil Properties in Southern Rwanda. Soil Syst. 2021, 5, 59. https://doi.org/10.3390/soilsystems5040059.

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol. Reforestation in the tropics: implication of tree species selection on soil microbial processes in Rwanda (*Manuscript*).

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol. Tree species identity influences the abundance of ammonia-oxidizing bacteria and archaea and soil nitrification rates in a forest plantation, Southern Rwanda (*Manuscript*).

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol. Assessing the effects of agricultural terracing practices on soil properties and processes in Southern Rwanda (*Manuscript*).

Conference Abstracts

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol (2017). *Microbial properties and nutrients content under exotic and native tree species in Southern Rwanda*. 5th International SASA Conference, from 4–6 October 2017 in Kigali, Rwanda.

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol (2019). *Influence of land terracing on bioindicators of soil quality in an agricultural ecosystem, Southern Rwanda*. Transforming Rwanda's Development by investing in people's skills; Kigali Convention Center, 26th – 27th September 2019, Kigali –Rwanda.

P. Rwibasira, F.X. Naramabuye, D. Nsabimana, M. Carnol (2019). *Effects of forest restoration tree species on soil properties in Southern Rwanda*. Transforming Rwanda's Development by investing in people's skills; Kigali Convention Center, 26th – 27th September 2019, Kigali –Rwanda.

GENERAL RESEARCH CONTEXT

General Research Context

Soils are vital for maintaining all forms of life on Earth through supporting, provisioning, and regulating ecosystem services and goods; yet anthropogenic pressures on soil resources are rising to critical limits (Poesen, 2018). Human activities, including changes in land use and inappropriate management practices, affect terrestrial ecosystem functioning and the soil's potential for providing important ecosystem services and functions (Fentie et al., 2020) . Soil quality has been defined as the "capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health", with animal health including human health, (Doran & Parkin, 1994; Karlen et al., 2003). Soil functions (i.e., element cycling, soil structure maintenance) can be considered as an assembly of soil processes, linked to soil-based ecosystem services (Kibblewhite et al., 2008; Bünemann et al., 2018), such as biomass production, sustaining biological diversity and activity, regulating elements and energy flows, storing and transforming substances, providing raw materials, and being physical support for plants and human-made infrastructure. More recently, the concept of soil health has been developed from the soil quality concept. Although much controversy and discussions are available in the literature on the different terms and concepts used (soil quality, fertility, health, services, functions, processes) (Blum, 2005; Baveye et al., 2016), a recent review distinguishes soil health from soil quality as "extending beyond human health to broader sustainability goals that include planetary health" (Lehmann et al., 2020). Within this thesis we use the terms soil functions and quality, as its scope has been defined as focusing on ecosystem functions and services (Bouma, 2014).

Changes in soil conditions often influence soil functions, which can be evaluated using indicators of its physical, chemical, and biological characteristics (Paz-Ferreiro & Fu, 2016). The loss of soil resources and the associated adverse effects are a global issue, since soil formation or restoration processes are often too slow compared to the current rates of soil erosion and degradation (Pulleman et al., 2012). This issue can be solved by adopting a sustainable use of soils, which may promote soil quality, enhance ecosystem services, and ensure climate change mitigation (Roy et al., 2022). According to the recent World Soil Charter endorsed in 2015, sustainable use of soils involves maintaining or

enhancing the supporting, provisioning, regulating, and cultural services provided by soil without significantly impairing either the soil functions that enable those services or biodiversity (FAO & ITPS, 2015).

Major threats to soil and ecosystem functioning include soil erosion, loss of soil organic carbon, nutrient depletion and imbalance, soil acidification, soil and water contamination, and loss of soil biodiversity (Sanaullah et al., 2020). Although several management practices are commonly proposed to deal with soil threats, some of them can adversely affect soils depending on climate, land use, biophysical, and initial soil conditions (Tarolli et al., 2014). In the context of maintaining and/or restoring soil quality, the ultimate goal is to adopt a holistic and integrated approach that will reduce soil losses, enhance soil C sequestration, promote nutrient cycling and availability, and sustain soil biodiversity (Lal, 2015). To achieve this goal, there is not a single strategy that can globally fit all conditions, because of differences in climate, degradation extent, land use, socio-economic, and biophysical factors that determine the effectiveness of the adopted strategy (Ghosh et al., 2021; Baradwal et al., 2022). In the context of restoration of degraded soils, the extent of soil degradation or recovery can be evaluated and interpreted as physical (e.g., structure, texture, aggregate stability, hydrology, and erosivity), chemical (e.g., pH, soil organic carbon, nutrient elements, salinity, toxicity), and biological (e.g., biodiversity, element cycling processes, abundance, activity, and biodiversity of soil organisms) indicators, involved in soil ecosystem functioning (Lal, 2015). Microbial soil parameters give insights into the living component of the soil and are directly related to many soil processes and functions mediated by soil microorganisms (Pulleman et al., 2012). Many biological soil properties are dynamic and can be more sensitive to changes in soil conditions than most physico-chemical soil properties (van Bruggen and Semenov, 2000). Despite their importance for soil functioning, the assessment of biological parameters is relatively recent and infrequent, especially very few data exist for tropical soils (Joergensen, 2010). Changes in land use and management practices can have negative, neutral, or positive effects on soil microorganisms and their activities (Paz-Ferreiro and Fu, 2016). Because soil degradation may often continue unnoticed by anticipating only positive outcomes from soil restoration actions (Maetens et al., 2012), the assessment of the responses of soil properties and processes to implemented soil conservation measures is essential to ensure timely intervention to maintain soil functioning (Paz González et al., 2014).

Rwandan soils are among the world's most degraded soils (Fenta et al., 2020; Wuepper et al., 2020), owing to an increasing population with limited financial resources (Bizoza, 2014), land scarcity, and overexploitation of agricultural fields. The estimated mean soil loss rate of 250 t ha^{-1} $year^{-1}$, due to water erosion (Fenta et al., 2020), is much higher than the average global rate of soil erosion, which is about 30 t ha^{-1} $year^{-1}$ (Stanchi et al., 2015). Only 52% of the entire territory can be used for cultivation, forcing farmers to cultivate high-sloping terrains which may increase the risk of soil erosion (Karamage et al., 2017; Nsengiyumva et al., 2019). To reverse the increasing soil degradation, Rwanda set a goal of land degradation neutrality by 2030 and was the first African nation to pledge to repair 2 million ha (76%) of its lands and forests under the Bonn Challenge in 2011 (REMA, 2021). According to the UN Decade on Ecosystem Restoration Program (2021-2030), forests and landscapes need to be restored with the goal of restoring ecological functions, such as biodiversity and soil functions that improve human well-being (Lewis & Nyamulinda, 1996; Fagan et al., 2020; Winowiecki et al., 2021).

Within this context, the Rwandan government undertook significant afforestation and terracing programs as strategies to conserve and/or restore degraded lands, (Kagabo et al., 2013), resulting in two common land use forms, forest plantations and farming terraces, characterizing the entire biophysical features of Rwanda. Forest trees can influence soil properties through various mechanisms, including litter quality, microclimate, and microbial activity linked with various key soil processes (Prescott & Vesterdal, 2013), and recent studies have shown that tree species identity is an important factor driving changes in soil physical, chemical, and biological properties (Dawud et al., 2017; Augusto & Boča, 2022). Although the establishment of forest plantations on degraded land has been considered as an ultimate strategy for the restoration of degraded soils (Binkley and Menyailo, 2004), the long-term effects of afforestation on soil properties and functions remains uncertain, especially on soil microbial functions in tropical regions (Wright et al., 2010).

Land terracing can help prevent soil erosion, improve water infiltration, and provide a stable ground for agricultural activities on steep terrains (Rutebuka et al., 2021). However, terracing can also alter soil properties through disturbance or disruption of soil structure and accelerated loss of soil organic carbon, resulting in potential negative effects on nutrient availability, retention, and activity of microbial communities (Deng et

al., 2021). The outcome of a terracing action in a specific region depends not only on the local environmental factors, but also on the terracing method applied. Furthermore, there is a lack of understanding of the effects of terracing on soil quality in Rwanda, especially on soil microbial functioning.

This thesis addresses the long-term effects of forest plantations and the influence of land terracing, the two dominant soil conservation and restoration approaches in Rwanda, on soil physico-chemical and microbial properties, with the aim to understand how these restorative approaches have changed soil quality.

Objectives and outline of the thesis

The overall aim of this study was to assess the effects of forest tree species in planted monospecific forest stands and agricultural land terracing on soil physico-chemical properties and microbial processes in southern Rwanda. Accordingly, we evaluated the following general hypotheses:

i) The plantation of exotic species, particularly Eucalyptus, negatively affect the soils while native trees improve soil properties and microbial processes.
ii) Ammonia oxidizing archaea (AOA), rather than ammonia oxidizing bacteria (AOB), drive nitrification in these acidic tropical soils and their activity is influenced by soil conditions related to the effects of tree species.
iii) Land terracing and hillslope position influence the aggregate stability and soil organic carbon contents which can indicate changes in soil quality.

This thesis dissertation comprises six chapters. **Chapter 1** introduces relevant information on the specific features of tropical soils, how changes in land use and management practices affect soil functioning and how these changes can be assessed through soil physico-chemical and microbial indicators. In this introduction, I also describe the state of land use changes in Rwanda and the potential implications of land terracing and forest plantation for soil functioning. Chemical soil properties including pH, exchangeable cations, and labile C and N fractions are essential for soil functioning, therefore their response to tree species identity was explored in **Chapter 2**. We hypothesized that the exotic eucalyptus species would reduce the chemical quality of the soils in comparison to native species and that labile C and N fractions would be more sensitive to a change in tree species than soil organic matter. Based on the observed effects of tree species on changes in chemical soil properties and considering that the biomass and activity of soil microorganisms often reflect soil conditions, **Chapter 3** focused on microbial properties and soil processes in response to tree species. We hypothesized that microbial processes would be most intense in the uppermost soil layer, lower under Eucalyptus species, and that differences in microbial activity between tree species would be explained by the availability of labile carbon substrate. **Chapter 4** explored the abundance and activity of AOA and AOB and their relationship with soil properties. Considering the low pH in this soil, we hypothesized a dominance of AOA over AOB, measured through the abundance of amoA gene, that would also reflect the

rates of nitrification, with higher values of amoA gene abundance and potential nitrification rates under tree species which improved soil. In **Chapter 5**, the effect of land terracing on soil properties and microbial processes is evaluated through a comparative assessment of soils from terraced and non-terraced farms to provide insights into land terracing effectiveness in improving soil quality. Due to the disturbance of soil structure and a potential destruction of soil aggregates during the construction of terraces, we expected changes in soil properties and processes, particularly those linked to C and N transformations at different locations of the slope. It was also hypothesized that soil microbial properties and processes would increase at the bottom hillslope position due to the accumulation of organic matter due to potential erosion and fertile soil displacement. Finally, **Chapter 6** discusses key findings of the thesis and provides overall conclusions. We briefly discuss the potential implications for the long-term effects of afforestation species and land terracing and highlight soil characteristics that could help in the evaluation of these conservation measures. At the end of this chapter, future perspectives and research directions are also briefly addressed.

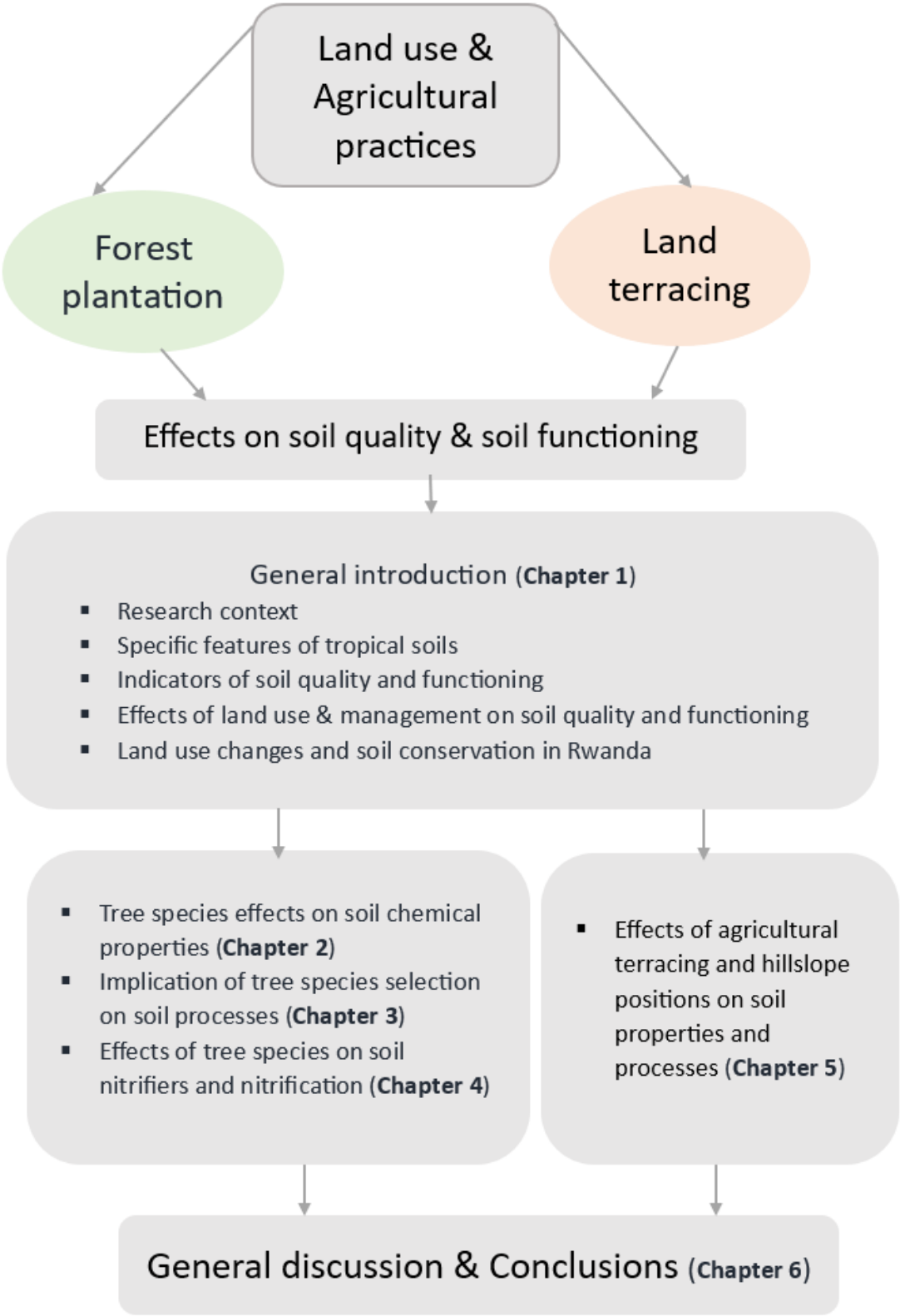

Fig. 1. 1. Schematic Outline of the Thesis

CHAPTER 1 – GENERAL INTRODUCTION

Chapter 1 - General Introduction

1.1. Specific features of tropical soils

Tropical soils are widespread around the equatorial belt between the Tropic of Cancer and the Tropic of Capricorn (Labrière et al., 2015), and they are generally considered as old, deep, highly weathered, and chemically impoverished soils (Schulte and Ruhiyat, 1998). Tropical soils are dominated by Ferralsols/Oxisols (Harter, 2007), characterized by intense weathering which results in high concentrations of iron and aluminum oxides (de Carvalho et al., 2015). The clay minerals of Ferralsols are predominantly composed of varying proportions of kaolinite, hematite, goethite, and gibbsite (Silva et al., 2021). However, some of these soils might originate from volcanic activities and hence exhibit different properties. Clay particles in reddish-colored tropical soils are mostly made of kaolinite and iron- and aluminum-oxide, and they have a limited ability to become sticky or to expand and contract when wet and dry (Foth, 1990).

The high microbial activity in tropical soils is facilitated by optimum temperature, moisture conditions throughout the year (Krishna and Mohan, 2017), and the continued supply of organic materials resulting from plant growth (Lynch, 1995; Bauhus & Khanna, 1999). In the tropical forests, the rapid decomposition of aboveground litter, root exudates, and other detritus, ensure quick internal cycling of nutrients , mainly driven by microbial processes which are thus essential for ensuring nutrient supply to the trees. High microbial activity and soil organic matter content are generally restrained to a thin upper soil layer (Bauters et al., 2017b). Most tropical agricultural soils are acidic and nutrient depleted, with high risks of Al toxicity (Kunito et al., 2016).

Given the impoverishment of the tropical soils and the rapid decomposition of organic matter, resulting in a very thin upper fertile layer, these soils are particularly prone to degradation through inappropriate soil management actions (Nyssen et al., 2009).

1.2. Indicators of soil quality and functioning

Assessing the responses of soil properties and microbial processes to environmental changes can be a valuable tool to understand the effects of land use changes and management practices on ecosystem functioning (R. D. Bardgett, 2011). Although the importance of soil quality for supporting life on Earth is widely acknowledged, there is

no consistency in how to analyse and interpret the term (Sposito and Zabel, 2003). The development of relevant soil quality indicators has been proven challenging, as soil quality is linked to holistic approaches of managing natural resources, which are hard to define objectively, because soils are naturally variable, and the definition of their quality depends on their intended use (Blum, 2005). Soil quality and functioning have been assessed using soil parameters such as pH, aggregate stability, nutrient and organic matter contents, bulk density, microbial abundance, diversity, and activity (Smith et al., 2021), although the consideration of microbial parameters is relatively recent (Schloter et al., 2018). According to Bünemann et al. (2018), it is important when choosing an indicator of change in soil quality to select a parameter with high sensitivity to changes in soil conditions and consider its relationship to others soils variables.

Interactions between soil chemical, physical and biological properties provide soils with the ability to perform multiple functions and services including pollutant and water filtration, biodiversity habitat, providing support, and cycling nutrients (Greiner et al., 2017a). For example, C and N transformation processes are used to reflect soil functions related to organic matter decomposition and nutrient cycling (Lima et al., 2013), and are assessed through measurement of soil microbial biomass C and N, nitrogen mineralization, nitrification, respiration, and their interactions with other soil properties (Turco et al., 2015; Schloter et al., 2018). Other soil properties such as pH and exchangeable base cations can also be used as proxies of soil fertility (Koch et al., 2013). Therefore, the effects of environmental changes on soil quality and functioning may vary depending on the extent to which relevant soil quality indicators are altered in a particular soil type and land use (Vogel et al., 2019a).

1.3. Effects of changes in land use and management on soil quality and functioning

Changes in soil characteristics and processes can have major effects on ecosystem functions and services (Chen et al., 2021). These changes might result from changes in land use and agricultural practices. In many locations at high risk of land degradation due to soil erosion, terracing and reforestation have been recommended as the most potentially successful soil conservation and restoration practices (Deng et al., 2021; Rutebuka, 2021). To ensure the sustainable use of soil resources, it is imperative to quantify the long-term effects of these changes.

1.3.1. Effects of tree species identity on soil properties and processes

Tree species influence soils through their specific traits, such as the quantity and quality of litter input and root exudates (Grayston et al., 1997; Augusto et al., 2002), nutrient requirements and acquisition mechanisms, canopy, and root architecture. These traits, influence soil structure and texture (Loranger et al., 2002), soil moisture and temperature, the presence of understory vegetation (Falkengren-Grerup et al., 2006), nutrient return to the soil by litter and throughfall (Carnol and Bazgir, 2013), soil biota (Aponte et al., 2013; Jozefowska et al., 2016), parameters which in turn influence soil properties and microbial processes.

Afforestation/reforestation may contribute to the restoration or the degradation of soil via tree species-specific litter traits and chemical composition (Wardle et al., 2004; Tajik et al., 2019), resulting from various nutrient acquisition strategies of related to tree species identity (Lambers et al., 2008). For instance, a global meta-analysis assessing the long-term effects of eucalyptus species on soil properties and processes (Mallen-Cooper et al., 2022) demonstrated that eucalyptus species decrease soil nutrients contents (Zhang et al., 2015) and water resources (Christina et al., 2017). Particularly, this synthesis showed a significant decline in soil moisture, microbial abundance, nitrogen, and cation contents under eucalypts, an increase soil carbon, and inconsistent effects on soil pH (Mallen-Cooper et al., 2022).

Eucalyptus tree species are commonly grown in the tropical regions such as in Rwanda (Figure 2), where they dominate forest plantations (89 %), to provide for the country's increasing need for firewood, charcoal, building materials, and other non-timber forest products. They also contribute significantly to environmental preservation by fighting frequent landslides and reducing soils erosion in the steep lands (Mugunga, 2016; IUCN, 2020).

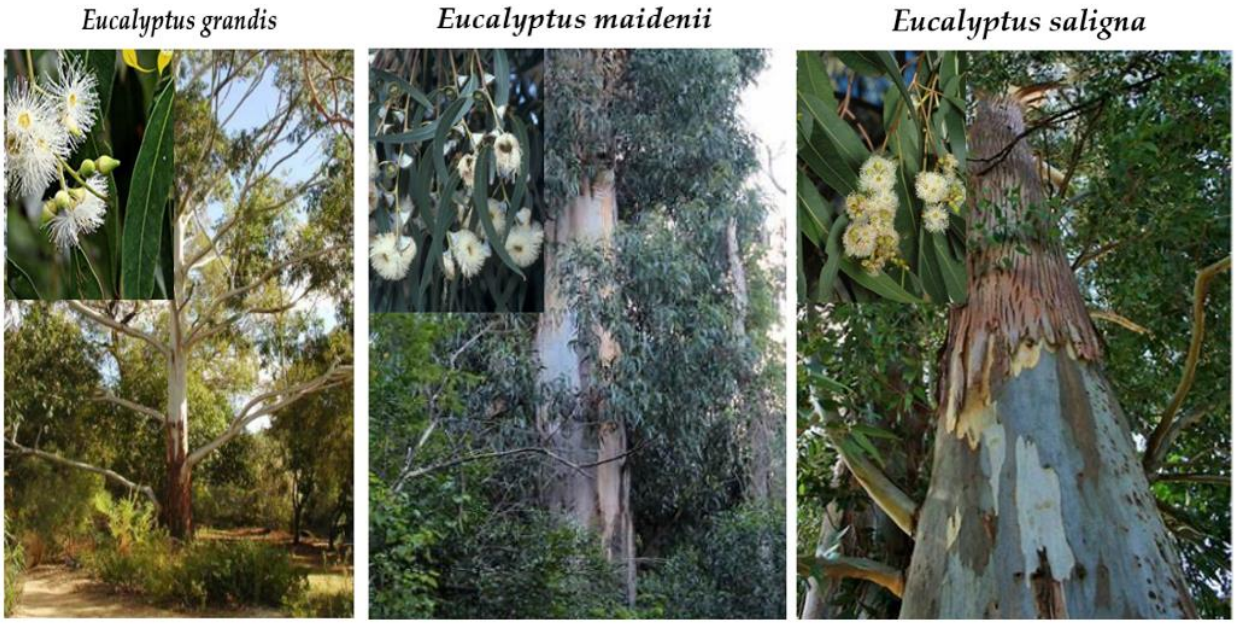

Fig. 1. 2. Commonly planted Eucalyptus tree species included in this study

Other species included in the present study (Figure 3), such as *Calliandra calothyrsus*, *Grevillea robusta*, and *Cedrela serrata,* are used in agroforestry (trees on farms) to improve soil fertility (Ndoli et al., 2021). *Calliandra calothyrsus* is commonly used as fodder for livestock (Kisaka et al., 2023). It is a N_2-fixing tree, in association with rhizobium bacteria, and has been reported to promote soil nitrogen accumulation, improve soil physical properties and topsoil organic matter content (Koutika et al., 2005). *Grevillea robusta* and *Cedrela serrata* are mainly planted not only for their provision of wood products, but also to improve soils through nutrient cycling, stabilizing the soils, reducing nutrient leaching, and producing leaf mulch that improves soil moisture and organic matter contents (Ndoli et al., 2021; Musongora et al., 2023). For example, *Grevillea robusta,* with its proteoid root structure, can easily adapt to and improve soils with poor nutrient contents (Kalinganire, 1996; Watt and Evans, 1999). Proteoid root mats, characterizing plants in the Proteaceae family, were reported to be prominent in nutrient-poor soils and to exude chemical compounds that mobilize unavailable nutrients bound to metal cations (e.g., Fe^{2+} and Al^{3+}) and enhance nutrient uptake (Dinkelaker et al., 1997).

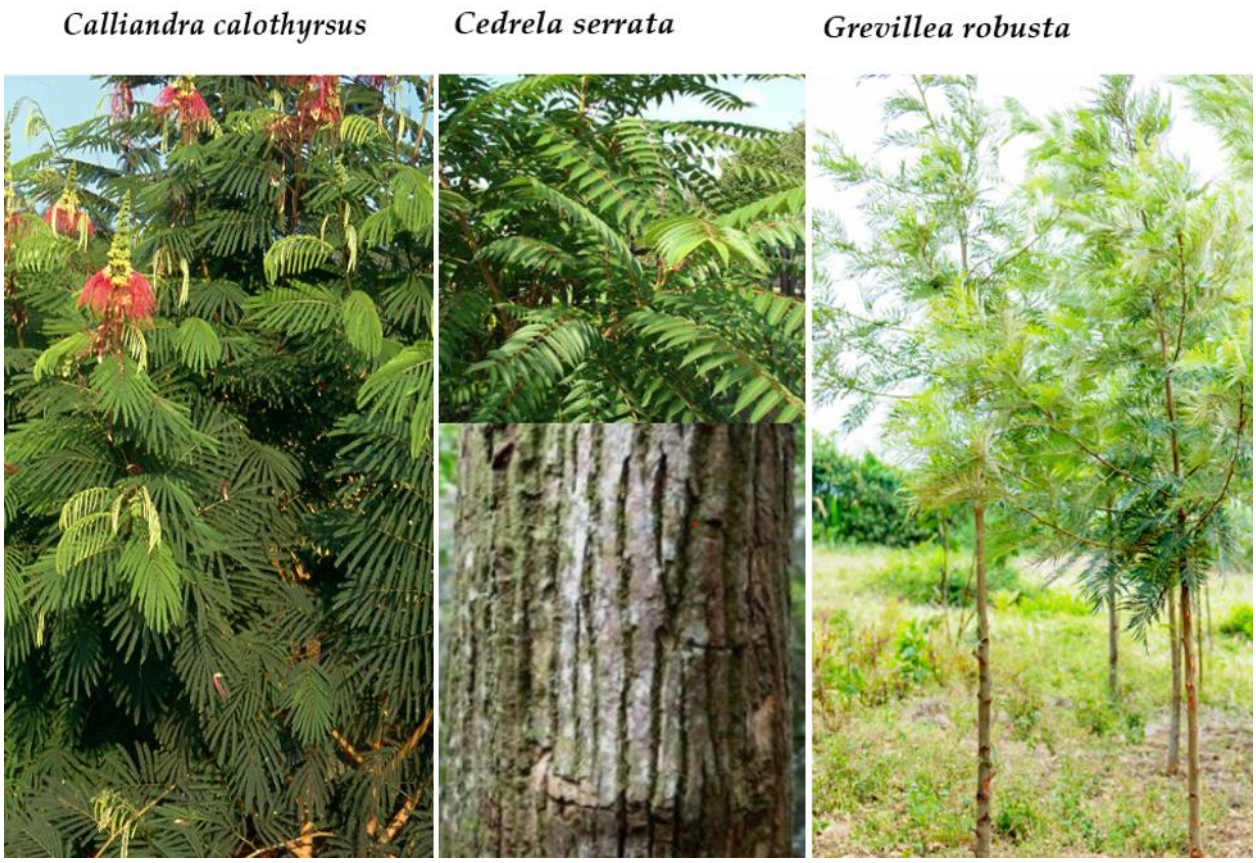

Fig. 1. 3. Studied agroforestry tree species

Among the native tree species used in this study, *Entandrophragma excelsum* and *Polyscias fulva* (known in Kinyarwanda as *Umuyove* and *Umwungo*, respectively) are common in Rwanda as well as in various natural forests of the tropical east and central African region (Figure 4). Being a late successional native species, *Entandrophragma excelsum* grows faster vertically at young age until it overtops other trees to successfully compete for light, then slowly increases in height and diameter of stem, buttress, and branches (Hemp et al., 2017; Mujawamariya et al., 2021). In the wood industry, *Entandrophragma excelsum* is categorized in "African Mahogany" species known as premium timber with high economic value on local and international market (Orwa et al., 2009; Styles and White, 1991). *Polyscias fulva* is an evergreen early successional tree species commonly found in tropical montane forests (Mujawamariya et al., 2023). This tree species is traditionally very known in Rwanda for multiple uses; mainly for its potential medicinal value and making wooden royal instruments (e.g., drums, chairs, beehives, music instruments, utensils, etc.).

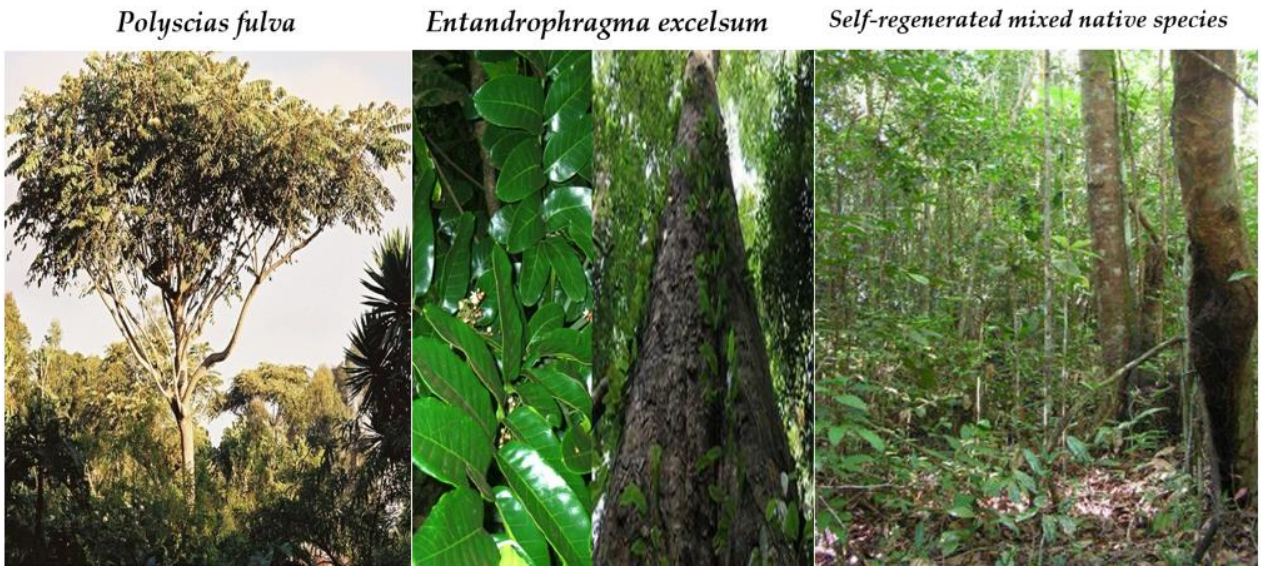

Fig. 1. 4. Native tree species included in this study

Antimicrobial properties of *Polyscias fulva* have been reported in many studies (Plunkett et al., 2001; Winnie et al., 2019; Ashmawy et al., 2020). The structure of its thick foliage and wide-spread parasol canopy makes *Polyscias fulva* to be preferred in agroforestry systems where mulch and shade are needed (ICRAF, 2015).

1.3.2. Effects of land terracing on soil properties and processes

Land terracing is one of the oldest techniques for increasing the arable surface area on steep hillslopes through conservation of water and soil (Cao et al., 2013; Arnáez et al., 2015). The technique consists of creating flat surfaces, generally used for cultivation, separated by a 'vertical riser' protected by a wall of dry stones, soil, grass, or trees (Deng et al., 2021). Terrace farming systems include bench terracing, contour, and parallel terraces. Bench terraces comprise steps and flat areas arranged in regular intervals, and its construction is labor consuming and implies soil disturbance. Contour terracing follows the relief contour, requires less labor and causes less soil disturbance than bench terracing. Parallel terracing, resulting in parallel constructions, is achieved through heavy labor and costs and causes severe soil disruptions, as it may imply land-leveling operations (Stanchi et al., 2012).

The conversion of steep farmlands into terraced fields can increase the arable surface by 20%–40%, with an increase in crop yield of the same magnitude (Hu et al., 2005; Posthumus and De Graaff, 2005). Although land terracing is practiced for protecting soils against degradation by erosion, it may disturb soil structure, leading to reduced stability

of soil aggregates (Tobiašová et al., 2023), while accelerating SOM and nutrient loss via increased SOM degradation (Caravaca et al., 2002). As a result, soils with poor structure and unstable aggregates are prone to nutrient leaching, compaction, and high runoff with low infiltration (Deng et al., 2021). The mechanical the construction of terraces has a non-negligible effect on soil properties and processes. First, excavation and changes in slope topography have a significant negative effect on soil structure. Also, the fertile topsoil may be removed, and subsoil may be upturned causing redistribution of soil layers and associated characteristics. As terraced fields vary in shapes and sizes, as well as in the intensity of the mechanical disruption (from manually created terraces to the use of heavy machinery), implications for soil quality and functioning may differ greatly between terraced sites.

1.4. Land use changes and soil conservation in Rwanda

Human-induced land degradation adversely affects 29% of global land, threatening the livelihood of more than 2.6 billion people with soil erosion by water being the major cause of land degradation in the world (Wuepper et al., 2020). The wellbeing of humans and all terrestrial life depends on soil resources, but these are globally subjected to degradation as a result of both natural events and anthropogenic activities (FAO & ITPS, 2015). Land degradation was described as the reduction or loss in the quality and the amount of land resources, required to support ecosystem services and functions and enhance food security within specific temporal and spatial scales and ecosystems (Borrelli et al., 2020). Land use and agricultural practices are important factors that influence the characteristics of soils through the alterations of the soil physical, chemical, and biological properties (García-Orenes et al., 2010). The decline in soil fertility is a major concern in Sub-Saharan Africa, where approximately 67% of the total land is degraded to varying degrees of severity (Sileshi et al., 2019).

Rwanda is one of the countries facing high risks of soil degradation due to its climate characterized by frequent heavy rains and the typical mountainous landscape, exposing soils to erosion (Rutebuka, 2021). Between 1990 and 2015 soil loss by erosion increased by about 54%, with an annual average soil loss of 62 tons per hectare (Karamage et al., 2016; Nyesheja et al., 2019). Specifically, soil degradation is primarily attributable to the exploitation of soils, frequently on sloped farms, because of limited land resources and the increasing need to feed an ever-growing population.

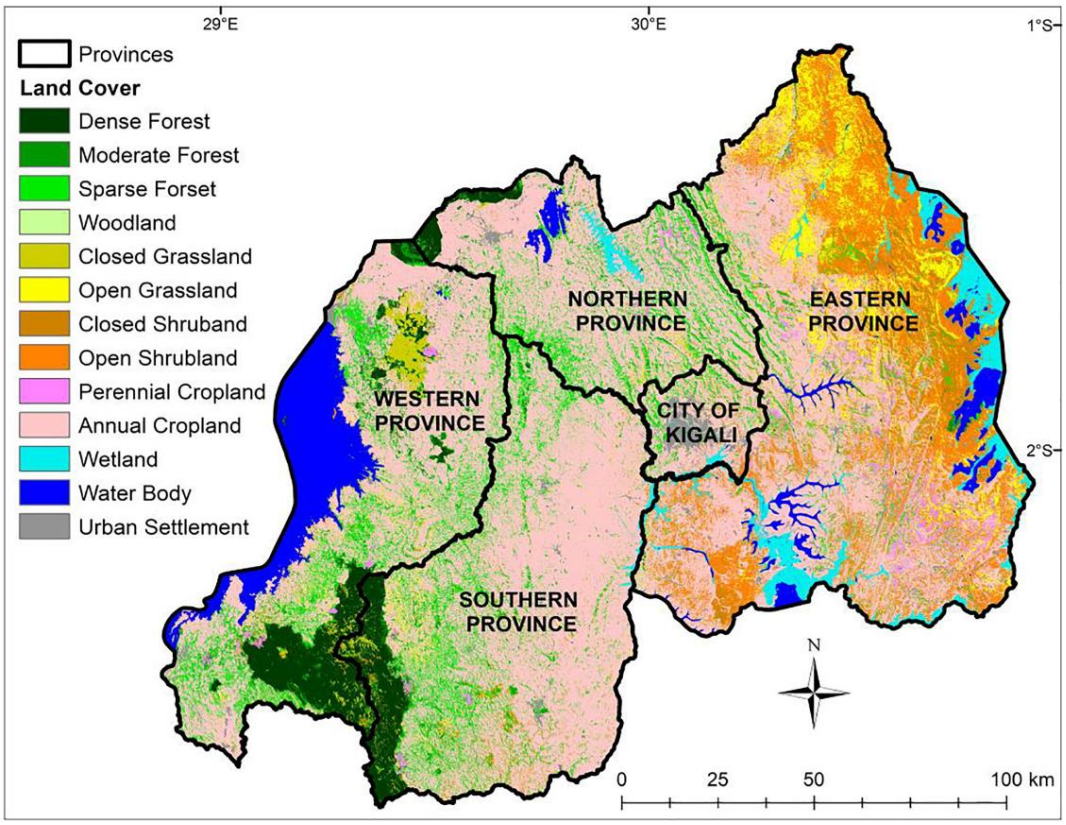

Fig. 1. 5. Land use – land cover map for Rwanda, 2015.
Source: (Banerjee et al., 2020).

According to the Rwandan fifth Population and Housing Census (RPHC, 2022), the country's population increased from 10.5 million in 2012 to counted 13.2 million in August 2022 and it is projected to hit 16.3 million by 2032 (NISR, 2023). With this rapid population growth in a small country (26,338 km^2), Rwanda continues to be one of the most densely populated nation in Africa, with a population density of 535 habitants per km^2. With 72% of people living in rural areas and 69% of households daily engaged in agricultural activities, pressures on agricultural lands are high. About 90% of agricultural activities are conducted on steep slopes, leading to country's reliance on vulnerable terrains for agricultural productivity across the country (Rutebuka, 2021).

Recognizing the threats of environmental degradation and their effects on the global and country's economic development, the government of Rwanda, in its current vision 2050, places a strong emphasis on the necessity of environmental sustainability, resource management, and climate change adaptation (REMA, 2021). According to this vision, research should play an important role as a tool for tracking progress on socio-economic, environmental and natural resource indicators that bring together information on resource stocks and flows, uses, scarcities. This vision states that the improvement of

sustainable economic growth results from the integration of this information into development plans (Bagstad et al., 2019). Based on specific local factors, associated with its topography, climate, and socio-economic conditions, Rwanda has been making significant efforts in restoring degraded land through establishing new forest plantations and terracing agricultural lands (Bucagu et al., 2013). Further, with Rwanda's long-term Vision 2050 and as a signatory of global commitments such as the Bonn Challenge 2030, Paris Agreement, SDGs, UN land degradation neutrality (LDN), and Africa Agenda 2063, there is need for assessing the responses of soils to restoration measures that have been implemented in past decades, including tree plantation and terracing lands. Both forests and agricultural activities are crucial in daily livelihood of the Rwandan population. Forests provide 86% of the primary energy source, mainly as domestic cooking energy (RFA, 2021). They hold the base for the country's tourism opportunities, protect watersheds, downstream wetlands and rivers, as well as support agriculture, which accounts for 36% of GDP (NISR, 2019).

1.4.1. Agricultural land terracing in Rwanda

Rwanda has used a variety of terracing techniques to specifically reduce the detrimental effects of intensive cultivation of steep slopes on soil quality and soil loss (Kagabo et al., 2013). In Rwanda, it is estimated that 1,080,168 ha of land (45%) are at high risk of erosion across the country (IUCN, 2022). About 71 941 ha (7%) of this total risk area are at extremely high risk, while 190, 433 ha (18%) are at very high risk and 300,805 ha (28%) at moderately high risk of erosion respectively. Although efforts have been invested in controlling accelerated soil erosion, especially in agricultural systems, only 282,352 ha (26%) of the total land at high risk of erosion are protected either by contour bank terraces, commonly known as progressive terraces, covering 28,870 ha (10%), and bench terraces, protecting 15% of hillslope lands at high risk of erosion.

The land consolidation program, focusing on production of specified crops in different agroecological zones of Rwanda, has enabled extended farming terraces via financial support from the government and development NGOs (Byamukama et al., 2011; Bizoza, 2014). Although the main goal of this program was to allow people to increase arable land surface, work together to produce more, and be more connected to markets, some farmers have been resisting this process, with the claims that terraced land were quickly degraded after just a few productive seasons. Consequently, it is very common in Rwanda

to observe adjacent farms with terraced and unterraced agricultural plots owned by the same farmer (Figure 5).

These claims are the main catalyst behind the need for understanding the response of soil characteristics to land terracing practices (Rushemuka et al., 2014). Land terracing is not a particular agricultural practice to Rwanda, as it existed for many years in other areas of the world.

Fig. 1. 6. Common agricultural practice showing paired terraced – unterraced plots in Rwanda

The benefits of terracing include increased arable land on sloping farms, soil conservation by minimizing soil loss by erosion, improved productivity, reduce sedimentation and pollution of water streams (Karamage et al., 2017). However, there are some disadvantages of terrace farming related to reduced productivity in terraced lands due to the mechanical disruption of soil structures, increased oxidation and loss of organic matter stocks and associated benefits, financial cost to maintain terraces and their fertility (Deng et al., 2021).

1.4.2. Reforestation and afforestation in Rwanda

One of the main targets in the Vision 2020 launched in 2000 was to reach 30% of forest cover by 2020 in Rwanda. According to the forest cover map of 2019, the total forested

land area is 724,695 ha, representing 30.4% of the country land among which plantation forests occupy 387,425 ha (16.2%), natural mountain rainforests are 130,850 ha (5.5%), wooded savannah occupy 161,843 ha (6.8%) and shrubs occupy 43,963 ha representing 1.8% of the total country land (Rwanda forest authority; RFA, 2021).

Fig. 1. 7. Arboretum of Ruhande (study site) serving as gene bank of forest germplasm and planting materials in Rwanda

Although the target was reached, forests are unevenly distributed and not diversified. For example, the Eastern province is the least afforested, and it is the only rural area in Rwanda where deforestation surpasses afforestation (MoE, 2019a). The campaigns to plant more forests will continue, but it remains unclear how different tree species may affect soil processes and soil quality in Rwanda, especially in regard to introduced exotic species.

CHAPTER 2 – Long-term effects of forest plantation species on chemical soil properties

Chapter 2 - Long-Term Effects of Forest Plantation Species on Chemical Soil Properties in Southern Rwanda

Adapted from: Rwibasira, P.; Naramabuye, F.X.; Nsabimana, D.; Carnol, M. Long-Term Effects of Forest Plantation Species on Chemical Soil Properties in Southern Rwanda. Soil Syst. 2021, 5, 59. https://doi.org/ 10.3390/soilsystems5040059

Abstract

Understanding the long-term effects of tree species on soil properties is crucial for the development of forest restoration policies in relation to the choice of species that meet both environmental and local livelihood needs. This study was performed in the Arboretum of Ruhande, Southern Rwanda, where monocultures of 148 deciduous and 56 conifer species have been established in 0.25 ha replicated plots from 1933 onwards. We investigated the effects of six exotic and two native tree species planted in monoculture plots and native species mixed within one self-regenerated plot on soil properties in two layers (0–5 cm and 5–10 cm depth). We measured general soil properties (pH, SOM, exchangeable base cations) and water-soluble C and N as a proxy for soil functioning. Changes in soil properties were observed in the upper soil layer for all tree species. Planting Eucalyptus species caused soil acidification, whereas soil exchangeable cations and pH were higher under native species (*Entandrophragma excelsum* and *Polyscias fulva*) and mixed native species. The effects of tree species were more pronounced for hot water-extractable C and N than for other soil properties. Their analyses could be used for detecting changes in soil functioning linked to vegetation types.

Keywords: *soil quality; soil functions; Eucalyptus species; soil acidity; exchangeable cations; water-extractable C and N; Ruhande Arboretum; Rwanda*

2.1. Introduction

Plants and soils are key components of terrestrial ecosystems, and changes in vegetation cover may lead to changes in soil properties, especially in the forest topsoil (Binkley and Menyailo, 2005; Carnol and Bazgir, 2013). Soils provide important ecosystem functions, such as nutrient cycling, carbon sequestration in soil organic matter (Bauters et al., 2015), and provision of fiber and food through the supply of water and nutrients to the vegetation (Lathwell and Grove, 2011). In turn, trees are an important soil-forming factor, and tree species can affect soils through various mechanisms, including nutrient uptake and return to the soil, soil organic matter dynamics, changes in soil acidity via root–soil exchange, and protection from erosion (Carnol and Bazgir, 2013; Hobbie et al., 2006; Prescott and Vesterdal, 2013). As a result, physical, chemical, and biological properties as well as the related processes may be affected by tree species (Veldkamp, 2000) and thus influence the nutrient supply capacity of the soils to the trees. In tropical forests, soil fertility relies heavily on the internal cycling of nutrients through the rapid decomposition of above- and belowground litter from vegetation, taking place in the thin upper soil horizon (Sayer & Banin, 2016; Schulte & Ruhiyat, 1998). Understanding the effect of tree species is particularly important in tropical forest ecosystems for the long-term preservation of soil quality and for promoting soil functioning.

Recently, there has been much interest and debate about the delimitation of the concepts of soil quality, health, fertility, and ecosystem services (Baveye et al., 2016b; Bünemann et al., 2018; Karlen et al., 1997; Salomé et al., 2016), with sometimes overlapping or contradicting views, leading to confusion across disciplines. Karlen et al. (Karlen et al., 1997) defined soil quality as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation''. They also recommended that soil quality should be evaluated based on soil function without, however, providing a specific definition of soil functions. Greiner et al. (Greiner et al., 2017b) indicated that soil functions result from the interaction of soil properties and processes and that they are related to ecosystem services and human benefits, as illustrated in the "Cascading framework" (Haines-Young and Potschin, 2008). Soil functions can be measured through physical, chemical, and biological soil properties and

processes, which are used as the basic tools to evaluate soil quality under different land-use systems (Turco et al., 2015; Vogel et al., 2019b).

Among many soil properties, soil organic matter and/or carbon (SOM, SOC), pH, and base cations are frequently used as primary indicators of forest soil quality (Augusto et al., 2002; Bünemann et al., 2018). Given the importance of soil organic matter for soil functioning (Franzluebbers, 2002), several studies investigated tree species-induced changes on total soil organic carbon (SOC) after afforestation(Wellock et al., 2011). The findings differed, with some studies showing no change (Degryze et al., 2004; Peri et al., 2010), increased SOC (Del Galdo et al., 2003; Mao et al., 2010), and decreased SOC (Ross et al., 1999; Wellock et al., 2011). Numerous factors may govern these contradictory results, and, in a review of 43 afforestation studies, Paul et al. (Paul et al., 2002) identified, in order of importance, previous land use, climate, and forest tree species as key factors influencing forest soil organic matter dynamics. While SOM is recognized as an important global indicator of soil quality, its slow dynamics does not allow for early detection of changes (Nyberg et al., 2002). Further, most SOM might not be available for microbial breakdown; therefore, total SOM might not be a relevant indicator of soil functioning (Curtin et al., 2021). For example, in a grassland, 60% of SOM was shown to be a recalcitrant pool (Paul, 2016). SOM undergoes continuous changes that generate distinct chemical and physical organic matter fractions with different turnover rates, from readily available labile to recalcitrant carbon and nitrogen fractions (Choudhary et al., 2013; Haynes, 2005). Labile SOM fractions have recently gained interest as indicators of soil quality because they are more sensitive to changes in vegetation cover and land use than the total organic matter (Strosser, 2011; Zhang et al., 2011). Additionally, being the main substrate and energy source for soil microorganisms, labile carbon and nitrogen fractions such as water-extractable C and N are linked to soil nutrient cycling and thus to soil functioning (Curtin et al., 2021; Haynes, 2005; Wang et al., 2008).

Rwanda experienced the loss of its natural forest cover from 30% in 1920 to 8% in 1998 (Habiyaremye et al., 2011). This deforestation in a country whose topography is dominated by steep sloping hills with heavy precipitation has led to accelerated soil erosion and to the decline of soil fertility (Clay and Lewis, 1990). A tree plantation program was initiated in 2010 to promote "in situ soil conservation through agroforestry and forest landscape restoration" (MINILAF, 2018) and halt the decline of forest cover,

counter soil erosion, and land degradation as well as to meet increasing demands for wood. Within this program, the government of Rwanda has mobilized its entire population and non-governmental organizations to plant trees, mainly fast-growing exotic species, and to maintain remaining forests, whereby a target was set in 2010 to restore the country's forest cover from 19.6% to 30% by 2030 (Warnest et al., 2012). This target was reached in 2020 with 724,695 ha (30.4%) forest cover in the country (RFA, 2021). This forest cover is composed of the following: 387,425 ha (53.5%) forest plantations, wooded savannahs in the east cover 161,843 ha (22.3%), natural montane forests occupy 130,850 ha (18.1%), shrublands cover 43,963 ha (6.1%), and 613 ha are occupied by bamboo (MoE, 2019b). Of the forest plantations, eucalyptus species are dominant with 89%, followed by 6.5% pines, 3.1% mixed exotic forests, and 1.4% being plantations of native species (IUCN, 2020). While the effects of tree species on soils were extensively studied for temperate ecosystems, data on tropical soils are scarce (Bauters et al., 2017b). The results of most studies may therefore have limited relevance within the context of tropical soils (Bauters et al., 2017a). Additionally, numerous studies were performed in relatively short-term common garden experiments (Bauters et al., 2017b). We need an in-depth understanding of the effects of the planted species on soil quality in tropical ecosystems. Such expertise for local conditions is important for selecting suitable species promoting soil functioning in these tropical forest ecosystems.

The general aim of this study was to assess the long-term effects of tree species planted in Rwanda on chemical soil quality, including water-soluble labile C and N fractions, as a proxy for soil functioning. Specific aims were to (i) determine the differences in soil chemical properties between tree species in two soil layers (0–5 cm and 5–10 cm depth); (ii) characterize hot and cold water-extractable mineral N and organic C and N in soils under different plantation species, and (iii) investigate the relationships between labile C and N fractions and other soil properties in response to tree species. We hypothesized that the exotic eucalyptus species would reduce the chemical quality of the soils in comparison to native species and that labile C and N fractions would be more sensitive to a change in tree species than SOM.

2.2. Materials and Methods

2.2.1. Study site and soil sampling

Soils were sampled in the Arboretum of Ruhande (Southern Rwanda, 2°36′S, 29°44′E, Figure 1) located at 1638–1737 m elevation on a flat plateau of the Ruhande hill (Nsabimana et al., 2008). This site is characterized by a mean annual rainfall of 1230 mm and a temperature between 17.5 °C and 19 °C. The rainfall has a bimodal regime with irregular short rains from September to December and a short dry season (January to February), followed by a heavy rainy season from March to May and a long dry season from June to August (Meteorwanda, 2021). The soil is classified as Ferralsol (also known as Oxisols in USDA soil taxonomy), a red-brown colored soil with a sandy loam texture and diffuse horizons (Nsabimana et al., 2009). It is developed from weathered Precambrian phyllite, and granitic batholith parental rocks coated with a mixture of quartzites and mica schists (Moeyersons, 2003; Steiner, 1998).

The site was established in 1933 on cultivated land under the request of the colonial leaders of Rwanda-Urundi territory for forestry research, wood, and seed provision to the rest of the country (Kalinganire, 1995). The size of the arboretum was progressively increased to currently reach 200 ha with 143 hardwood tree species, including 126 introduced exotic species of which 69 are eucalyptus species and 17 are native species. It also contains 57 deciduous tree species and 3 bamboo species, of which two are native to Rwanda (Nsabimana et al., 2008). Trees are planted in replicated monoculture stands of 0.25 ha (50 m × 50 m), resulting in 504 numbered plots (with 454 plots of exotic species) separated by inter-plot paths 6–10 m wide (Figure 2). Thinning and removal of shrubs and other invading vegetation is performed annually on all plots, except on an undisturbed plot (4 ha) of self-regenerated mixed native species (Mns). Plots are managed to maintain a constant density of the main tree species by planting in replacement of dead plants. From the 24 selected plots (see below), six were completely re-established, but they were aged minimum 30 years at the time of this study (Table A1). Neighbouring local households are allowed to collect dry wood each Friday for cooking. Given that trees were planted on the same site with similar (agricultural) land-use history and climatic conditions, we expect current differences in soil characteristics to reflect the influence of the planted tree species.

The uniqueness of Arboretum of Ruhande in terms of design, landscape, tree species composition, and presence of other living organisms lies in its multiple roles as a global site for forestry conservation, research, educational activities, and a gene bank of forestry germplasm in addition to being the country's main source of forest planting materials (Burren, 1995). This botanical garden was recently (May 2018) awarded international recognition through its enrolment into the "Queen's Commonwealth Canopy" projects. This is a network of forest conservation initiatives within Commonwealth countries aiming at forest and biodiversity conservation for future generations (Commonwealth, 2018).

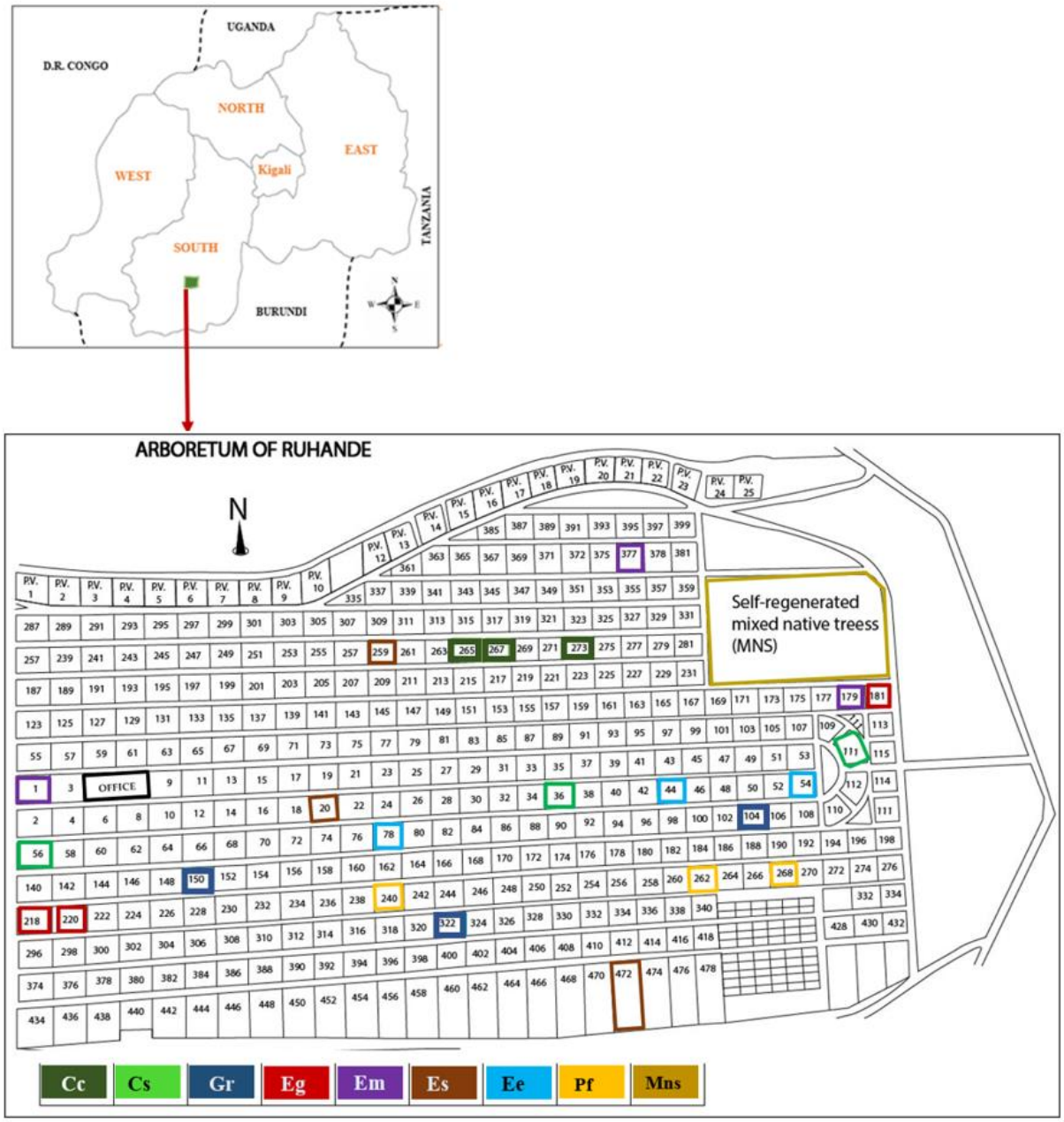

Fig. 2. 1. Location and map of the Arboretum of Ruhande, Rwanda. Studied plots are indicated in colour.

Tree species: **Cc**= *Calliandra calothyrsus*; **Cs**= *Cedrela serrata*; **Gr**= *Grevillea robusta*; **Eg**= *Eucalyptus grandis*; **Em**= *Eucalyptus maidenii*; **Es**= *Eucalyptus saligna*; **Ee**= *Entandrophragma excelsum*; **Pf=** *Polyscias fulva*; **Mns**= Mixed native species (self-regenerated).

2.2.2. Soil Sampling and Chemical Analyses

Based on the records of forestry seed demands and species adaptability in different regions of the country (Iiyama et al., 2018; Ndayambaje, 2016), eight species were selected considering three plots per species (Fig. 2.1; Table 2.A1). These included three eucalyptus species (*Eucalyptus grandis*, *Eucalyptus maidenii*, and *Eucalyptus saligna*), three agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*), two native species (*Entandrophragma excelsum* and *Polyscias fulva*), and a self-regenerated plot of native forest (mixed native species = Mns).

Each plot was divided into two sub-plots (25 × 50 m), where soil samples were collected under the trees' canopy at a distance of 1 to 1.5 m from the tree base (Bini et al., 2013). One composite sample was taken in each sub-plot by mixing five soil cores (X-shaped sampling) collected using a 30 × 30 cm frame and a shovel. Samples were taken at two soil depths—0–5 cm and 5–10 cm—the most active layers in tropical forest soils with a high rate of organic matter decomposition and nutrient cycling (Zalamea et al., 2016). Thus, we took two composite soil samples per plot at two soil depths. Soils were sieved fresh (4 mm) and stored at 4 °C until analyses.

Gravimetric water content, soil organic matter (SOM), and pH were determined as described by Allen et al. (Allen, 1989). Briefly, moisture was calculated as the difference between fresh and oven-dried soil at 105 °C for 3 h; SOM was calculated as a weight loss from oven-dry soil after overnight ignition at 550 °C in a muffle furnace. Soil organic carbon (SOC) was estimated by dividing SOM by 1.724 (Van Bemmelen factor), assuming that organic matter contains 58% of organic carbon (Périé and Ouimet, 2008). The pH_{KCL} was determined in a soil solution (1:2.5 *v*/*v*) with 1 M KCl and measured using a pH meter (HI2550 Multiparameter pH Benchtop meter, HANNA® Instruments-USA). Soil water holding capacity (WHC) was determined using Shaw's method according to Jenkinson & Powlson, (1976) as the difference between the volume of water (50 mL) added to 25 g of fresh soil and the volume drained after 30 min of saturation in addition to the initial soil moisture content.

Exchangeable cations (Al^{3+}, Ca^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, and Zn^{2+}) were extracted from fresh soil with 0.1 M $BaCl_2$ (1:5 *w*/*v*) by agitation for 30 min, followed by centrifugation at 180 rpm (Hendershot and Duquette, 1986). Chemical analysis of the filtered (Macherey

Nagel MN 6151/4. Ø 150 mm, Germany) and the acidified (1% HNO_3 Suprapur) $BaCl_2$ extracts was performed using ICP-AESS (Varian, Australia). The sum of exchangeable cations (∑cations) was calculated as the sum of all measured cations, and exchangeable base cations (EBC) were calculated as the sum of Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+}; expressed in c mol$_c$ kg^{-1}.

Water-extractable C and N were determined using the method of Ghani et al. (Ghani et al., 2003). Fresh soil was extracted with distilled water (1:6, *w*/*v*), shaken (120 rpm, 30 min), centrifuged (4000 rpm, 10 min), and filtered (Whatman #42), representing water-soluble C and N (WSC, WSN) fractions. Hot water-extractable C and N (HWC, HWN) were subsequently extracted from the remaining wet soil, mixed with distilled water (30 mL), and placed in the oven for 16 h at 80 °C. Organic C in the cold (WSC) and the hot water (HWC) extracts was measured using a Total Organic Carbon analyzer (LabToc, Pollution and Process Monitoring, UK). Cold ("WS…") and hot water-extractable ("HW…") nitrogen forms (N-NH_4: $WSNH_4$, $HWNH_4$; N-NO_3: $WSNO_3$, $HWNO_3$) and total nitrogen (WSNtot, HWNtot) were measured colorimetrically using a continuous flow autoanalyzer equipped with a UV digestor (Autoanalyser3, BranLuebbe, Germany). Organic nitrogen in the extracts (WSN_{org}, HWN_{org}) was calculated as the difference between total nitrogen and mineral nitrogen. Given that most of the mineral N is extracted with cold water, and as ammonium N in hot water extracts comes from hydrolysis of organic N (Gregorich et al., 2003), we assumed that HWNtot was entirely deriving from organic N and thus included WSN_{org}, WSNtot, HWN_{org}, HWNtot, WSC:WSN_{org}, and HWC:HWNtot in our analyses.

2.2.3. Statistical analyses

We used linear mixed-effects models (LMM) to investigate the differences in soil chemical properties between tree species and soil layers, using *lme4* package and *lmer* function (Bates et al., 2015) in R, version 3.5.1 (Venables and Ripley, 2012). The model used "Species" (9 levels: *C. calothyrsus*, *C. serrata*, *G. robusta*, *E. grandis*, *E. maidenii*, *E. saligna*, *E. excelsum*, *P. fulva*, and Mixnatives, with three replicates per species), "Layer" (with two levels: upper and lower soil layers), and the interaction between tree species and soil layer (species*layer), which were included in models as fixed effects. "Plot" was included as a random effect to account for the non-independence of the two samples collected within the same plot and the tree age differences between plots. Normality was tested

using the Shapiro–Wilk test and/or visual inspection of plotted residuals. Homoscedasticity of random errors was tested using *Levene* test function, part of the Car package in R. Where necessary, response variables were transformed to improve normality and homoscedasticity of errors. Significance of tree species and soil layer effects were analysed using the model's estimated marginal means (*EMMeans*) function, part of the *multcompView* package in R, using Tukey–Kramer honestly significant difference range post-hoc test to compare all measured parameters across levels at a significant probability of $\alpha = 0.05$. The prediction of response variables explained by the model was determined using a multi-model inference (MuMIn-v1.42.1) package and *r.squaredGLMM* function in R (Barton, 2018).

Pearson's coefficient of correlation was used to determine the correlation between measured variables. Principal component analysis (PCA: using *FactoMineR* and *ggplot2* packages) was used to describe the patterns of variation explained by soil parameters of interest (pH, SOM, EBC, WSC, WSN_{org}, WSNtot, WSC:WSN_{org}, HWC, HWN_{org}, HWNtot, and HWC:HWNtot) between tree species. All statistical analyses and tests were carried out using R software, version 3.5.1 (Venables and Ripley, 2012).

2.3. Results

2.3.1. Chemical soil properties in two topsoil layers

Values for all soil parameters (Fig. 2.2; Table 2A.3) were significantly higher in the upper (0–5 cm) soil layer compared to the lower (5–10 cm) layer under all tree species (except for Al^{3+}, Fe^{2+}, and Na^{+}). pH, SOM, and EBC were 14%, 57%, and 78% higher in the upper compared to the lower soil layer (4.9, 22%, and 36.3 $cmol_c\ kg^{-1}$ versus, 4.2, 9.6%, and 7.8 $cmol_c\ kg^{-1}$, respectively). Base cations dominated the sum of exchangeable cations, representing 78% (Ca^{2+}), 19.4% (Mg^{2+}), and 2.3% (K^{+}) in the upper soil layer and 65% (Ca^{2+}), 16.5% (Mg^{2+}), and 1.7% (K^{+}) in the lower soil layer (Table 2A.2; 2A.3). In contrast to the other soil parameters, the contribution of Al^{3+}, Fe^{2+}, and Na^{+} to the sum of exchangeable cations was less in the upper soil layer (0.002% Fe^{2+}, 0.2% Al^{3+}, 0.4% Na^{+}) compared to the lower soil layer (0.1% Fe^{2+}, 13.5% Al^{3+}, 2% Na^{+}).

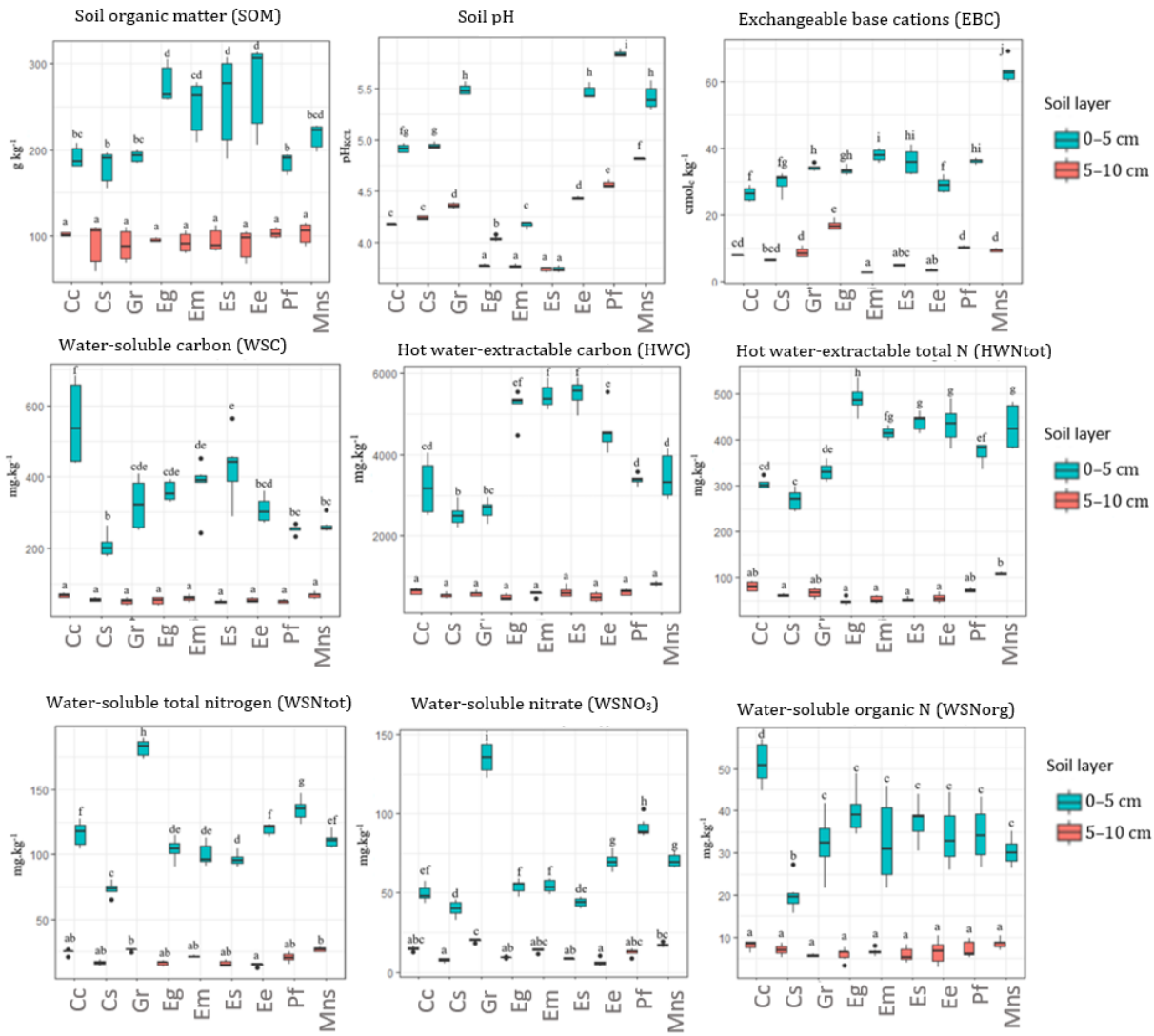

Fig. 2. 2. Predicted (LMM) soil properties under nine treatment (Tree species) and two soil layers (0–5 cm and 5–10 cm).

Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species. The horizontal black line in the box shows the estimated sample median, while the lower and the upper box boundaries show the first and the third percentiles, respectively. The dots outside the whisker boundaries show observations outside the 5th–95th percentile range. Different letters denote significant differences between tree species and soil layer (mixed linear models, Tukey's HSD, $p<0.05$).Figure 5th–95th percentile range. Different letters denote significant differences between tree species and soil layer (mixed linear models, Tukey's HSD, $p < 0.05$).

Labile (water-soluble and hot water-extractable) carbon and nitrogen also differed between soil layers (Fig. 2.2). The amounts of water-soluble C (WSC) and hot water-extractable C (HWC) were about seven times higher in the upper than in the lower soil layer. Different components of water-extractable N also varied significantly with soil depth (Table 2.1). In the upper soil layer, across tree species, cold water extractable N comprised nitrate ($WSNO_3$, 52%), ammonium ($WSNH_4$, 13.4%), and organic nitrogen (WSN_{org}, 34.6%) (Table 2A.4). In the lower soil layer, these proportions accounted for 58.4% nitrate ($WSNO_3$), 6.7% ammonium (WSNH4), and 34.9% organic nitrogen (WSN_{org}). The proportions extracted by hot water also differed with soil depth where nitrate, ammonium, and organic nitrogen accounted for 3.5%, 18.7%, and 77.8%, respectively, in the upper soil layer against 3.4%, 12.6%, and 84%, respectively, in the lower soil layer (Table 2A.4).

Table 2. 1. *Measured waters' C and N under different tree species in the Arboretum of Ruhande (means ± SEM). Different letters within one parameter denote significant differences between tree species and soil depths (mixed linear models, Tukey's HSD, p < 0.05.*

Labile C and N Fractions	Soil Layer (cm)	*Calliandra calothyrsus*	*Cedrela serrata*	*Grevillea robusta*	*Eucalyptus grandis*	*Eucalyptus maidenii*	*Eucalyptus saligna*	*Entandrophragma excelsum*	*Polyscias fulva*	*Mixed natives*
Water-soluble C and N fractions										
WSC (mgkg^{-1})	0–5	550 ± 49 ^{f}	210 ± 13 ^{b}	320 ± 30 ^{cde}	360 ± 12 ^{cde}	380 ± 29 ^{de}	430 ± 38 ^{e}	310 ± 15 ^{bcd}	250 ± 5 ^{bc}	260 ± 8.9 ^{bc}
	5–10	67 ± 2.5 ^{a}	56± 2.3 ^{a}	50 ± 3.4 ^{a}	52 ± 3.9 ^{a}	60 ± 3.4 ^{a}	50 ± 1.7 ^{a}	53 ± 2.6 ^{a}	50 ± 1.9 ^{a}	68± 3.3 ^{a}
WSNtot (mgkg^{-1})	0–5	120 ± 3.8 ^{f}	73 ± 2.2 ^{c}	180 ± 2.8 ^{h}	100 ± 3.4 ^{de}	100 ± 3.8 ^{de}	96 ± 2 ^{d}	120 ± 1.7 ^{f}	130 ± 3.5 ^{g}	110 ± 2.3 ^{ef}
	5–10	25 ± 0.81 ^{ab}	17 ± 0.73 ^{ab}	26 ± 0.36 ^{b}	16 ± 0.73 ^{ab}	22 ± 0.3 ^{ab}	16 ± 0.99 ^{ab}	15 ± 0.46 ^{a}	21 ± 1.4 ^{ab}	27 ± 0.69 ^{b}
WSN_{org} (mgkg^{-1})	0–5	51 ± 2 ^{d}	20 ± 1.6 ^{b}	32 ± 2.8 ^{c}	40 ± 2.2 ^{c}	33 ± 4.1 ^{c}	37 ± 1.9 ^{c}	34 ± 2.9 ^{c}	35 ± ±2.7 ^{c}	30 ± 1.3 ^{c}
	5–10	8.1 ± 0.44 ^{a}	7.1 ± 0.5 ^{a}	5.8 ± 0.12 ^{a}	5.9 ± 0.64 ^{a}	6.6 ± 0.37 ^{a}	5.9 ± 0.69 ^{a}	6.6 ± 1.2 ^{a}	7.2 ± 0.83 ^{a}	8.6 ± 0.49 ^{a}
$WSNH_4$ (mgkg^{-1})	0–5	15 ± 0.66 ^{d}	13 ± 0.45 ^{cd}	15 ± 0.75 ^{d}	9.9 ± 0.92 ^{b}	13 ± 1.1 ^{cd}	15 ± 0.53 ^{d}	15 ± 1.2 ^{d}	8.7 ± 0.3 ^{b}	10 ± 0.24 ^{bc}
	5–10	1.8 ± 0.14 ^{a}	2 ± 0.27 ^{a}	0.52 ± 0.01 ^{a}	0.76 ± 0.06 ^{a}	0.9 ±0.07 ^{a}	1.2 ± 0.2 ^{a}	2.2 ± 0.2 ^{a}	0.82 ± 0.03 ^{a}	0.8 ± 0.05 ^{a}
$WSNO_3$ (mgkg^{-1})	0–5	50 ± 2.1 ^{ef}	40 ± 2 ^{d}	130 ± 4.1 ^{i}	54 ± 1.8 ^{f}	54 ± 1.7 ^{f}	44 ± 1.2 ^{de}	70 ± 2.2 ^{g}	91 ± 2.6 ^{h}	70 ± 1.8 ^{g}
	5–10	15 ± 0.49 ^{abc}	7.6 ± 0.55 ^{a}	20 ± 0.39 ^{c}	9.3 ± 0.17 ^{ab}	14 ± 0.54 ^{abc}	8.7 ± 0.21 ^{ab}	6.1 ± 0.93 ^{a}	13 ± 0.89 ^{abc}	17 ± 0.53 ^{bc}
WSC/WSNtot	0–5	4.8 ± 0.5 ^{f}	2.9 ± 0.2 ^{abcd}	1.9 ± 0.2 ^{a}	3.5 ± 0.1 ^{bcde}	3.8 ± 0.3 ^{def}	4.5 ± 0.4 ^{ef}	2.9 ± 0.1 ^{abc}	1.9 ± 0.1 ^{a}	2.4 ± 0.0 ^{ab}
	5–10	2.7 ± 0.2 ^{abcd}	3.4 ± 0.1 ^{bcde}	1.9 ± 0.1 ^{a}	3.3 ± 0.3 ^{bcde}	2.8 ± 0.1 ^{abc}	3.2 ± 0.1 ^{bcd}	3.6 ± 0.2 ^{cdef}	2.5 ± 0.2 ^{abc}	2.6 ± 0.1 ^{abc}
WSC/WSN_{org}	0–5	11 ± 1 ^{a}	11 ± 0.9 ^{a}	11 ± 1.8 ^{a}	9.2 ± 0.5 ^{a}	12 ± 1.5 ^{a}	12 ± 1.3 ^{a}	9.3 ± 0.9 ^{a}	7.5 ± 0.6 ^{a}	8.7 ± 0.2 ^{a}
	5–10	8.6 ± 0.83 ^{a}	8 ± 0.34 ^{a}	8.8 ± 0.58 ^{a}	9.6 ± 1.5 ^{a}	9 ± 0.2 ^{a}	8.9 ± 0.79 ^{a}	9.6 ± 1.7 ^{a}	7.4 ± 0.81 ^{a}	8 ± 0.55 ^{a}
Hot water-extractable C and N fractions										
HWC (mgkg^{-1})	0–5	3200 ± 280 ^{cd}	2500 ± 110 ^{b}	2700 ± 97 ^{bc}	5200 ± 150 ^{ef}	5400 ± 120 ^{f}	5500 ± 140 ^{f}	4600 ± 210 ^{e}	3400 ± 51 ^{d}	3500 ± 230 ^{d}
	5–10	640 ±32 ^{a}	540 ± 25 ^{a}	590 ± 25 ^{a}	500 ± 29 ^{a}	580 ± 25 ^{a}	630 ± 51 ^{a}	500 ± 46 ^{a}	620 ± 31 ^{a}	830 ± 16 ^{a}
HWNtot (mgkg^{-1})	0–5	300 ± 4.5 ^{cd}	270 ± 9.4 ^{c}	330 ± 8 ^{de}	490 ± 13 ^{h}	420 ± 5.2 ^{fg}	440 ± 8 ^{g}	430 ± 17 ^{g}	370 ± 8.9 ^{ef}	430 ± 20 ^{g}
	5–10	80 ± 4.6 ^{ab}	61 ± 1.2 ^{a}	66 ± 4.4 ^{ab}	48± 3.1 ^{a}	52 ± 3.2 ^{a}	51 ± 1.7 ^{a}	55 ± 3.8 ^{a}	72 ± 1.5 ^{ab}	110± 1.3 ^{b}
HWN_{org} (mgkg^{-1})	0–5	240 ± 1.9 ^{cd}	220 ± 7.5 ^{c}	240 ± 5.5 ^{cd}	400 ± 11 ^{g}	330 ± 4.8 ^{ef}	360 ± 6.2 ^{f}	340 ± 13 ^{ef}	270 ± 6 ^{d}	320 ± 18 ^{e}
	5–10	65 ± 4.3 ^{ab}	50 ± 1.7 ^{a}	57 ± 3.8 ^{ab}	41 ± 2.9 ^{a}	44 ± 2.9 ^{a}	43 ± 1.6 ^{a}	45 ± 3.9 ^{a}	62 ± 1.7 ^{ab}	91 ± 1.2 ^{b}
$WSNH_4$ (mgkg^{-1})	0–5	50 ± 2.4 ^{b}	43 ± 2.1 ^{b}	73 ± 3.1 ^{cde}	77 ± 2.8 ^{de}	63 ± 1.8 ^{c}	68 ± 2 ^{cd}	82 ± 3.7 ^{e}	94 ± 4.9 ^{f}	97 ± 2.1 ^{f}
	5–10	12 ± 0.35 ^{a}	9.1 ± 0.75 ^{a}	6.4 ± 0.42 ^{a}	5.2 ± 0.15 ^{a}	5.1 ± 0.35 ^{a}	5.9 ± 0.81 ^{a}	9.3 ± 0.59 ^{a}	8.2 ± 0.43 ^{a}	14 ± 0.42 ^{a}
$WSNO_3$ (mgkg^{-1})	0–5	12 ± 0.98 ^{cd}	8 ± 0.19 ^{bc}	17 ± 0.7 ^{de}	17 ± 1.4 ^{de}	21 ± 0.97 ^{e}	14 ± 1.3 ^{d}	14 ± 1.2 ^{d}	12 ± 0.92 ^{cd}	12 ± 3.2 ^{cd}
	5–10	3 ± 0.35 ^{ab}	1.6 ± 0.11 ^{a}	2.8 ± 0.25 ^{ab}	2 ± 0.26 ^{a}	2.2 ± 0.29 ^{a}	1.9 ± 0.03 ^{a}	0.99 ± 0.03 ^{a}	2.4 ± 0.29 ^{a}	3.1 ± 0.21 ^{ab}
HWC/HWNtot	0–5	11 ± 0.9 ^{bcd}	9.4 ± 0.3 ^{abc}	8 ± 0.1 ^{a}	11 ± 0.1 ^{bcd}	13.9 ± 0.1 ^{e}	13 ± 0.2 ^{de}	11 ± 0.3 ^{bcd}	9.1 ± 0.2 ^{ab}	8 ± 0.1 ^{a}
	5–10	8 ± 0.1 ^{a}	8.9 ± 0.5 ^{ab}	9 ± 0.4 ^{ab}	10 ± 0.3 ^{bcd}	11 ± 0.8 ^{cde}	12 ± 0.6 ^{de}	9.1 ± 0.5 ^{ab}	8.5 ± 0.4 ^{ab}	7.7 ± 0.1 ^{a}
HWC/HWN_{org}	0–5	13 ± 1.2 ^{defg}	12 ± 0.4 ^{abcdef}	11 ± 0.2 ^{abcde}	13 ± 0.2 ^{defg}	16 ± 0.2 ^{h}	15 ± 0.3 ^{gh}	14 ± 0.4 ^{efgh}	13 ± 0.3 ^{cdefg}	11 ± 0.2 ^{abcde}
	5–10	9.8 ± 0.17 ^{abc}	11 ± 0.85 ^{abcde}	10 ± 0.54 ^{abcd}	12 ± 0.38 ^{bcdefg}	13 ± 0.95 ^{defg}	14 ± 0.77 ^{fgh}	11 ± 0.6 ^{abcde}	10 ±0.5 ^{ab}	9.1 ± 0.16 ^{a}

Analyzed soil variables (WSC = water-soluble C; WSNtot = water-soluble total N; WSNorg = water soluble organic N; WSNH4 = water-soluble ammonium; $WSNO_3$ = water-soluble nitrate; WSC/WSNtot = water soluble C/N ratio; WSC/WSNorg = water soluble organic C/N ratio; HWC = hot water-extractable C; HWNtot = hot water-extractable total N; HWNorg = hot water-extractable organic N; HWC:HWNtot= hot water-extractable C/N ratio; and HWC/HWNorg= hot water-extractable organic C/N ratio).

2.3.2. Effects of tree species on water-extractable C and N and other soil properties

Most differences in soil properties between tree species were found in the upper 0–5 cm soil layer (Table 2A.3). In this layer, pH was highest under *Polyscias fulva* (pH_{KCL} = 5.8), followed by the two native species stands (Mns and *Entandrophragma excelsum*) and *Grevillea robusta* (Figure 2). Soils under *Calliandra calothyrsus* and *Cedrela serrata* had an intermediate pH (pH_{KCL} = 4.9), while all eucalyptus species showed the lowest soil pH values (*Eucalyptus saligna* < *Eucalyptus grandis* < *Eucalyptus maidenii*). The SOM content was significantly higher under most eucalyptus species and *Entandrophragma excelsum*, while it was not different between the other species.

Water-extractable labile C and N (Table 2.1, Fig. 2.2) also differed under tree species. Water-soluble organic carbon (WSC) was significantly higher under *Calliandra calothyrsus*, followed by some eucalyptus species. Hot water-extractable carbon (HWC) showed the highest values under eucalyptus species and *Entandrophragma excelsum,* while values were not significantly different under the other tree species. Water-soluble total nitrogen (WSN_{tot}) was highest under *Grevillea robusta* followed by native species (*Entandrophragma excelsum* and *Polyscias fulva*) and *Calliandra calothyrsus* with intermediate values under eucalyptus species and lowest concentration under *Cedrela serrata*.

Unlike WSNtot, hot water-extractable total nitrogen (HWNtot) showed similar differences between tree species as HWC, with the highest values under eucalyptus species and *Entandrophragma excelsum* and similar values under the other tree species. The highest percentage of water-soluble mineral nitrogen relative to total water-soluble nitrogen was measured under *Grevillea robusta* (WSNmin = 82%; $WSNO_3$ = 73% + $WSNH_4$ = 9%), while the lowest percentage was measured under *Entandrophragma excelsum* (WSNmin = 39%; $WSNO_3$ = 12% + $WSNH_4$ = 27%). The proportion of water-soluble organic nitrogen (WSN_{org}) was highest under *Entandrophragma excelsum* (WSN_{org} = 61%) and lowest under *Grevillea robusta* (WSN_{org} = 18%). The proportions of WSN_{org} under the other tree species ranged between 26% and 44%. In the hot water N extracts, organic nitrogen dominated fractions for all species. The highest proportion of mineral nitrogen was measured under *Polyscias fulva* (HWNmin, 28%; $HWNO_3$ = 3% + $HWNH_4$ = 25%), while the lowest proportion was measured under *Eucalyptus saligna* (HWNmin, 18%; $HWNO_3$ = 3% + $HWNH_4$ = 15%). Consequently, WSN_{org} was higher under *Eucalyptus*

saligna (WSN_{org} = 82%) followed by *Polyscias fulva* (HWN_{org} = 72%). WSC/WSN_{org} ranged from 7.5 to 12, with no significant difference between tree species (Table 2.1).

The sum of exchangeable base cations (EBC: Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) was significantly higher under mixed native species, followed by eucalyptus species, *Polyscias fulva*, and *Grevillea robusta* compared to *Calliandra calothyrsus*, *Cedrela serrata,* and *Entandrophragma excelsum*. Soil base cations such as Ca^{2+}, Mg^{2+}, and K^{+} dominated with nearly 97% of the total exchangeable cations, and they generally showed the higher concentrations under native and Eucalyptus species.

In the lower soil layer (5–10 cm), there were no significant differences between tree species for SOM, WSC, WSN_{org}, $WSNH_4$, HWC, $HWNH_4$, and $HWNO_3$. Significant differences between species were observed for pH, EBC, ∑cations, and individual cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Al^{3+} (Table 2A.3). There was also a significant effect of tree species for WSNtot, $WSNO_3$, WSC/WSN, HWNtot, HWN_{org}, and HWC/HWNtot (Table 2.1). The highest pH (pH_{KCL} = 4.8) was measured under the Mns, followed by the plot of monospecific native species and agroforestry species (*Polyscias fulva* > *Entandrophragma excelsum* = *Grevillea robusta* > *Calliandra calothyrsus* = *Cedrela serrata*), while the lowest pH (pH_{KCL} = 3.7) was measured under eucalyptus species. EBC ranged from 2.8 ± 0.02 $cmol_c kg^{-1}$ (*Eucalyptus maidenii*) to 17 ± 0.6 $cmol_c kg^{-1}$ (*Eucalyptus grandis*); this trend was similar to ∑cations, which ranged from 5.4 ± 0.07 $cmol_c kg^{-1}$ under *Eucalyptus maidenii* to 17 ± 0.2 $cmol_c kg^{-1}$ under *Eucalyptus grandis*. Exchangeable Ca^{2+} was significantly higher under *Eucalyptus grandis*, intermediate under Mns, *Polyscias fulva*, *Grevillea robusta*, and *Calliandra calothyrsus*, and lower values were measured under *Cedrela serrata*, *Eucalyptus maidenii*, *Eucalyptus saligna*, and *Entandrophragma excelsum*. Mg^{2+} was higher under *Eucalyptus grandis*, *Polyscias fulva*, and Mns, whereas the values of Mg^{2+} were lower under *Eucalyptus maidenii*, *Eucalyptus saligna*, and *Entandrophragma excelsum*, with intermediate values under agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata,* and *Grevillea robusta*). Similar to Ca^{2+} and Mg^{2+}, the concentration of K^{+} was also significantly higher under *Eucalyptus grandis* and Mns but not different for the remaining tree species. There was high variability in the exchangeable Al^{3+} concentration between tree species in the lower soil layer. The concentration of Al^{3+} in the soil classified tree species in the following order: *Eucalyptus maidenii* > *Entandrophragma excelsum* >

Eucalyptus saligna > *Cedrela serrata* **>** *Grevillea robusta* **=** *Calliandra calothyrsus* > Mns > *Polyscias fulva* > *Eucalyptus grandis*.

The proportions of water-soluble nitrogen fractions in the lower soil layer (Table 2A.4) showed that the mineral nitrogen was dominant with the highest percentage under *Grevillea robusta* (WSNmin = 78%; $WSNO_3$ = 76% + $WSNH_4$ = 2%) and the lowest percentage under *Entandrophragma excelsum* (WSNmin = 56%; $WSNO_3$ = 41% + $WSNH_4$ = 15%). The other species had WSNmin percentages ranging between 57% and 69%. The water-soluble organic nitrogen ranged between 22% (*Grevillea robusta*) and 44% (*Entandrophragma excelsum*). Hot water-extractable fractions contained mostly organic N ranging from 72% to 82% of the HWNtot and 81% to 86% in the 0–5 cm and the 5–10 cm soil layers, respectively. The hot water-extractable mineral N forms were dominated by $N\text{-}NH_4^+$ (15% to 25%) in the 0–5 cm soil layer and 10% to 17% in the 5–10 cm soil layer. The less abundant hot water-extractable mineral N fraction was $N\text{-}NO_3$- that ranged from 2% to 5% in both 0–5 cm and 5–10 cm soil layers.

2.3.3. Relationships between water-extractable elements (C, N) and other soil properties

The correlation between soil properties (pH, SOM, and EBC) and water-extractable C and N fractions (WSC, WSN_{org}, WSNmin, $WSNO_3$, WSC/N_{org}, HWC, HWNtot, and HWC/HWNtot) showed significant correlations within each of the two soil layers (Fig. 2.3).

In the upper soil layer (0–5 cm), soil pH was negatively correlated with SOM, all water-soluble and hot water-extractable C and N fractions, and HWC/HWNtot, except WSNmin and $WSNO_3$, which were positively correlated with pH. There was a significant positive correlation between SOM and all the above-mentioned water-extractable C and N fractions, except WSNmin and $WSNO_3$ (r = -0.2). The strongest positive correlation was found between SOM and HWC (r = 0.8), HWNtot (r = 0.7), and HWC/HWNtot (r = 0.5). EBC showed a weak positive correlation with HWNtot and a weak negative correlation with WSC, WSN_{org}, and HWC/HWN; no significant correlation was found with the other water-extractable C and N fractions. In the lower soil layer (5–10 cm), soil pH was positively correlated with HWC, HWNtot, WSN_{org}, WSNmin, and WSNO3, while it was negatively correlated with HWC/HWN. The relationship patterns between SOM and water-extractable C and N fractions showed a positive correlation with HWC, HWNtot,

and WSN_{org}, while it was negatively correlated with WSC/WSN_{org}. The strength of the correlation between SOM and water-extractable C and N fractions was comparatively lower compared to the upper soil layer, and there was no significant correlation between EBC and water-extractable C and N fractions (Fig. 2.3).

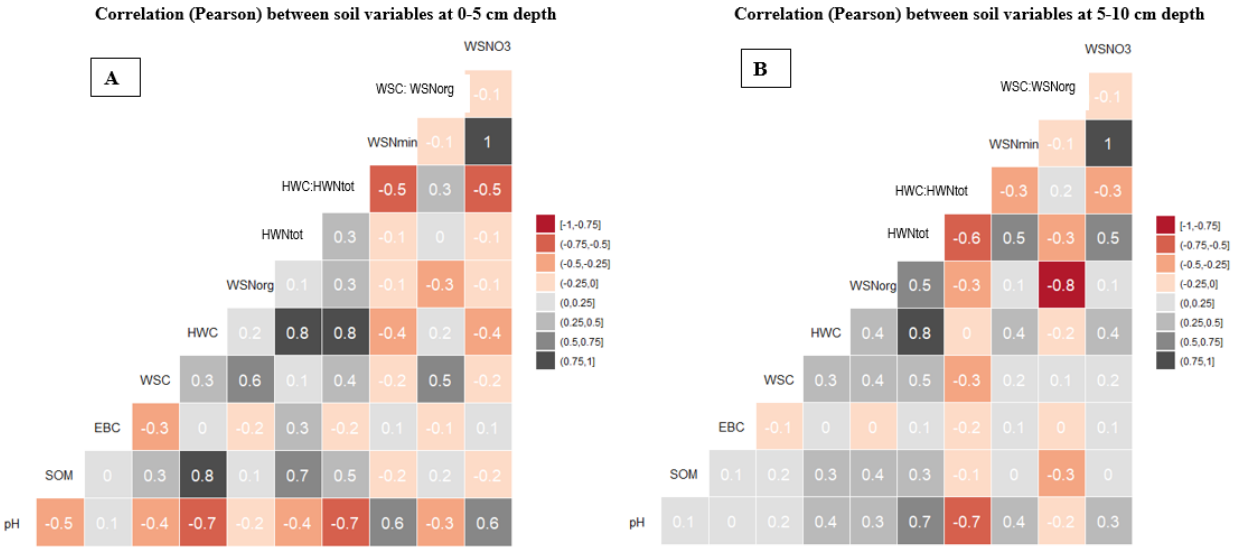

Fig. 2. 3. Pearson correlation matrices showing the relationship between soil properties and water-extractable C and N fractions.
Soil layers: A=upper (0–5 cm); B=lower (5–10 cm) Relationships between parameters are indicated by the values at the intersection of parameters and interpreted within color contrast as shown in the legends.

Principal component analysis (PCA) of soil properties (pH, SOM, and EBC) and water-extractable C and N fractions (WSC, WSN_{org}, WSNtot, $WSC:WSN_{org}$, HWC, HWN_{org}, HWNtot, and HWC/HWNtot) for the upper and the lower soil layers showed differences in the patterns of the tree species clustering based on these soil properties (Fig. 2.4). In the upper soil layer (0–5 cm), the total variance explained by the first two principal components was 62%. SOM, HWC, HWN_{org}, HWNtot, and C/N ratio of hot water extracts (HWC/HWNtot) had the highest positive loadings on PC1 (43%), while pH and WSNtot showed the highest loading to the negative side of PC1 (Figure 2.4-A).

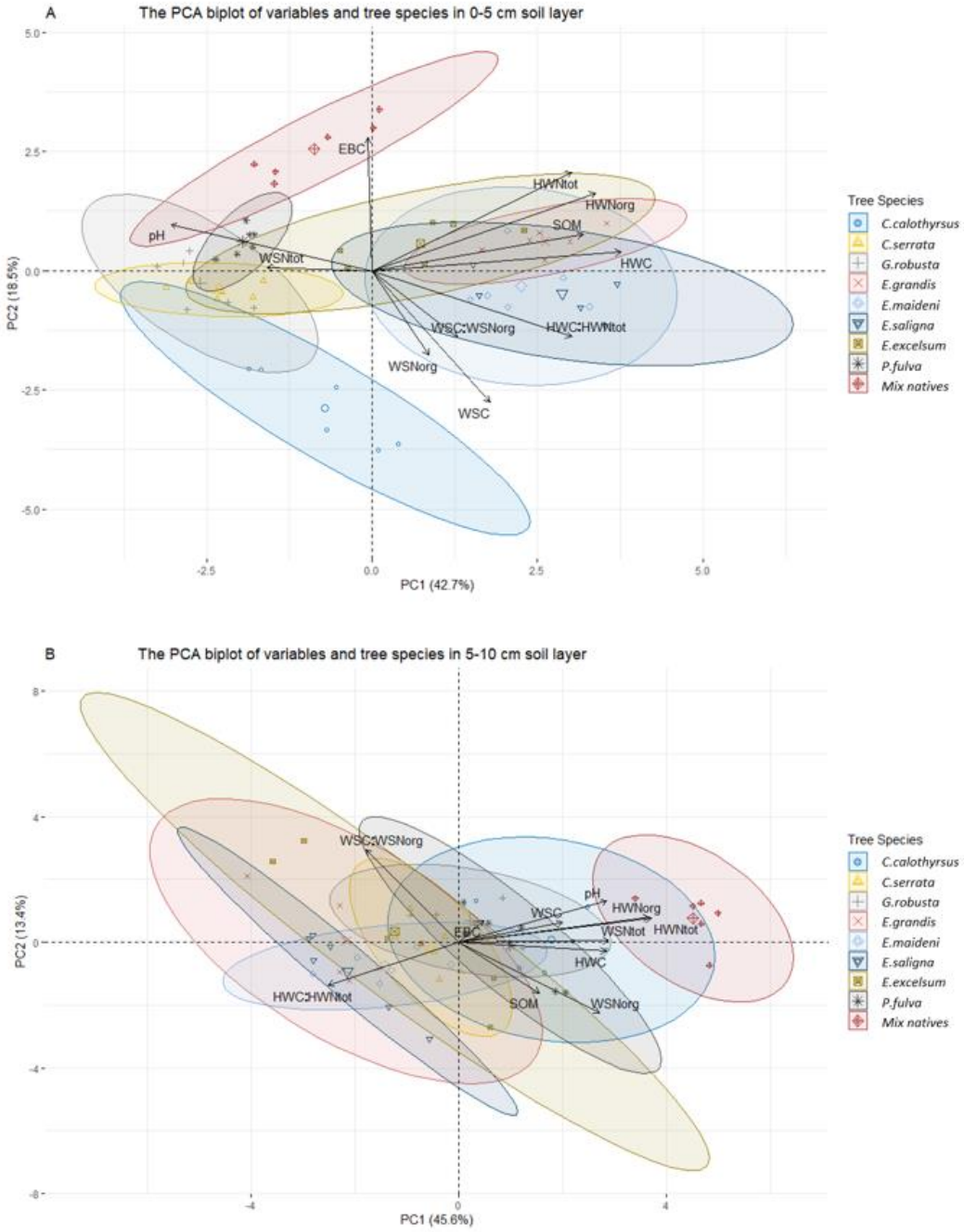

Fig. 2. 4. PCA biplot of soil chemical properties and tree species for the upper (A) and the lower (B) soil layers.

The first two principal components explained 62.5% of the combined variation in soil parameters at 0–5 cm soil depth and 54.9% at 5–10 cm soil depth between tree species. Statistical ellipses at 95% confidence level group tree species (represented by different symbols and colours) based soil variables depicted by vectors (pH; SOM = soil organic matter; EBC = exchangeable basic cations; WSC = water-soluble C, WSN_{org} = water soluble organic N; WSNtot = water-soluble total N, WSC:WSN_{org} = water soluble organic C/N ratio; HWC = hot water-extractable C, HWN_{org} = hot water-extractable organic N, HWNtot = hot water-extractable total N, and HWC:HWNtot= hot water-extractable C/N ratio).

Eucalyptus species and *Entandrophragma excelsum* clustered separately along with the positive side of PC1, while species such as *Polyscias fulva* and *Grevillea robusta* clustered along its negative side. EBC positively loaded highest on the second PC (19%), while WSC, WSN_{org} C/N ratio of water-soluble C, and organic N (WSC/WSN_{org}) had their negative loading to PC2. Mns plot clustered separately from the other plots along the positive side of the second axis, and *Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta* overlapped on its negative side (Table 2.2, Fig. 2.4-A).

In the lower soil layer (5–10 cm), the first two principal components explained 59% of the combined variation in PCA input variables between tree species (Table 2.2, Fig. 2.4-B,). The positive loadings on PC1 (46%) were observed for pH, WSN_{org}, WSNtot, HWNorg, HWC, HWNtot, and EBC, while the HWC/HWNtot was highly loaded on its negative side. On the PC2 (13%), WSC/WSN_{org} showed a positive loading, while SOM showed a negative loading. In this soil layer, most of the tree species clustered around the center of biplot quadrants with a tendency for the plots of Mns, *Polyscias fulva*, *Grevillea robusta*, and *Calliandra calothyrsus* to overlap on the positive side of the PC1. Eucalyptus species overlapped with both the negative side of the PC1 and the positive side of the PC2. The clustering patterns of species such as *Entandrophragma excelsum* and *Cedrela serrata* showed a stretching of statistical ellipses across the intersection of PCA axes towards both sides of PC2.

Table 2. 2. Results of principal component analysis (PCA) for 11 selected soil chemical properties measured in 108 samples under nine treatments (tree species) at two soil layers.

	Upper Soil Layer (0–5 cm)					Lower Soil Layer (5–10 cm)				
Principal Components	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
Eigenvalues	4.69	2.03	1.57	1.03	0.71	5.02	1.472	1.19	1.02	0.89
% variance	42.66	18.52	14.27	9.43	6.48	45.64	13.38	10.85	9.34	8.16
Cumulative % of the total variance	42.66	61.19	75.46	84.89	91.38	45.64	59.03	69.88	79.22	87.39
	Loadings (weight) of variables on PCs (%)									
pH_{KCL}	−0.77	0.24	0.22	0.25	−0.15	0.73	0.33	−0.21	−0.22	−0.09
SOM	0.81	0.19	0.07	0.17	−0.25	0.40	−0.41	0.04	0.50	0.38
WSC	0.45	−0.70	0.24	0.34	0.29	0.51	0.16	0.50	−0.01	0.59
WSN_{org}	0.22	-0.45	0.80	−0.20	0.21	0.70	−0.58	−0.04	−0.19	0.18
WSNtot	−0.40	0.017	0.63	0.48	−0.23	0.74	0.01	0.08	−0.07	−0.20
$WSC:WSN_{org}$	0.33	−0.35	−0.55	0.66	0.06	-0.46	0.76	0.35	0.19	0.13
HWC	0.96	0.10	0.05	−0.05	−0.08	0.73	−0.07	0.41	0.30	−0.39
HWN_{org}	0.86	0.41	0.14	0.04	−0.05	0.94	0.20	0.04	0.08	−0.14
HWNtot	0.77	0.53	0.23	0.14	−0.05	0.95	0.19	0.04	0.05	−0.09
HWC:HWNtot	0.77	−0.35	−0.11	−0.24	−0.03	−0.64	−0.35	0.46	0.29	−0.32
EBC	−0.01	0.71	0.02	0.14	0.64	0.12	0.18	−0.59	0.67	0.04
	Contribution of variables to PCs (%)									
pH_{KCL}	12.96	2.98	3.34	6.25	3.21	10.82	7.78	3.90	4.79	0.94
SOM	14.30	1.85	0.35	3.09	9.37	3.20	11.63	0.18	24.70	16.20
WSC	4.43	24.50	3.79	11.33	12.24	5.33	1.81	21.35	0.03	38.75
WSN_{org}	1.03	10.07	40.84	4.15	6.21	9.79	22.89	0.15	3.78	3.92
WSNtot	3.54	0.01	26.03	22.46	7.58	11.08	0.01	0.55	0.50	4.67
$WSC:WSN_{org}$	2.35	6.33	19.55	42.61	0.58	4.25	39.38	10.45	3.68	1.98
HWC	19.85	0.49	0.19	0.24	0.89	10.72	0.34	14.47	9.28	17.71
HWN_{org}	15.96	8.55	1.34	0.22	0.42	17.77	2.88	0.15	0.64	2.44
HWNtot	12.76	13.79	3.63	2.01	0.37	18.31	2.62	0.16	0.32	1.06
HWC:HWNtot	12.77	6.21	0.84	5.56	0.19	8.36	8.37	18.14	8.36	12.02
EBC	0.01	25.16	0.05	2.02	58.90	0.30	2.23	30.45	43.87	0.25

Variable loadings higher than 0.6 are in bold, expressing a significant weight of variables on PC, and the first five principal components explaining 87%–91% of the cumulative total variance are presented. The sign on variable loadings indicates the direction of the variable on PC axes. Analyzed soil variables (pH; SOM = soil organic matter; EBC = exchangeable basic cations; WSC = water-soluble C, WSN_{org} = water soluble organic N; WSNtot = water-soluble total N, $WSC:WSN_{org}$ = water soluble organic C/N ratio; HWC = hot water-extractable C, HWN_{org} = hot water-extractable organic N, HWNtot = hot water-extractable total N, and HWC:HWNtot= hot water-extractable C/N ratio).

2.4. Discussion

Given that trees species were planted on the same site with similar land-use history and climatic conditions, the Arboretum of Ruhande provided a unique set-up for investigating the effects of tree species used for forest plantations in Rwanda on soil chemical properties. We thus base the interpretation of the results on the assumption that the current differences in soil characteristics reflect the influence of the planted trees.

2.4.1. Importance of the thin upper soil layer (0–5 cm Depth)

The present study showed higher values for all analysed soil properties in 0–5 than 5–10 cm soil layers (except for Al^{3+} and Fe^{2+}), regardless of tree species, although the two soil layers were visibly indistinguishable under most species. SOM, EBC, water-soluble, and hot-water-extractable C and N were two to nine-fold higher compared to the 5–10 cm layer. This vertical distribution was particularly marked for parameters related to soil organic matter content and water-extractable C and N. The water-soluble fractions represent the amount of the readily mineralizable C and N in soil (Gelsomino and Azzellino, 2011) and have been linked to soil functions which provide nutrients for the trees. Physical protection and the preservation of soil properties and processes of this layer are therefore of utmost importance (FAO, 1998).

In a previous study conducted at the same site, Nsabimana et al. (2008) showed that planting trees increased the levels of soil carbon, nitrogen, base saturation, and exchangeable cation pools in the upper 10 cm of the soil compared to agricultural lands in the same agroecological zone. In the present study, we observed that planted trees influenced soil fertility only in the uppermost soil layer (0–5 cm), with higher values of SOM and exchangeable base cations than the values reported by Nsabimana et al. (Nsabimana et al., 2008) a decade before at this site and compared to those reported for other tropical forest soils (Adugna and Abegaz, 2015; Bauters et al., 2017b).

In contrast to high Al saturation and low amounts of exchangeable cations generally characterizing highly weathered and acidic tropical soils dominated by kaolinitic clays (Cleveland et al., 2003), we observed that the sum of exchangeable cations was relatively high and dominated by calcium (75%), whereas aluminum represented only 3% of the sum of exchangeable cations. Similarly, high base saturation (87%) with a dominance of Ca^{2+} was reported at this site (Nsabimana et al., 2008) and for other sites in the same

agroecological zone with base saturations between 45% and 85% (Mbonigaba et al., 2009; Yamoah et al., 1990). The high proportion of Ca^{2+} could be related to plant litter Ca content, soil pH, and the nature of clay minerals at this site. In tropical nutrient-poor soils, organic acidity is promoted by plants (and soil microorganisms) through the production and the release of organic acids into the soil solution as a "nutrient acquisition strategy" (Fujii, 2014). This may lead to an exchange acidity dominated by protons, allowing for high base saturation events at certain pH values (Duchaufour, 1994). Further, the presence of interstratified kaolinite-smectite, as reported for soils from some subtropical and tropical climates (Dudek et al., 2006; Ryan and Huertas, 2013), may explain the relatively high exchange capacity measured in this study.

2.4.2. Effects of tree species on chemical soil properties

Tree species effects were mostly observed in the upper soil layer (except for Al and Fe). This may indicate that the changes in aboveground litter quality and quantity, rather than mineral weathering and root exudation, most likely influenced soil chemical properties. In contrast to Bauters et al. (Bauters et al., 2017b), who found a significant effect of tree species on soil pH and carbon content until about 30 cm deep in tropical forest plantations, our results highlighted the importance of this thin uppermost 0–5 cm layer in these highly weathered tropical forest soils.

Planting trees is one of the key strategies for restoring degraded forests and soils, especially in tropical soils with inherently poor chemical properties (Celentano et al., 2011). In our study, the pH under eucalyptus species was 0.6 pH units lower than under exotic agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*) and 1.7 pH units lower than under native species (*Entandrophragma excelsum*, *Polyscias fulva*, and self-regenerated mixed natives) in the upper layer. Soil acidification under eucalyptus species was reported in previous studies conducted at this site (Nsabimana et al., 2008), in forest plantations near this site (Mugunga et al., 2015), and in other tropical (Behera & Sahani, 2003; Laclau et al., 2010) and non-tropical regions (Rhoades and Binkley, 1996). The relatively higher concentrations of exchangeable Al^{3+} and Fe^{2+} measured in soils under *Eucalyptus saligna* and *Eucalyptus grandis* compared to other species in this study could be related to the acidifying effect of these species, leading to Al^{3+} and Fe^{2+} release (Bauters et al., 2017b) with potential toxic effects for plant roots (Fujii, 2014). Two main mechanisms were suggested for the effects of tree species on soil

pH: (1) input of organic acids from litter decomposition and root exudates, (2) increased proton release in the soil to compensate for the high plant uptake and storage of base cations (Augusto et al., 2002; Jobbágy and Jackson, 2003). We measured higher pH and exchangeable base cations under mixed native species (Mns) plots compared to other plots. The Mns plots were characterized by high tree density and vegetation diversity dominated by mature native trees accompanied by shrubs and grasses. All species together might have contributed to high quality and quantity of litter as a natural regeneration setup (de Medeiros et al., 2017) compared to other adjacent monoculture plots. Therefore, we suggest that soil pH, SOM content, water-extractable C and N, and exchangeable cations were likely influenced by the species-specific litter chemical quality.

In the upper soil layer, the clear grouping by tree species and high loadings of variables such as SOM, pH, and hot water-extractable C and N fractions (HWC, HWNorg, HWNtot, and HWC:HWNtot) on the first principal component (PC1 = 43%) may indicate that these properties were the most influential set of variables in explaining the variation between species. A previous study (Nsabimana et al., 2008) associated eucalyptus plantations with soil organic matter accumulation and decreased pH. This is in line with our PCA results, where the first PC representing soil organic matter-related properties and pH were associated with a cluster of eucalyptus species (*E. grandis*, *E. maidenii*, and *E. saligna*). The high loadings of pH and WSNtot associated with *Grevillea robusta, Polyscias fulva,* and mixed native species indicate increased soil pH and N availability under these species. The second set of influential variables included EBC, WSC, and WSN_{org} loading high on the second principal component (PC2, 18.5%). It was described that these variables represent the quality and the bioavailability of mineralizable organic matter and related nutrient cycling processes (Wang et al., 2020). The high positive loading of EBC associated with Mns plots may be due to the capacity of this undisturbed self-regenerated native forest containing highly dense and diverse vegetation (trees, shrubs, and grasses) for improving soil chemical quality in terms of nutrients cycling. The observed relationship of water-soluble C and organic N (WSC and WSN_{org}) with *Calliandra calothyrsus* may be due to the characteristics of this plant used in agroforestry as an N-fixing tree (Koutika et al., 2014).

In the 5–10 cm soil layer, the two axes of the PCA explained 59% of the variation between tree species. *Calliandra calothyrsus* and Mns plots grouping was explained by pH, WSN_{org}, WSNtot, HWC, HWNorg, HWNtot, and HWC:HWNtot (PC1, 46%). The remaining species overlapped around the center of the biplot, indicating the lack of species influence on selected soil variables. The multivariate analysis of covariation between chemical properties and tree species in this study suggests that the influence of tree species is mainly limited to the upper soil layer (0–5 cm). This first principal component could be interpreted as a measurement of soil acidity and bioavailability of hot water-extractable C and N fractions, reflecting the quality of SOM and its mineralization process in this soil layer. The results from the present study allowed us to consider this upper layer as a highly sensitive layer to vegetation changes in this tropical forest ecosystem.

2.4.3. Differences in water-extractable c and n between tree species

Soil organic matter has been used for many years as one of the major indicators of soil quality, given its important role in controlling soil chemistry as well as physical and biological processes (Wang et al., 2011). However, it may take many decades to detect a change in the total soil organic C pool, given its slow rate of change (Bankó et al., 2021). Water-soluble and hot water-extractable C and N analysed in this study are labile components of soil organic matter that could reflect early changes in soil–plant interactions (Gregorich et al., 1994). Water-soluble fractions contain dissolved organic components almost similar to those measured directly in the soil solution using lysimeters and suction devices (Ostrowska et al., 2010). Hot water-extractable fractions consist of an easily decomposable pool of SOM, including microbial biomass, that serves as the source of energy and substrate to soil microorganisms, and its decomposition provides nutrients to plants (Bankó et al., 2021; Ghani et al., 2003). This implies that labile fractions of SOM, especially those extracted with hot water, might be used as a proxy for soil microbial biomass and activity (Curtin et al., 2021; Ghani et al., 2003). The influence of tree species on soil function, as represented by water-soluble and hot water-extractable C and N, was observable through the discrimination of tree species and also through the correlation of these fractions with other soil properties. These fractions are closely related to the decomposability of the plant's detritus, which is influenced by the litter chemistry (Russell et al., 2007) and might thus be used as a proxy for soil functioning (Curtin et al., 2021). Labile C and N fractions were significantly correlated to

SOM, EBC, and pH in both upper and lower soil layers, and correlation between HWC and soil organic matter was greater than that for WSC, as also observed by Ghani et al. (Ghani et al., 2003). While the mineralizable organic N decreased with soil depth, nitrates increased with soil depth. This is likely due to water solubility and leaching of nitrates towards the lower soil horizons (Landgraf et al., 2006) and the fact that water-soluble and hot water-extractable C and N fractions originate mainly from above-ground litter rather than root exudates (Binkley and Menyailo, 2004). The dominance of organic N compared to other nitrogen forms may be explained by the fact that most of the mineral N was already extracted by the previous cold-water extraction. Hot water (80 °C) extracts the organic matter not only from decomposing plant litter but also from soil microorganisms (Ćirić et al., 2016).

2.5. Conclusions

The present study was conducted to evaluate the effects of forest tree species on chemical soil quality in Rwanda. The most important changes in soil pH, SOM, water-extractable labile C and N fractions, and base cations were observed in the thin upper soil layer (0–5 cm) across tree species, which made it possible to recognize the importance of this thin upper soil layer for soil fertility. Eucalyptus species led to soil acidification while soil pH and nutrients increased under native species (*Entandrophragma excelsum* and *Polyscias fulva*) and Mns plots. Hot water-extractable C and N fractions strongly correlated with most of the analysed soil parameters and were more sensitive in discriminating tree species effects than other soil properties analysed. This reflects the suitability of this methodological approach for detecting subtle changes that might be linked to forest trees and its potential to be used as a proxy to SOM analysis. In selecting forest trees, priority should be given to the species which do not negatively alter chemical soil quality.

Author Contributions

Conceptualization, P.R., F.X.N., D.N., and M.C.; Data curation, P.R.; Formal analysis, P.R.; Funding acquisition, D.N. and M.C.; Investigation, P.R.; Methodology, P.R. and M.C.; Project administration, M.C.; Resources, M.C.; Software, P.R.; Supervision, F.X.N., D.N., and M.C.; Validation, M.C.; Visualization, P.R.; Writing—original draft, P.R., M.C.; Writing—review & editing, P.R., F.X.N., D.N., and M.C. All authors have read and agreed to the published version of the manuscript.

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CHAPTER 3 – Reforestation in Rwanda: the choice of tree species and implications for soil microbial processes

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Abstract

Although afforestation tree species may have a pronounced effect on soils, the long-term consequences of planted trees on soil microbial properties remain unclear, especially in tropical acidic soils. We investigated the long-term effects (>30 years) of tree species on soil microbial properties and processes in the Ruhande Arboretum in Rwanda. Two soil layers (0–5 cm and 5–10 cm) were analysed under eight tree species planted in replicated monoculture plots (3 eucalyptus sp., 3 agroforestry sp., and 2 native sp.) and under a stand of mixed self-regenerated native species. We measured soil microbial biomass carbon and nitrogen, soil respiration potential, net N mineralization, metabolic quotient, and microbial quotient. We also assessed how differences in soil properties related to changes in soil microbial biomass and processes under the different tree species. Our results showed that soil microbial parameters differed significantly between soil layers, with 2–12 times higher values in the upper 0–5 cm layer. Soil microbial biomass generally increased in soil under Eucalyptus species and decreased under agroforestry species: values were highest under *Eucalyptus grandis* (2010 mg C kg^{-1}), and lowest under *Grevillea robusta* (1120 mg C kg^{-1}). In the 0–5 cm soil layer, the major driving factor of soil microbial properties and processes under different tree species was hot water-extractable carbon, while the sum of exchangeable base cations was the major driving factor of soil microbial properties and processes in the 5–10 cm soil layer. Overall, tree species significantly affected soil microbial biomass and activity, and species effects were more pronounced at 0–5 cm than 5–10 cm soil depth. Furthermore, the observed sensitivity of soil microbial parameters to changes in tree species suggests that they could be used in monitoring programs, in addition to other soil quality indicators, and guide the selection of tree species for afforestation/reforestation.

Keywords: *Soil quality; microbial processes; tropical soils; Eucalyptus species; Ruhande Arboretum; Rwanda.*

3.1. Introduction

The interactions between trees, soils, and soil microorganisms play an essential role in controlling biogeochemical cycling and nutrient supply to plants (Aponte et al., 2013; Lathwell and Grove, 2011). These relations are of particular importance in the highly weathered, often nutrient-poor and acid, tropical forests, which have adapted through species-specific nutrient conservation mechanisms and associations with bacteria and fungi, as well as efficient nutrient cycling processes (Vitoussek & Sanford, 1986; Veldkamp et al., 2020). While deforestation in Africa showed an increasing deforestation rate for the last three decades with a net loss estimated at 3.9 million hectares (FAO, 2020), reforestation and afforestation initiatives aim to rehabilitate degraded soils to improve soil quality and support the sustainable use of forest soils (Berthrong et al., 2009; Smal et al., 2019). The selection of tree species for such reforestation/afforestation programs is crucial for determining the long-term effects on ecosystem functioning. Programs frequently focus on economic and social aspects, such as productivity and benefits for local communities, but little attention has been paid to the long term-implications for soil nutrient cycling processes. Such an understanding of the responses of soil microbial processes to different tree species is essential for the assessment of long-term consequences and supporting sustainable reforestation/afforestation practices. While tree species' effects on soils have been extensively studied in temperate systems (Augusto et al., 2002), and soil chemical parameters in response to deforestation and reforestation studied and reviewed for tropical soils (Nsabimana et al., 2008; Townsend et al., 2008; Veldkamp et al., 2020), few studies have addressed the effects of tree species in reforestation/afforestation management on soil microbial parameters in tropical areas (Xu et al., 2007).

In tropical forests, plant productivity and the nutritional properties of the highly weathered and inherently nutrient-poor soils are mainly driven by the rapid decomposition of aboveground and belowground litter, root exudates, and other detritus, ensuring quick internal cycling of nutrients (Laclau et al., 2010; Sayer et al., 2020; Doetterl et al., 2021). Through tree species-specific characteristics of litter quantity and chemical composition (Tajik et al., 2020), resulting from differing nutrient acquisition strategies (Lambers et al., 2008), tree species identity influences the fertility of tropical

forest soils through the role of microbial processes of re-mineralizing nutrients during litter decomposition (Schulte and Ruhiyat, 1998; Sayer and Tanner, 2010). Tree species also influence, directly or indirectly, soil properties, such as pH, moisture, and organic matter, which in turn are key drivers of changes in soil microbial parameters in forest ecosystems. The exchange of nutrients between soils and plants might be mainly concentrated in the topsoil layer due to the substantial quantities of litter continuously produced and rapidly decomposing on the soil surface (Sayer et al., 2006), and supplying a significant proportion of plants' nutrient requirements. Although most of the tree species included in this study are locally preferred for their high biomass production, the differences in litter quality in terms of leaf contents of macro- and micronutrients, lignin, tannin, leaf C/N ratio, and other organic compounds determine the decomposition rates (Ge et al., 2013). For example, recent global meta-analyses confirmed reduced moisture, nutrients, microbial abundance, nitrogen and increased carbon under Eucalyptus species, and reported inconsistent effects on soil pH (Zhang et al., 2015; Christina et al., 2017; Mallen-Cooper et al., 2022). *Calliandra calothyrsus*, *Grevillea robusta*, and *Cedrela serrata* are commonly used in agroforestry (trees on farms) to improve soil fertility. *Calliandra calothyrsus* is a N_2-fixing tree reported to improve soil fertility (Kisaka et al., 2023). *Grevillea robusta* is mainly planted for soil stabilization and fertility improvement by its proteoid root structure that can easily intercept leaching nutrients (Kalinganire, 1996; Watt and Evans, 1999), mobilize unavailable nutrients bound to metal ions (e.g., Al and Fe) and enhance nutrient uptake (Dinkelaker et al., 1997). *Entandrophragma excelsum*, a late successional evergreen, and *Polyscias fulva*, an early successional evergreen, are known in Kinyarwanda as Umuyove and Umwungo, respectively and they are planted for their valuable timber and their multiple uses in traditional medicine (Mujawamariya et al., 2021; Ndoli et al., 2021). Litter traits of some of studied afforestation tree species can be found in Table 3.2.

Microbial biomass is the soil's living constituent, making up 1 to 5 % of the total SOM, and responding relatively rapidly to changes in soil conditions (Carter et al., 1997). Understanding the biomass and activity of microorganisms in forest soils is crucial because they are significant drivers of soil processes (Bardgett, 2005), which are in turn essential in maintaining ecosystem functions and environmental change (FAO & UNEP, 2020; Babur et al., 2021). High levels of soil microbial biomass are often seen as favourable, whereas a reduction in microbial biomass may be considered to be

deleterious to soils, particularly when it adversely affects their functioning (Gonzalez-Quiñones et al., 2011). However, the size of the microbial biomass does not necessarily reflect the activity of microbial community. Microbial activity can be assessed by soil processes, i.e., soil respiration and N mineralisation (Temesgen et al., 2019; Babur et al., 2021). Further, eco-physiological indices, such as the ratio of soil respiration to microbial biomass carbon (qCO_2; metabolic quotient), may be used to evaluate the efficiency of the soil microbial community to utilize organic carbon substrates (Tang et al., 2020; Babur et al., 2021a).

A primary goal of assessing the effects of forest tree species on soil functions is to ensure the sustainable use and management of forest soils (Bolte et al., 2019), by predicting the consequences for maintaining or improving the soil's capacity to (i) sustain plant productivity (ii) maintain in balance the cycling of water, carbon, and other nutrients, and (iii) preserve ecological forest functions (Burger et al., 1998). The choice of soil parameters to be analysed should consider management goals and the fact that some properties better reflect changes in the soil ecosystem than others (Schloter et al., 2003; Muscolo et al., 2015). Soil microbial parameters are increasingly considered to be good indicators for shifts in soil functioning because major biogeochemical processes are driven by soil microorganisms (Ratcliffe et al., 2018; Schloter et al., 2018). Although many recent studies have demonstrated the effects of forest tree species on soil microbial properties and processes (Ribbons et al., 2018a; Zhang et al., 2020; Zheng et al., 2022), the mechanisms and extent to which species affect soils vary with climate and soil specificities which does not allow findings to be largely generalized. A meta-analysis by Zhang et al. (2017) showed that local climate and soil factors play a major role in determining the differences in soil characteristics attributed to tree species. In addition, the integration of soil microbial parameters as sustainability indicators for management of forest plantation is hindered by the scarcity of soil microbial data, especially in tropical African regions (Vitoussek and Sanford, 1986; Sloan and Sayer, 2015).

In Rwanda, factors such as hilly topography, inherently nutrient-poor and highly weathered soils, and heavy rains intensifying soil erosion, lead to land degradation and declining soil fertility (Clay and Lewis, 1990). Over recent decades, the country has recognized the importance of forest plantations, not only as an effective land restorative strategy but also for improving population livelihood that highly depends on wood

products (Habiyaremye et al., 2011). Through the synergy of government institutions, non-governmental organizations, and individual citizens, the target to increase the forest cover from 19.6% in 2010 to 30% by 2030 has been reached in 2020, with 724,695 ha (30.4%) forest cover (RFA, 2021). Forest plantations, mainly with introduced exotic tree species, occupy now 53.5% of the country's forests. The main species of these plantations are Eucalyptus (89%), pine (6.5%), mixed stands of exotic species (3.1%), and plantations of native species (1.4%) (IUCN, 2020). Although the effects of different tree species on soil properties and processes are well established in temperate and boreal ecosystems, to our knowledge, there are limited studies examining how tree species affect soil microbial properties and processes in tropical forest plantations. A few studies have investigated the responses of soil physical and chemical properties to introduced tree species in tropical soils (Nsabimana et al., 2009; Rwibasira et al., 2021). Understanding the effects of these species on microbial properties and processes related to soil functioning is crucial to inform forest managers on the selection of suitable species that meet both ecological and local livelihood needs.

In this study, we investigated soil microbial properties and processes in the upper soil layers (0-5 and 5-10 cm) under replicated planted monoculture plots of eight tree species and in a self-regenerated mixed species plot, within the arboretum of Ruhande in Rwanda. Tree species *Eucalyptus grandis*, *Eucalyptus maidenii*, *Eucalyptus saligna*, *Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*, *Entandrophragma excelsum* and *Polyscias fulva* were selected, according to their use and adaptability to the different regions of the country. The main aim of this study was to assess the effects of tree species commonly used in afforestation/reforestation programs in Rwanda on soil functioning, assessed through microbial parameters related to C and N cycling, as well as to determine key soil chemical properties influencing the response of microbial parameters to tree species. We hypothesized that microbial processes would be most intense in the upper soil layer, lower under Eucalyptus species, and that differences in microbial activity between tree species would be explained by the availability of labile carbon substrate.

3.2. Materials and Methods

3.2.1. Study site

The study was conducted at the Arboretum of Ruhande (2°36' S, 29°44' E), located in the Huye District, Southern Province of Rwanda (Fig. 1.5). The site has a humid tropical climate characterized by a mean annual air temperature between 17.5 °C and 19 °C, and approximately 1230 mm rainfall with a bimodal distribution pattern of two rainy seasons (heavy rains: March-May; and irregular rains: September–December) and two dry seasons (short: January–February; and long: June–August) (Meteorwanda, 2021). The soil is characterized by a brown-red colour with a sandy loam texture and diffuse horizons, classified as Ferralsols or Oxisols according to FAO and USDA Soil Taxonomy, respectively (Verdoodt et al., 2006). Detailed soil characteristics of the studied plots can be found in Rwibasira et al. (2021).

The site was established in 1933 on the flat plateau of Ruhande hill (1737 m asl), previously cultivated through a typical traditional organic cropping system. Its area has progressively increased to reach the present size of 200 ha (Nsabimana et al., 2008). The site currently contains 204 tree species, with 144 deciduous, 57 conifers, and 3 bamboo species planted on 477 plots (50 m x 50 m) of replicated monoculture stands (Fig. 2.1). Among all species, exotic trees represent 84% (172 species, of which 69 are eucalyptus species), while native species represent 13% (32 species) (RFA, 2021). All plots are regularly managed by the removal of invading vegetation and planting young trees in replacement of dead plants, to maintain a constant density of the initial tree species in the plots, except on an undisturbed plot (4 ha) of self-regenerated mixed native species, dominated by *Polyscias fulva* trees (Mugunga et al., 2022). No fertilization, weed control treatments, clear-cut, or fire events have occurred since the establishment of the site.

Table 3. 1. Tree species ranking (in decreasing order) based on soil chemical properties in the 0–5 cm soil layer

Rank	Soil $pH_{(KCl)}$			SOM (%)			HWC (mg kg^{-1})			HWNtot (mg kg^{-1})			EBC (cmolc kg^{-1})		
1	Pf	5.8 ±.01	i	Em	27.6 ±.09	d	Es	5500 ±140	f	Eg	490 ±13	h	Mns	63 ±1.4	j
2	Ee	5.5 ±.02	h	Mns	27.5 ±2.2	d	Em	5400 ±120	f	Es	440 ±8	g	Em	38 ±0.7	i
3	Gr	5.5 ±.02	h	Ee	25.8 ±2.2	d	Eg	5200 ±150	ef	Ee	430 ±17	g	Pf	36 ±0.3	hi
4	Mns	5.4 ±.04	h	Es	25.0 ±1.3	d	Ee	4600 ±210	e	Mns	430 ±20	g	Es	36 ±1.6	hi
5	Cc	4.9 ±.01	fg	Eg	21.6 ±0.6	bcd	Mns	3500 ±230	d	Em	420 ±5.2	fg	Gr	34 ±0.3	h
6	Cs	4.9 ±.01	fg	Gr	19.2 ±0.2	bc	Pf	3400 ±51	d	Pf	370 ±8.9	ef	Eg	33 ±0.4	gh
7	Em	4.2 ±.01	c	Cc	19.1 ±0.5	bc	Cc	3200 ±280	cd	Gr	330 ±8	de	Cs	30 ±1.2	fg
8	Eg	4.0 ±.01	b	Pf	18.5	b	Gr	2700 ±97	bc	Cc	300 ±4.5	cd	Ee	29 ±1.0	f
9	Es	3.7 ±.01	a	Cs	18.1 ±0.8	b	Cs	2500 ±110	b	Cs	270 ±9.4	c	Cc	26 ±0.9	f

The Different letters within the column indicate significant differences ($p<0.005$) based on Tukey's HSD test. Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species. EBC: sum of exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}); HWC: Hot water-extractable carbon; HWNtot: total hot water-extractable nitrogen. Source: (Rwibasira et al., 2021).

Table 3. 2. Traits of different tree species included in the study

Tree species	Family	Height (m)	DBH (m)	Wood density (g cm^{-3})	Litter C (%)	Litter N (%)	Lignin (g kg^{-1})	Litter C/N	Growth rate, crown, and roots system	Other functional traits	References
Calliandra calothyrsus	Fabaceae	6–9	0.2–0.3	0.51–0.78	45–49	2.7–4.6	85–145	12–18	Fast growth; Tap & lateral roots	N fixing; litter proteins (22%), condensed tannins (13%)	[1]; [2]; [3]; [4]
Cedrela serrata	Meliaceae	25–30	2.0–3.0	0.26–0.52	47–49	1.2–1.6	198–285	32–36	Medium growth; Deep rooting system; Valuable timber	High phenolics and flavonoids with antimicrobial activity and insect repellent smell	[1]; [5]; [6]; [7]
Grevillea robusta	Proteaceae	30–40	2.0–3.0	0.54–0.72	37–43	5.6–7.5	35–40	52–78	Medium growth; Deep and proteoid root mat, thick leaf mulch	Leaves rich in Aluminum; Cyanogenic, and tannins (2.6 %); good fuelwood	[8]
Eucalyptus grandis	Myrtaceae	45–55	2.0–3.5	0.60–0.75	49–50	0.5–1.0	190–243	29–33	Fast growth; deep roots up to 5-6 m and lateral fine roots; thin crown	Cellulose (40–50%), Hemicellulose (25%); good fuelwood	[9]
Eucalyptus maidenii	Myrtaceae	40–55	1.5–2.0	0.65–0.80	49–55	0.7–1.3	133–189	43–68	Fast growth; deep roots and lateral fine roots	Antimicrobial, Essential oil (1% of DM), Cineole content (62% of DM), good fuelwood	[10]; [11]
Eucalyptus saligna	Myrtaceae	30–50	2.0–2.5	0.85–1.07	47–53	1.0–2.0	147–186	26–49	Fast growth; Deep, wide-spreading, and dense root system	Aromatic smell, alpha-pinene (71–84%), Essential oil (0.3-0.5% of DM); good fuelwood	[10]; [12]
Entandrophragma excelsum	Meliaceae	50–60	3.0–4.0	0.70–0.85	22–30	1.1–1.4	291–373	12–16	Slow growing; Surface buttress and deep roots; dense crown	Triterpenoid; Antimicrobials properties; toxic limonoids; Valuable timber	[2]; [13]; [14]
Polyscias fulva	Araliaceae	25–30	1.0–1.5	0.33–0.45	-	-	-	-	Slow growing; Tap & lateral roots; parasol crown; leaf fall forms good mulch	Antimicrobial saponins; triterpene glycosides; soil fertility improver; poor fuelwood quality;	[15]; [16]; [17];

[1]: (D. Nsabimana et al., 2009); **[2]**: (Orwa et al., 2009); **[3]**: (Kisaka et al., 2023); **[4]**: (Cobo et al., 2002); **[5]**: (Scherer-Lorenzen et al., 2007); **[6]**: (Russell et al., 2004); **[7]**: (Almubayedh and Ahmad, 2020); **[8]**: (Musongora et al., 2023b); **[9]**: (Bini et al., 2013); **[10]**: (Demessie et al., 2012); **[11]**: (Cizungu et al., 2014); **[12]**: (Sausen et al., 2014); [13]: (Loranger et al., 2002); **[14]**: (Rachmawati et al., 2019); **[15]**: (Ashmawy et al., 2020); **[16]**: (Plunkett et al., 2001); **[17]**: (Winnie et al., 2019).

3.2.2. Soil sampling and analyses

Based on the use and species' adaptability in the different regions of the country (Iiyama et al., 2018), eight species were selected (Fig. 2.1). These included three Eucalyptus species (*Eucalyptus grandis*, *Eucalyptus maidenii*, and *Eucalyptus saligna*), three agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*), two native species (*Entandrophragma excelsum* and *Polyscias fulva*), and a self-regenerated plot of native forest (Mixed native species = Mns). Three plots per species were selected and each plot was divided into two sub-plots (25 × 50 m). One composite soil sample was taken in each sub-plot for two soil layers (0-5 cm and 5-10 cm) by mixing five soil samples (X-shaped sampling) collected by using a 30x30 cm frame and a shovel under the tree's canopy at 1–1.5 m from the tree base (Bini et al., 2013). Soils were sieved fresh (4 mm) and stored at 4 °C until analyses.

Microbial biomass (MBC, MBN)

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined with the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Fumigation of 10 g soil was carried out for 3 days in a vacuum desiccator with alcohol-free chloroform. Fumigated and non-fumigated samples were extracted with 50 ml 0.5 M K_2SO_4 (1 h shaking at 180 rpm and filtration through Whatman filter #42). Organic carbon in the extracts was measured with a Total Organic Carbon analyser (Lab Toc, Pollution, and Process Monitoring, UK), and total nitrogen were analysed colorimetrically using a continuous flow analyser equipped with a UV digestor (AutoAnalyzer 3, BranLuebbe, Germany). Soil microbial biomass C and N were calculated as the difference between fumigated and non-fumigated samples with a conversion factor of 0.45 for biomass C (Joergensen and Mueller, 1996), and 0.54 for biomass N (Brookes et al., 1985).

Soil respiration potential

The soil respiration potential (RP) was measured at 20 °C as CO_2–C accumulation in the headspace (125 ml) of an amber bottle (Supelco, USA) from 20 g of fresh soil adjusted to 60% water holding capacity (see below), after an overnight pre-incubation at 20 °C in the dark (Robertson et al., 1999). Gas samples (4 ml) were taken at 0, 120, 150, 180, and 210 minutes with an airtight syringe (Hamilton Model 1005) and analysed with an infrared

absorption gas analyser (EGM-4, Ppsystem, UK). The respiration potential was estimated by linear regression of CO_2–C against time (μg CO_2–C g^{-1} h^{-1}).

Microbial and metabolic quotient

The microbial quotient (qmic) is an indicator of the availability of soil C for microorganisms (Anderson and Domsch, 1990). It was calculated by dividing soil microbial biomass carbon by the total soil organic carbon content, estimated as 58% of soil organic matter (Allen, 1989). The metabolic quotient (qCO_2) was calculated by dividing the respiration potential by the microbial biomass carbon (Anderson, 2003). It is an indicator of microbial maintenance energy requirement (Dilly and Munch, 1998).

Nitrogen transformation rates

Net nitrogen mineralization (Nmin) was determined from 15 g of fresh soil adjusted to 60% water holding capacity and incubated at 20 °C for 28 days (Hart et al., 1994). Extraction of inorganic nitrogen (NH_4^+–N and NO_3^-–N) was performed on sub-samples at the beginning and the end of the incubation period using 1 M KCl (1:5; w:v), after 1 h agitation at 180 rpm and centrifugation at 4000 rpm (Allen, 1989). The water loss during incubation was monitored gravimetrically and compensated by adding distilled water as necessary. Extracts were analysed colorimetrically using a continuous flow analyser equipped with a UV digestor (AutoAnalyser3, BranLuebbe, Germany). Net nitrogen mineralization (Nmin) and relative nitrification (Nit_{rel}) rates were calculated as the ratio between the net increase in inorganic nitrogen (NH_4^+–N and NO_3^-–N), and the number of incubation days, and as the percentage of nitrate produced (NO_3^-– N), respectively.

Soil physico-chemical properties

Soil organic matter (SOM) content was calculated as weight loss from oven-dry soil after overnight ignition at 550 °C in a muffle furnace, and soil pH was determined in a soil solution (1:2.5 w/v) with 1 M KCl using a pH meter (HI2550, HANNA® Instruments, USA) as described by Allen (1989). Soil water holding capacity (WHC) was determined volumetrically by using the Haines-funnel system (Jenkinson and Powlson, 1976). Briefly, water (50 ml) was added to 25 g of fresh soil and kept for 1 hour to saturate the soil before draining and measuring the volume of water retained by the soil. Exchangeable base cations (EBC: Ca^{2+}, K^+, Mg^{2+}, and Na^+) were extracted from fresh soil using the barium chloride and expressed as their sum in $cmol_c$ kg^{-1} (0.1 M $BaCl_2$: 1:5 w/v) method

(Hendershot and Duquette, 1986), and the chemical analysis of the extracts was performed using ICP-AESS (Varian, Australia). Water-extractable C and N forms were sequentially extracted, first at room temperature and then at 80 ^{o}C, following the method of Ghani et al. (2003). Details on methods and tree species' effects on soil chemical properties can be found in Rwibasira et al. (2021).

3.2.3. Statistical analyses

All statistical analyses and data visualizations were carried out using the R software, version 4.1.3 (R Core Team, 2022). We used linear mixed-effects models (REML, package "*lme4*": Bates et al. (2015)) to test for differences between tree species ("Species"=9 levels), soil layers ("Layer"=2 levels), and their interaction (Species*Layer), included as fixed effects (formula: response ~ -1 + Species + Layer + Species * Layer). The plot ("Plot") was included as a random effect (formula: ~1 | Plot) to account for the non-independence of the two soil samples collected within the same plot. The coefficients of determination for generalized mixed-effect models were obtained by using a multi-model inference package (*MuMIn*: Barton, (2020)) which calculates the total variance explained by the model (Conditional R_GLMM^2=R^{2}c) and the variance explained by the fixed effects alone (Marginal R_GLMM^2=R^{2}m). Standardized parameters were obtained by fitting the model on a standardized version of the dataset, where parameters were scaled and centred to a mean of zero and standard deviation of one by using the basic built-in *scale()* function in R. As the interaction terms were significant for all analysed parameters, multiple comparisons between tree species were analysed separately for each soil layer. Confidence Intervals (CI at 95%) and p-values were computed using the estimated marginal means package (*Emmeans*: Lenth et al. (2018)) with Tukey–Kramer Honestly Significant Difference (Tukey's HSD). The assumptions of normality and homoscedasticity of the residuals were assessed by visual inspection of the Q–Q plots and plots of the normalized residuals against the fitted values.

Relationships between soil chemical properties and microbial parameters were tested for each layer separately using the correlation-based network analysis ("*Corrr*" package, Kuhn et al. (2020)), computing pairwise correlation coefficients (Pearson) between any two pairs of analysed variables, and generating a correlation matrix according to the strength of correlation. Additionally, we performed a Principal Component Analysis (PCA) (packages "*FactoMineR*", Husson et al. (2020) and package "*ggplot2*", Wickham et

al. (2022)) to explore relationships between soil chemical properties and microbial parameters in each layer separately.

3.3. Results

3.3.1. Soil microbial properties in 0–5 cm and 5–10 cm topsoil layers

Soil microbial parameters were significantly affected by tree species at soil layer with higher values in the upper 0–5 cm compared to the 5–10 cm layer (Table 3.3; Fig. 3.1). Mean MBC and MBN values were 5 and 6 times higher in the 0–5 cm than in the 5–10 cm soil layer. RP was 12 times higher, and Nmin was 8.5 times higher in the 0–5 cm than in the 5–10 cm soil layer. Both qmic and qCO_2 were 2 times higher in the 0–5 cm than in the 5–10 cm soil layer. Relative nitrification rates (Nitrel) showed no differences between layers, while MBC:MBN was lower in the 0–5 cm than in the 5–10 cm soil layer.

Table 3. 3. Variance analysis linear mixed-effects model predicting the effects of tree species and soil layer on soil microbial properties and processes.

Variables	**Tree species (S)**		**Soil layer (L)**		**Interaction of Species and Layer (S*L)**		**R squared**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**R^2m**	**R^2c**
MBC (mg C kg^{-1})	1260.7	<0.0001 ***	7338.9	<0.0001 ***	40.3	<0.0001 ***	0.98	0.98
MBN (mg N kg^{-1})	3222.9	<0.0001 ***	11894.2	<0.0001 ***	246.7	<0.0001 ***	0.99	0.99
RP (µg CO_2-C g^{-1} h^{-1})	13538.2	<0.0001 ***	127549.3	<0.0001 ***	1548.7	<0.0001 ***	0.99	0.99
Nmin (mg N kg^{-1} d^{-1})	672.7	<0.0001 ***	4348.5	<0.0001 ***	67.4	<0.0001 ***	0.97	0.98
Nitrel (%)	148960.0	<0.001 **	13.2	0.0005 ***	9.9	<0.001 **	0.57	0.61
MBC:MBN	213.5	<0.0001 ***	39.9	<0.0001 ***	48.5	<0.0001 ***	0.83	0.88
qCO2 (µg CO_2-C mg^{-1} MBC h^{-1})	661.2	<0.0001 ***	1926.4	<0.0001 ***	112.7	<0.0001 ***	0.96	0.97
qmic (mg MBC g^{-1} Ctot)	404.0	<0.0001 ***	902.4	<0.0001 ***	12.5	<0.0001 ***	0.89	0.92

MBC: microbial biomass carbon; MBC: microbial biomass carbon; RP=respiration potential; Nmin=net N mineralization; Nitrel=relative nitrification; MBC:MBN=microbial biomass C/N ratio; qCO2=metabolic quotient; qmic=microbial quotient.

3.3.2. Tree species effects on soil microbial biomass and soil microbial C:N ratios

Tree species significantly affected soil microbial biomass and microbial C:N ratio (Fig. 3.1) Most differences in MBC and MBN between tree species were pronounced in the upper 0–5 cm soil layer. In this layer, MBC was highest under *Eucalyptus grandis* (Eg: 2010 mg C kg^{-1}), *Eucalyptus maidenii* (Em: 1860 mg C kg^{-1}), and mixed native species (Mns: 1800 mg C kg^{-1}), while the lowest values were measured under the agroforestry species, *Calliandra calothyrsus* (Cc: 1220 mg C kg^{-1}) and *Grevillea robusta* (Gr: 1120 mg C kg^{-1}).

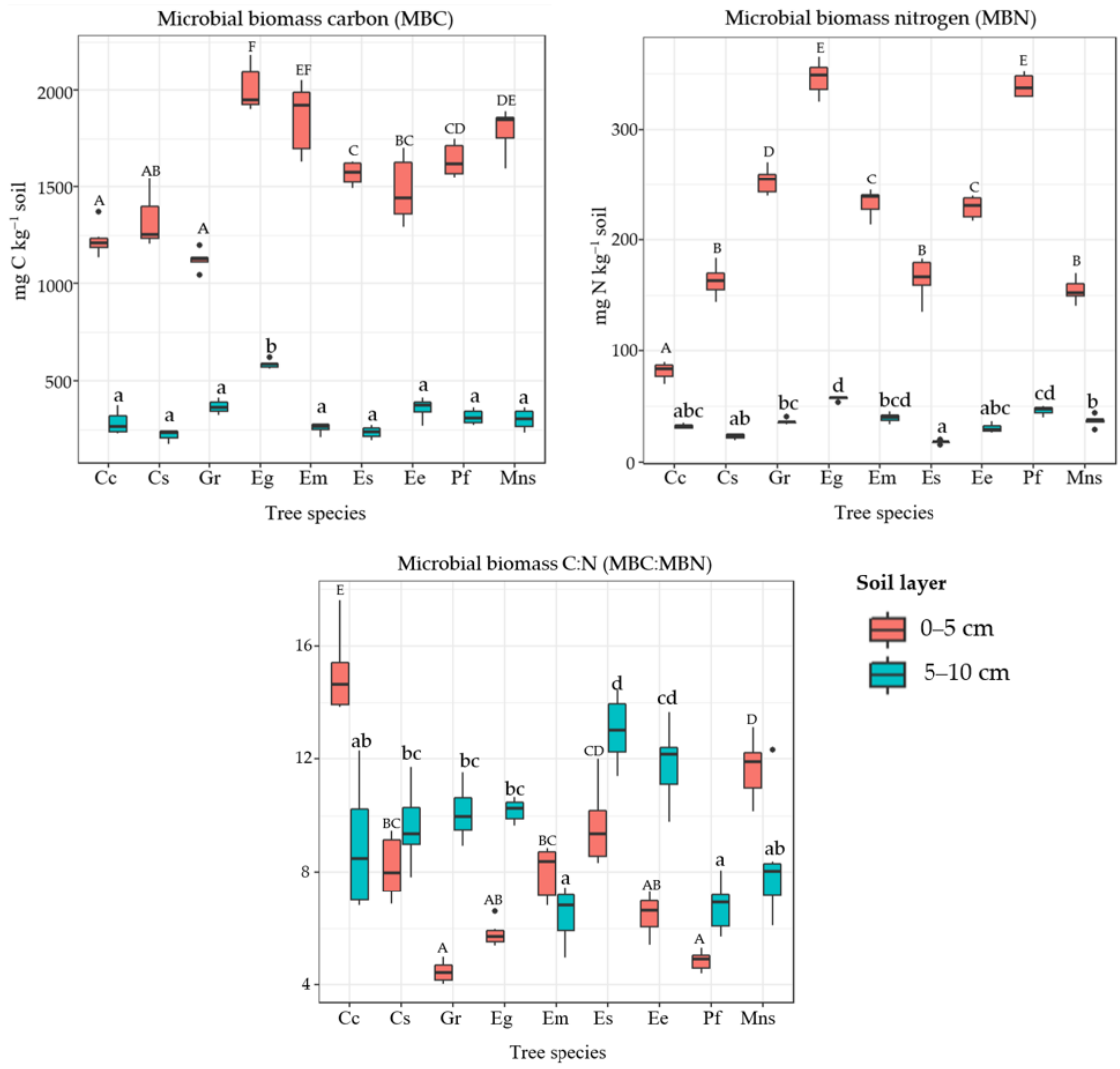

Fig. 3. 1. Predicted values (Linear Mixed-Effects Models) of soil microbial biomass across eight tree species in two soil layers.
The model was: response-variable ~ Species + Layer + Species*Layer + (1|Plot), where Plot is replicate PlotID and Layer (0–5 cm and 5–10 cm soil layers). As the interaction terms was significant for all analysed parameters, multiple comparisons between tree species were analysed separately for each layer. Red and blue boxes are model estimates in 0–5 cm and 5–10 cm soil layers, respectively. The horizontal black line in the box shows the estimated sample median, while the lower and the upper box boundaries show the first and the third percentiles, respectively. The dots outside the whisker boundaries show observations outside the 5th–95th percentile range. Different small letters (5–10 cm layer) and capital letters (0–5 cm layer) above the bars indicate statistically significant differences (LMM, Tukey's HSD, $p < 0.05$) between tree species in response variables. Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; and Mns= mixed native species.

Highest MBN values were measured under Eucalyptus grandis (346 mg N kg^{-1}) and *Polyscias fulva* (339 mg N kg^{-1}), and the lowest values were measured under *Calliandra calothyrsus* (81.9 mg N kg^{-1}). Highest soil MBC:MBN values were measured under

Calliandra calothyrsus (15), while the lowest values were measured under *Grevillea robusta* (4.4) and *Polyscias fulva* (4.8).

In the 5–10 cm soil layer, MBC (585 mg C kg^{-1}) and MBN (57.5 mg N kg^{-1}) were significantly higher under *Eucalyptus grandis*. MBC:MBN ratio (13) was also high under *Eucalyptus grandis*, while the lowest values were also measured under *Polyscias fulva* (6.7) (Fig. 3.1).

3.3.3. Tree species effects on soil microbial activities and ecophysiological indices

In the 0–5 cm soil layer, RP was highest under *Calliandra calothyrsus* (11.9 µg CO_2–C g^{-1} h^{-1}), followed by *Eucalyptus grandis* (10.1 µg CO_2–C g^{-1} h^{-1}), while the lowest values were measured under *Eucalyptus saligna* (10.1 µg CO_2–C g^{-1} h^{-1}) (Figure 3.2). In this soil layer, the highest values of Nmin were measured under *Eucalyptus maidenii* (5.7 mg N $kg^{-1}d^{-1}$) followed by *Entandrophragma excelsum* (4 mg N kg^{-1} d^{-1}) and *Calliandra calothyrsus*. In the 5–10 cm soil layer, there were no differences between tree species in soil RP and (Nmin). Relative nitrification was high (> 98%) under all species, and did not differ between soil layers, except for *Polyscias fulva* under which, the percentage of nitrates was significantly higher in the 0–5 cm than in the 5–10 cm soil layer (Figure 3.2).

In the 0-5 cm soil layer, metabolic quotient (qCO_2) values were higher under agroforestry and native species and lower under Eucalyptus species. The highest values were measured in soil under *Calliandra calothyrsus* (9.8 µg CO_2–C mg^{-1} MBC h^{-1}), *Grevillea robusta* (7.2 µg CO_2–C mg^{-1} MBC h^{-1}), and *Polyscias fulva* (6.2 µg CO_2–C mg^{-1} MBC h^{-1}), while *Eucalyptus maidenii* and *Eucalyptus saligna* showed the lowest values (3.0 and 2.8 µg CO_2–C mg^{-1}MBC h^{-1}, respectively).

In the 5–10 cm layer, soils under Eucalyptus species had the lowest values of the metabolic quotient (1.1 µg CO_2–C mg^{-1}MBC h^{-1}) while values were highest under *Polyscias fulva* and *Cedrela serrata* (4.7 and 3.5 µg CO_2–C mg^{-1}MBC h^{-1}, respectively) (Fig. 3.3). The microbial quotient (qmic) was highest under *Eucalyptus grandis* both in 0–5 cm and 5–10 cm soil layers (16.0 and 9.8 mg MBC g^{-1} C_{tot}, respectively). The lowest values of qmic at 0-5 cm were found under *Grevillea robusta* and *Entandrophragma excelsum* (10.0 mg MBC g^{-1} C_{tot}), and at 5–10 cm, *Polyscias fulva* showed the lowest qmic values (2.9 mg MBC g^{-1} C_{tot}).

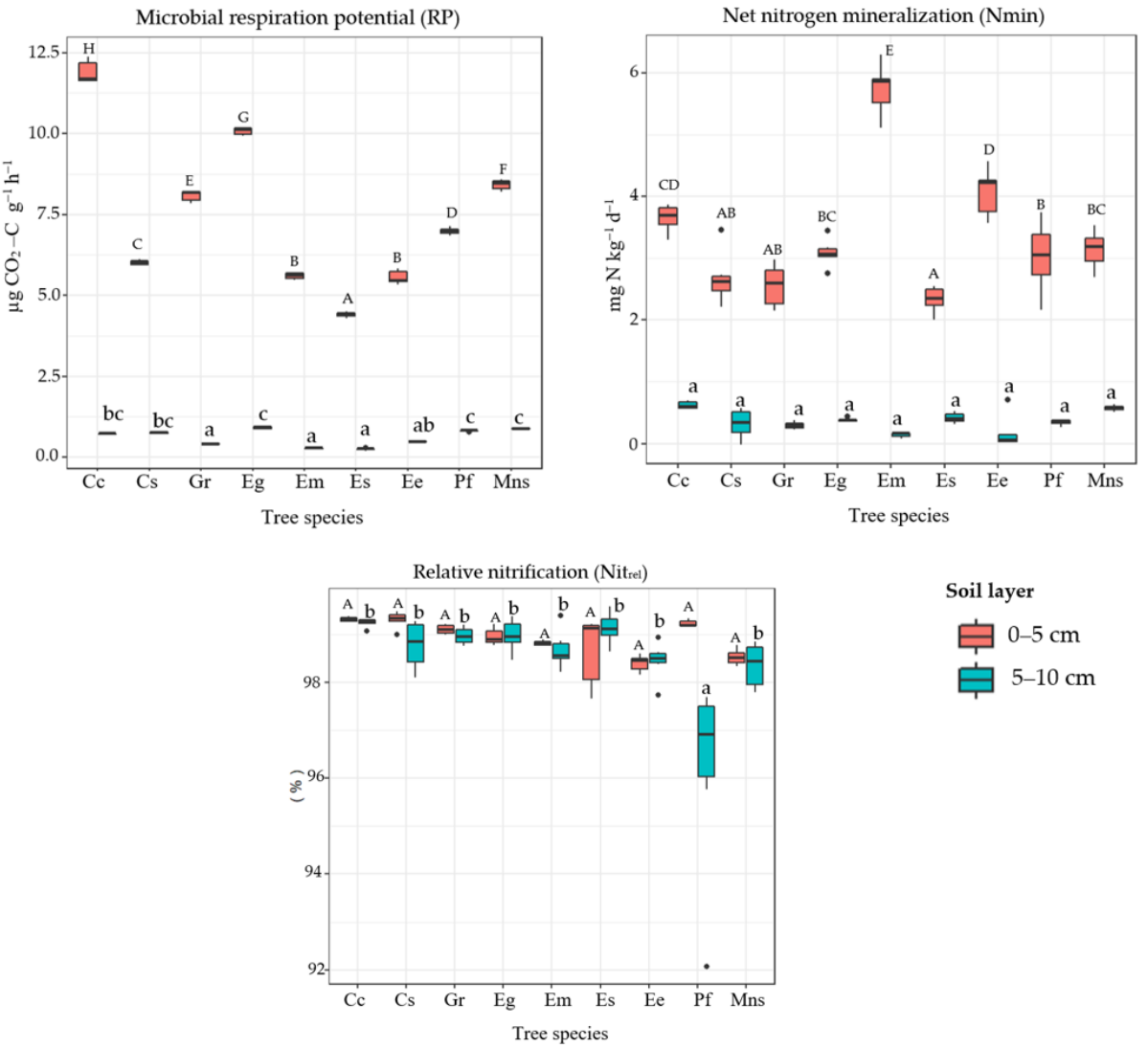

Fig. 3. 2. Predicted values (Linear Mixed-Effects Models) of soil microbial respiration, net nitrogen mineralization, and relative nitrification across eight tree species in two soil layers. The model was: response-variable ~ Species + Layer + Species:Layer + (1|Plot), where Plot is replicate PlotID and Layer (0–5 cm and 5–10 cm soil layers). Whenever the interaction of main effects was statistically significant, the multiple comparisons of tree species were separately analysed by soil layer. Red and blue boxes are model estimates in 0–5 cm and 5–10 cm soil layers, respectively. The horizontal black line in the box shows the estimated sample median, while the lower and the upper box boundaries show the first and the third percentiles, respectively. The dots outside the whisker boundaries show observations outside the 5th–95th percentile range. Different small letters (5–10 cm layer) and capital letters (0–5 cm layer) above the bars indicate statistically significant differences (LMM, Tukey's HSD, $p < 0.05$) between tree species in response variables. Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; and Mns= mixed native species.

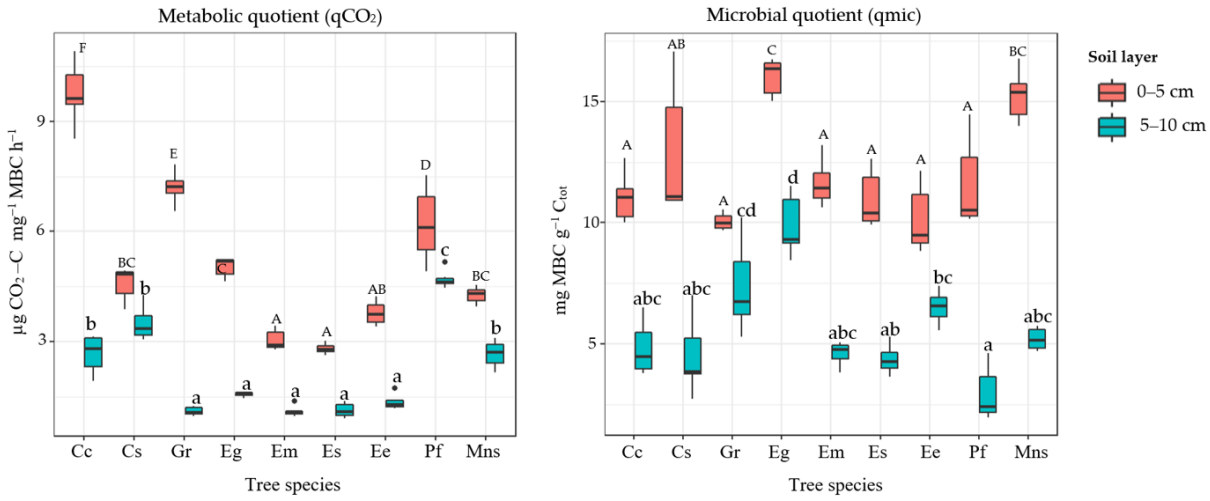

Fig. 3. 3. Figure 12. Predicted (Linear Mixed-Effects Models) values of soil microbial quotient and metabolic quotient across eight tree species in two soil layers.
The model was: response-variable ~ Species + Layer + Species:Layer + (1|Plot), where Plot is replicate PlotID and Layer (0–5 cm and 5–10 cm soil layers). Whenever the interaction of main effects was statistically significant, the multiple comparisons of tree species were separately analysed by soil layer. Red and blue boxes are model estimates in 0–5 cm and 5–10 cm soil layers, respectively. The horizontal black line in the box shows the estimated sample median, while the lower and the upper box boundaries show the first and the third percentiles, respectively. The dots outside the whisker boundaries show observations outside the 5th–95th percentile range. Different small letters (5–10 cm layer) and capital letters (0–5 cm layer) above the bars indicate statistically significant differences (LMM, Tukey's HSD, $p < 0.05$) between tree species in response variables. Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; and Mns= mixed native species.

3.3.4. Relationships between soil microbial parameters and soil chemical properties

Relationships between soil microbial parameters and soil chemical properties

Pearson's correlation-based network analysis between soil properties (pH, SOM, HWC, and EBC) and microbial parameters (MBC, MBC:MBN, RP, and Nmin) showed different patterns of association within each of the two soil layers (Figure 3.4). Generally, the strength of association in the 0–5 cm soil layer was higher than in the layer beneath (5–10 cm soil layer).

In the 0–5 cm soil layer, MBC showed a significant positive correlation with HWC (r= 0.65, p<0.001), SOM (r= 0.49, p<0.001), EBC (r= 0.42, p=0.002), and Nmin (r=0.30, p=0.02), while it showed a significant negative correlation with pH (r= -0.38, p=0.005). Soil respiration potential (RP) was positively correlated with MBC:MBN ratio (r=0.38, p=0.005) and negatively correlated with HWC (r=-0.31, p=0.01). Net N mineralization

had a positive correlation with HWC and SOM (r=0.38, p=0.005 and r=0.28, p=0.03, respectively).

In the 5-10 cm soil layer, MBC was positively correlated with EBC and RP (r= 0.76, p<001 and r=0.37, p=0.005, respectively), and negatively correlated with HWC (r=-0.25, p=0.05). RP had a positive correlation with EBC, pH, and Nmin (r=0.68, p<0.001, r=0.45, p<0.001, and r=0.43, p=0.001, respectively), and a negative correlation with MBC:MBN (r=-0.28, p=0.03). Net N mineralization was positively correlated with HWC and EBC (r=0.46, p<0.001 and r=0.29, p<0.02, respectively).

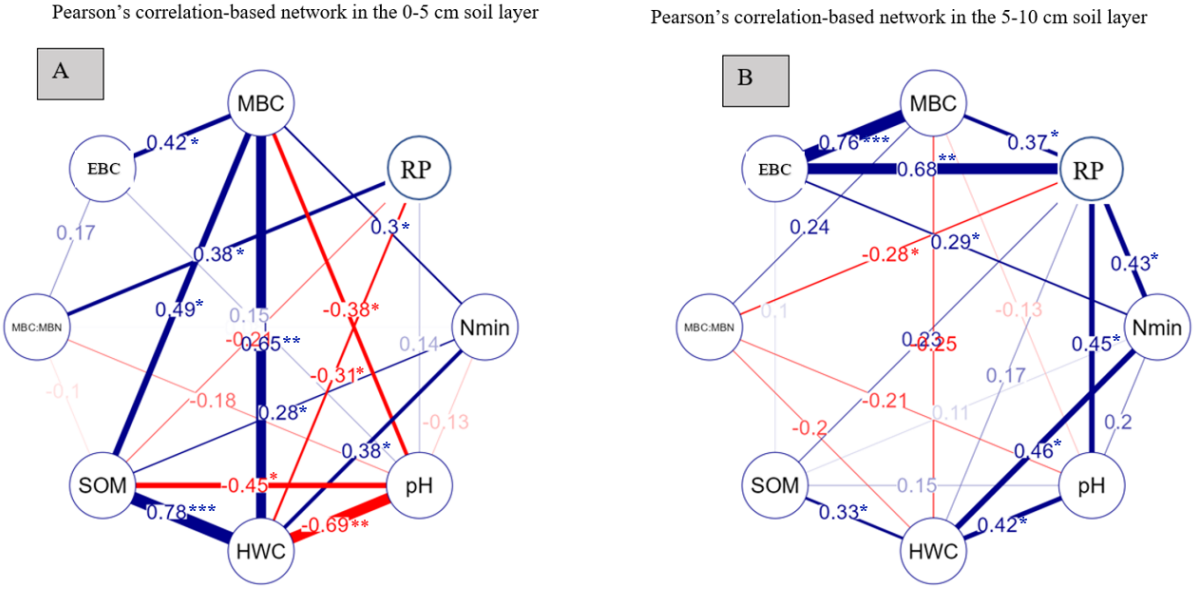

Fig. 3. 4. Pearson's correlation-based network shows the relationship between soil properties and microbial parameters in the upper (A: 0–5 cm) and lower (B: 5–10 cm) soil layers.
Correlation coefficients between parameters are indicated by the values on the path line connecting the variables and the strength of association is indicated by the thickness of the line. Lines' colour indicates the positive (Blue colour) and negative (Red colour) correlations between connected variables. The significance of relationships between variables are indicated by asterisks (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).

Tree species influence on relationships between microbial and chemical soil properties

To explore the relationships between soil microbial biomass and activity (MBC, MBC:MBN, RP, and Nmin) and soil chemical properties (pH, SOM, HWC, and EBC) as a response to tree species in the two soil layers, a principal component analysis (PCA) was performed. The first two principal components explained more than 55.4% of the total variation in the PCA input variables for each soil layer (Fig. 3.5 and Fig. 3.6).

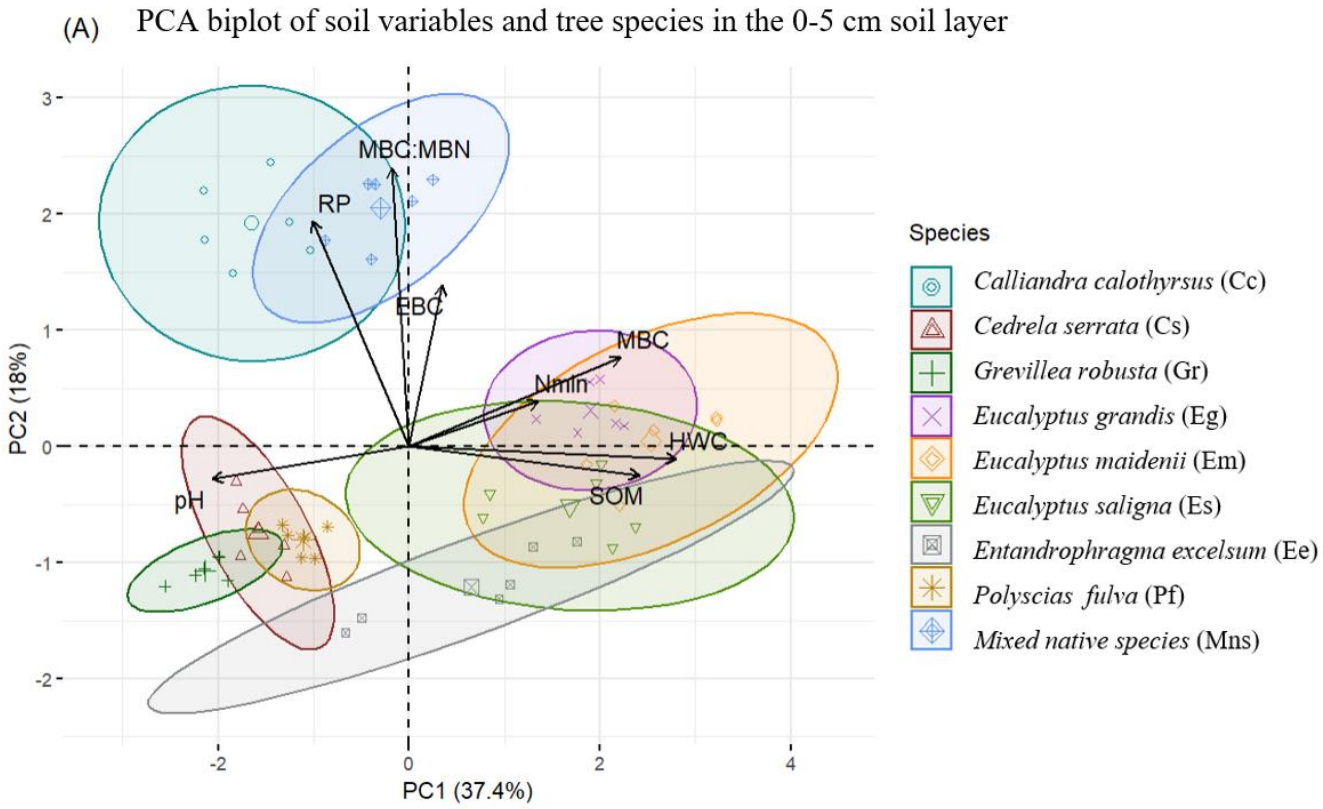

Fig. 3. 5. PCA biplot of soil microbial parameters and chemical properties across tree species for the upper (0–5 cm) soil layer.
The first two principal components explained 55.4% of the combined variation in analysed soil variables. Statistical ellipses at 95% confidence level group tree species (represented by different symbols and colours) based soil variables depicted by vectors (pH; SOM = soil organic matter; EBC = exchangeable basic cations; HWC = hot water–extractable C, MBC = microbial biomass carbon; RP = respiration potential, MBC:MBN = microbial C/N ratio; Nmin = net nitrogen mineralization.

In the 0–5 cm soil layer, the first two principal components explained 55.4% of the total variance (Fig. 3.5). PC1 (37.4%) had strong positive loading with HWC (31%), SOM (23%), MBC (19%), Nmin (8%) with clustering of Eucalyptus species and *Entandrophragma excelsum*, and negative loading with pH (16%) with clustering of species such as *Grevillea robusta*, *Cedrela serrata*, and *Polyscias fulva*. PC2 (PC2=18%) had a strong positive loading with MBC:MBN (46%), RP (30%) and EBC (16%) with a cluster of *Calliandra calothyrsus* and the mixed native species.

In the 5–10 cm soil layer, the total variance explained by the first two PC axes was 57.1% for which the PC1 and PC2 accounted 31.7% and 25.4, respectively. The significant positive contributions of variables to the first PC (Fig. 3.6) were observed for RP (32%),

EBC (21%), and Nmin (15%). MBC (34%) and EBC (19%) loaded positively on PC2, while HWC (21.5%) and pH (12.5%) loaded negatively.

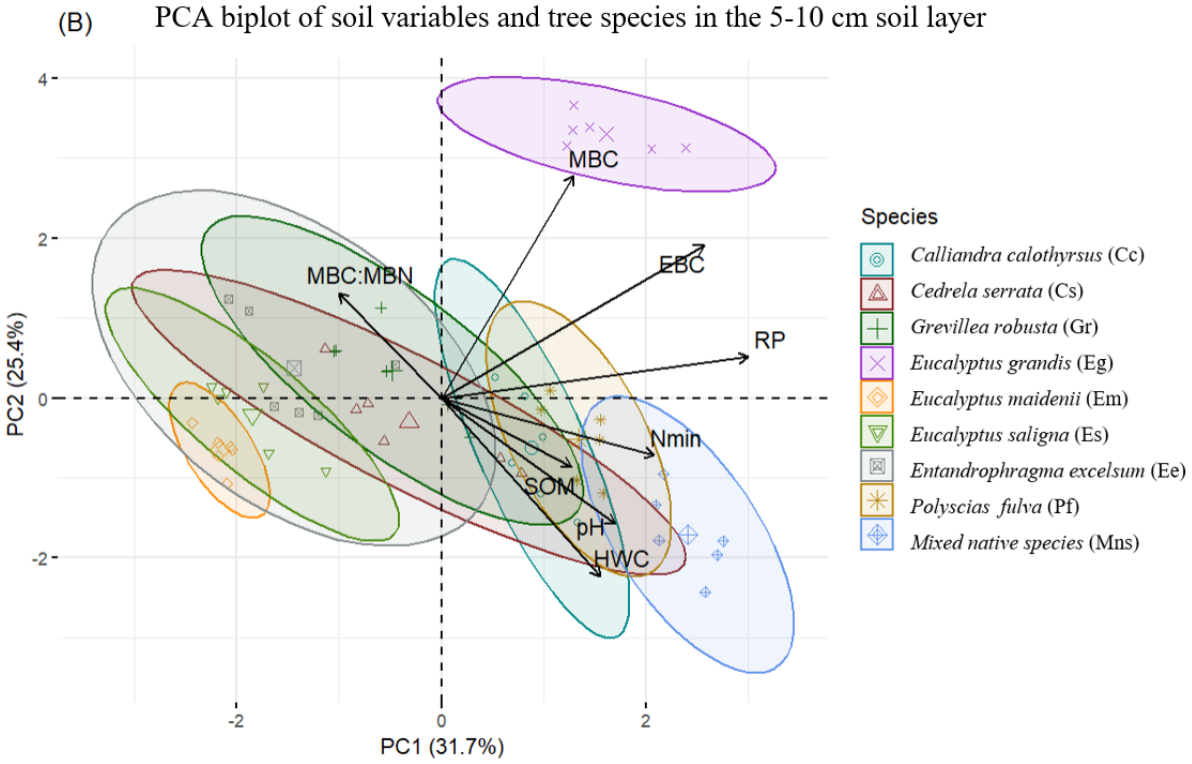

Fig. 3. 6. PCA biplot of soil microbial parameters and chemical properties across tree species for the upper (5–10 cm) soil layer.
The first two principal components explained 57.1% of the combined variation in analysed soil variables. Statistical ellipses at 95% confidence level group tree species (represented by different symbols and colours) based soil variables depicted by vectors (pH; SOM = soil organic matter; EBC = exchangeable basic cations; HWC = hot water–extractable C, MBC = microbial biomass carbon; RP = respiration potential, MBC:MBN = microbial C/N ratio; Nmin = net nitrogen mineralization.

In this soil layer, there were three main tree species clusters across the center origin of the biplot in association with PCA input variables. The first cluster in the right part of the biplot grouped *Calliandra calothyrsus*, *Polyscias fulva* and the mixed native species and these were associated with SOM, pH, HWC, and Nmin. The second cluster associated with MBC discriminated *Eucalyptus grandis* in the upper right quadrant of the biplot, and the remaining species were grouped in the left part of the biplot in association with MBC:MBN.

3.4. Discussion

Understanding the effects of tree species on soil functioning is essential for the selection of suitable tree species in afforestation/reforestation programs (Gere et al., 2022). Microbial parameters may provide an early estimation of changes in biogeochemical processes and an indication of potential changes in soil functioning and nutrient availability for trees (Schloter et al., 2003). In this study, we assessed the effects of 8 tree species, planted minimum of 30 years ago in an arboretum, on microbial processes in two topsoil layers of a tropical soil and their relationships with soil chemical properties. Since the investigated tree species were planted on the same site with similar land use history and climatic conditions, we interpret the observed changes in soil microbial parameters as a result of change in tree species.

3.4.1. The thin upper soil layer dominates microbial properties and soil functions

Highest values of microbial parameters, more significant differences between tree species, and strong relationships between microbial parameters and soil properties were found in the 0–5 cm compared to 5–10 cm soil layer. In the present study, the values of microbial parameters generally fell within the ranges reported in previous studies for tropical forest soils, although we observed relatively greater values in the upper 0–5 cm soil layer compared to previous studies that sampled a thicker (0–10 cm) soil layer. Our findings showed that, on average, microbial biomass C and N in the 0–5 cm layer (1558 mg C kg^{-1} and 218 mg N kg^{-1}, respectively) were in the same order of magnitude, but slightly higher than those measured in tropical forest plantations in the 0–10 cm layer ranging from 836–1135 mg C kg^{-1} and 50–148 mg N kg^{-1}, respectively (Barbhuiya et al., 2004; Temesgen et al., 2019). Values of soil microbial activity parameters of our study, including soil respiration and net N mineralization, as well as eco-physiological indices, were relatively low compared to values in the above-mentioned studies conducted in tropical plantation forests of India and highlands of Ethiopia. Such differences may be due to management practices; our site is less disturbed (no harvest) compared to those forests often harvested for commercial purposes (Groffman et al., 2001). Our findings highlight the importance of this thin (0–5 cm) topsoil layer in nutrient cycling and fertility of tropical soils, where fertility depends heavily on the efficient internal nutrient cycling resulting from the optimal conditions for microbial decomposition of plant litter (Pabst et al.2013; Sayer & Banin, 2016).

3.4.2. Tropical tree species identity effects on soil microbial processes

For sustainable forest management, it is important to predict whether planted trees will improve or adversely affect soil functioning (Bukoski et al., 2022). Our results demonstrated that tree species identity was related to changes in microbial biomass and activity. Given the low values and limited tree species effects in the 5 – 10 cm layer, the following discussion focuses on the upper 0– 5 cm soil layer.

Soil under *Calliandra calothyrsus,* an exotic N_2–fixing agroforestry species showed highest microbial activity, confirming that this species globally improves soil quality (Kisaka et al., 2023). However, it also caused lower MBC compared to other species and higher metabolic quotient, indicating high requirement of microbial maintenance energy as a result of physiological stress (Anderson and Domsch, 2010). The litter of this species might be recalcitrant due to the high content of condensed tannins in the leaves (Temesgen et al., 2019).

Our data suggest that we cannot generalize the effects of Eucalyptus species on soil microbial properties. While plantations of all three Eucalyptus species induced high soil MBC, *Eucalyptus grandis* plantation also resulted in higher MBN, RP, and qmic. Previous results showed high amounts of soil labile C and N, and low pH under this species (Rwibasira et al., 2021). The results from this study showed a significant positive relationship HWC and Nmin but HWC negatively related to RP, which partially contradict the previous studies which reported that soil microbial activity, particularly RP was driven by substrate C availability (Luo & Zhou, 2006; Pietri et al., 2008). Planting *Eucalyptus maidenii* increased Nmin but reduced RP and subsequently the qCO_2. In addition to lowest RP and qCO_2, planting *Eucalyptus saligna* also significantly reduced Nmin. The difference in effects of eucalyptus species may be attributed to their differing litter traits (Table 3.2). *Eucalyptus grandis* has leaf litter with high lignin content (Bini et al., 2013), while *Eucalyptus maidenii* was expected to reduce C and N mineralization rates given its high litter C/N ratios (Demessie et al., 2012; Cizungu et al., 2014).

The plantation of native tree species such as *Entandrophragma excelsum* and *Polyscias fulva* significantly increased soil microbial biomass, particularly MBN. The previous study on same samples showed that these species significantly increased soil pH, while *Polyscias fulva* particularly increased soil exchangeable base cations (Rwibasira et al., 2021). The low RP and qCO_2 with high values in qmic and Nmin also under these native

species are in the same range with the values measured under mixed native species (Mns), indicating high litter quality (Xu et al., 2007), which might reflect an improved soil quality under these species (Bauhus et al., 1998; Chen et al., 2018). We observed no differences between tree species in the rates of relative nitrification (Nit_{rel}), where nitrifying capacity was high (>98%) in soils under all tree species. This may be due to the tropical optimal temperature and moisture that favours the permanently high activity of microbial nitrifiers, implying potential N losses through the leaching of soluble nitrates and soil acidification of these tropical Ferralsols (Binkley and Menyailo, 2005).

In contrast to our hypothesis, we did not observe a reduced soil microbial biomass under Eucalyptus. It was reported in the previous studies that these species often produce allelopathic chemical compounds that are detrimental to soil microorganisms (Naidoo et al., 2014; Zhang et al., 2016). This is likely due to the fact that most of these allelopathic experiments used short-term laboratory germination or early plant growth in pots, which may differ from long-term field conditions (Damptey et al., 2020).

3.4.3. Relating changes in microbial processes to soil properties under tree species

Our results from multivariate and correlation analyses revealed that soil chemical properties were the main driving factors of soil microbial biomass and activity under different tree species. The PCA components and correlation matrix differed between the two upper layers, most likely due to the sharp vertical gradient in tropical soil properties influenced by the availability and quick mineralization of substrate (Cleveland et al., 2003; Gelsomino and Azzellino, 2011).

In the 0–5 cm soil layer, the correlation between hot water-extractable carbon (HWC) and MBC, Nmin, and RP was stronger than the correlation between SOM and the same microbial variables. Our results are consistent with previous studies (Ghani et al., 2003; Ćirić et al., 2016) indicating the higher importance of labile organic carbon compared to total SOM in driving soil biochemical processes (Coca-Salazar et al., 2021a). MBC was negatively related to pH and EBC, suggesting that the microorganisms' biomass may decline when soils acidify (Kunito et al., 2016). However, the results from the present study indicated significantly high MBC under eucalyptus species, despite the known effects of these species for soil acidification. We attribute this increased microbial biomass to (a) a higher amount of available carbon under Eucalyptus species (Rwibasira

et al., 2021) and (b) long-term forest management at this site (>80 years, no periodic harvest or rotation) that may be long enough to allow adaptive responses of soil microorganisms to soil conditions under these tree species (Degens et al., 2000).

PCA clustering also reflected the importance of soil variables such as labile organic pools, SOM concentration, and soil pH in influencing microbial biomass and organic matter mineralization. These results could suggest that the nutrient-rich substrate under species such as *Calliandra calothyrsus* and mixed native species plots could promote high microbial activity. These plots were also characterised by the presence of shrubs and understorey grasses (field observation) which could contribute to the diversity of nutrient inputs (Sayer and Banin, 2016), and previous studies have associated the high MBC:MBN ratios with fungal dominance and/or increased C mineralization under diverse substrate in undisturbed forest soils (Crowther et al., 2019).

In the 5–10 cm soil layer, exchangeable base cations, rather than HWC and/or SOM positively correlated with soil MBC and RP. This contrasts with our hypothesis, as we expected that soil microbial biomass and activity would be mainly related to labile soil carbon. The observed negative correlation between HWC and MBC and a positive correlation between HWC and Nmin may reflect the decreased availability of mineralizable substrate with increasing soil depth (Crowther et al., 2019). In this soil layer, RP was the only microbial parameter significantly correlated to pH. In the previous study on tropical forest soils, Doetterl et al. (2015), reported increasing carbon stabilization with decreasing soil pH in lower soil profiles through reduced respiratory C losses. Although this soil layer (5-10 cm) in the present study cannot be considered as a lower soil profile, it had more acidic soil compared to the 0–5 cm soil layer (Rwibasira et al., 2021). The PCA analysis in this soil layer revealed that species such as *Calliandra calothyrsus*, *Polyscias fulva* and self-regeneration of mixed native species increased EBC, SOM, pH, HWC, which positively loaded on PC1 associated with RP and Nmin. Specifically, the second PCA axis showed a distinctly positive effect of *Eucalyptus grandis* on microbial biomass, again contrasting with our hypothesis regarding the negative effects of planting Eucalyptus species on soil microbial properties. This demonstrated that we cannot generalize the effects of planting Eucalyptus species.

3.5. Conclusion

Understanding the effects of tree species on soil microbial properties and processes can provide important information not only on soil ecological functions but also can guide forest management decisions related to the choice of plantation species. Our results demonstrate that tree species significantly influenced soil microbial biomass, soil respiration and nitrogen transformations. Highest values and pronounced tree species effects in the 0–5 cm compared to 5–10 cm soil layer confirm the importance of this thin upper layer as an active layer and a hotspot of nutrient cycling and fertility of tropical forest soils. The present study also showed that Eucalyptus species were not adversely affecting microbial properties and processes. We suggest planting native species (i.e., *Entandrophragma excelsum*, and *Polyscias fulva*) as they showed potential to improve soil quality compared to eucalyptus and agroforestry species. Considering a relatively high variability of site characteristics in tropical soils, the results from the present study propose that (1) we cannot generalize the effects of Eucalyptus tree species on soils, (2) the differences in microbial activity between tree species would be explained by the availability of labile carbon substrate, and (3) combining analysis of soils microbial and physico-chemical indicators would better guide the policy related to the selection afforestation tree species for sustainable management of forest ecosystems.

Author Contributions

Peter Rwibasira: Conceptualization (equal); investigation (lead); formal analysis (lead); writing–original draft (lead), writing–review and editing (equal), visualization (lead). Donat Nsabimana: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Francois Xavier Naramabuye: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Monique Carnol: Conceptualization (equal); formal analysis (supporting); writing–original draft (equal); writing–review and editing (equal); resources (lead); funding acquisition (equal); project administration (lead); supervision (lead).

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CHAPTER 4 – Ammonia-oxidizing archaea are the main nitrifiers under different tree species in a tropical forest plantation, southern Rwanda

Chapter 4 - Ammonia-oxidizing archaea are the main nitrifiers under different tree species in a tropical forest plantation, Southern Rwanda

Adapted from: Rwibasira, P.; Naramabuye, F.X.; Nsabimana, D.; Carnol, M. Ammonia-oxidizing archaea are the main nitrifiers under different tree species in a tropical forest plantation, Southern Rwanda (Manuscript).

Abstract

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) naturally coexist in terrestrial ecosystems and play a major role in the global nitrogen cycle. Their abundance and relative contribution to soil nitrification and the influence of tree species remain less understood, especially in acidic tropical soils. We sampled two topsoil layers (0–5 cm and 5–10 cm) to investigate the long-term effects (>30 years) of eight tree species on the abundance of AOA and AOB and their contribution to soil nitrification, using quantitative PCR targeting the *amoA* gene and measuring potential nitrification rates (PNR). Significantly higher PNR and copy numbers of AOB *amoA* genes, as well as greater variation between species were observed in the upper compared to lower soil layer, confirming higher microbial activity and the importance of this thin layer for the fertility of tropical forest soils. We found a dominance of AOA over AOB in terms of abundance and contribution to nitrification activity across tree species, suggesting that soil acidity and low ammonia concentrations might have given a competitive advantage to AOA. Gene copy numbers of *amoA* showed a significant positive correlation with AOA activity and a negative relation with AOB activity in the 0–5 cm soil layer. Planting *Polyscias fulva*, *Eucalyptus grandis*, *Grevillea robusta*, and *Cedrela serrata* significantly increased the rate of both AOA and AOB nitrification. Tree species significantly influenced abundance of AOA and AOB and their nitrification rates via changes in soil pH and supply of N substrates.

Keywords: *Nitrification*; *ammonia-oxidizing archaea (AOA) and bacteria (AOB)*; *amoA gene*; *Tropical soils*, *tree species*; *Ruhande arboretum*; *Rwanda*.

4.1. Introduction

Ammonia oxidation is the first, rate-limiting, step of the nitrification process which significantly contribute to the global biogeochemical cycle of nitrogen (Kowalchuk & Stephen, 2001; Stein, 2019). Nitrification by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) is performed through two microbially driven processes consisting of ammonia (NH_3) oxidation to nitrate (NO_3^-) via nitrite (NO_2^-), though the recently discovered comammox bacteria can complete ammonia oxidation within a single organism (Dimitri Kits et al., 2017; Jung et al., 2022). Nitrogen is a vital component of all forms of life, and it is often the major growth–limiting macronutrient in terrestrial ecosystems (Maathuis & Diatloff, 2013; Kuypers et al., 2018). The availability of plant-available nitrogen forms (e.g., $NO_3^-$and NH_4^+) is limited (Gruber and Galloway, 2008), and (AOB) and archaea (AOA) play an important role in the biogeochemical cycle of nitrogen, controlling the form of available N, as well as N losses through their nitrifying activity (Laffite et al., 2020; Jung et al., 2022).

Many studies have used ammonia monooxygenase subunit A (*amoA*) as a functional and phylogenetic marker gene which is shared by AOA and AOB (Prosser and Nicol, 2008), and the assessment of *amoA* gene abundance was suggested as an important measure to characterize and quantify these ammonia-oxidizers in terrestrial ecosystems (Rotthauwe et al., 1997; Zeglin et al., 2011). While AOA and AOB naturally coexist in the environment, they differ in their ecological niches (Hink et al., 2018). Quantifications of the *amoA* gene indicate that AOA genes often numerically predominate over AOB in soil (Leininger et al., 2006; Chen et al., 2008), that they exhibit a greater tolerance to soil acidity and require lower amounts of ammonia, thus suggesting a greater contribution to nitrification compared to AOB in acid and N poor ecosystems (Nicol et al., 2008; He et al., 2012; Trivedi et al., 2019). It was reported that the abundance of ammonia oxidizers, rather than their species diversity, could explain changes in nitrification rates (Hou et al., 2013). However, other studies showed that, despite the dominance of archaeal *amoA* gene abundance, AOB were more important than AOA in driving soil nitrification (Sterngren et al., 2015). This shows that the abundance of ammonia-oxidizing microorganisms may not always reflect the high rates of soil nitrification process. Thus, it is critical to explore not only the relationship between gross nitrification rates and amoA, but also the distinctive role of AOA and AOB in nitrification rates as related to their abundance.

Vegetation types may differently affect many physico-chemical and microbial soil properties and processes (Mueller et al., 2012; Zhao et al., 2022). For example, the decomposition of plant litter can alter the concentration of soil nutrients, toxic elements, as well as cause a change of soil pH, which in turn may influence the composition and activity of soil microorganisms (Pajares and Bohannan, 2016). While N mineralization controls nitrogen substrates availability for plants and microorganisms in terrestrial ecosystems (Rütting et al., 2021; Fan et al., 2022) (Rütting et al., 2021), labile N fractions especially those extracted with hot water were also reported to be more closely linked to N cycling compared to the total organic N pools (Curtin et al., 2021; Haynes, 2005; Wang et al., 2008). Thus, both nitrogen mineralization (Nmin) and total hot water-extractable nitrogen (HWNtot) could be considered as proxies for N substrate availability for microorganisms involved in N cycling processes.

Tree species may influence soil nitrification directly through altering input of elements that in turn can affect availability of substrate for soil microorganisms (Ribbons et al., 2018b) or releasing substances that may inhibit growth and/or activity of nitrifiers (Lehtovirta-Morley et al., 2013; Laffite et al., 2020). Further, some native tree species might also recruit diverse types of microorganisms which are specific to certain environmental conditions and can simultaneously perform multiple ecological processes including N cycling (Shu and Huang, 2022). Despite that many studies have reported that the choice of planted tree species may strongly influence soil processes, knowledge on how tree species and environmental conditions influence the composition and activity of soil microorganisms carrying out nitrogen cycling processes remains scarce (Nelson et al., 2016). Particularly, it is still unclear how tree species affect the abundance and activity of AOA and AOB, especially in acidic tropical soils.

In Rwanda, about 724,695 ha (30.4%) of the land is forested; planted forests occupy 387,425 ha (16.2%) and are dominated by eucalyptus species, accounting for 89% of the forest plantations (IUCN, 2020). Although the target to reach 30% of the country's land covered by forests was reached in 2019 (MoE, 2019a), the campaigns to plant more forests are continuing because forests are not diversified and/or unevenly distributed across the country. Furthermore, harvested forests should be replaced to maintain the desired of forest cover (Rwanda forest authority; RFA, 2021). However, it remains

unclear how different tree species may affect soil processes and soil quality in Rwanda, especially in regard to introduced exotic species.

The general objective of this study was to assess the contribution of AOA and AOB to the nitrification process in acidic tropical forest soils. Specifically, we addressed the effects of tree species on the activity of AOB and AOA through the measurement of potential nitrification rates, as well as on their abundance through the measurement of bacterial and archaeal amoA gene. Given the role of soil pH in influencing abundance and activity of AOA and AOB, we hypothesized that (1) AOA would be more abundant and contribute most to the nitrification rates under acidifying Eucalyptus species, (2) AOB abundance and activity would be relatively more important in less acid soils under monospecific native tree species and self-regenerated native species.

4.2. Materials and Methods

4.2.1. Study site

The study was conducted at the Arboretum of Ruhande (2°36' S, 29°44' E), located in the Huye District, Southern Province of Rwanda (Fig. 1.5). The site has a humid tropical climate characterized by a mean annual air temperature between 17.5 °C and 19 °C, and approximately 1230 mm rainfall with a bimodal distribution pattern of two rainy seasons (heavy rains: March-May; and irregular rains: September–December) and two dry seasons (short: January–February; and long: June–August) (Meteorwanda, 2021). The soil is characterized by a brown-red colour with a sandy loam texture and diffuse horizons, classified as Ferralsols or Oxisols according to FAO and USDA Soil Taxonomy, respectively (Verdoodt et al., 2006). The site was established in 1933 on the cultivated flat plateau of Ruhande hill (1638–1737 m asl), where a typical traditional organic cropping system had been used. Its area has progressively increased to reach the current size of 200 ha (Nsabimana et al., 2008). The site currently contains 204 tree species, with 144 deciduous, 57 conifer, and 3 bamboo species planted on 477 plots (50m x 50m) of replicated monoculture stands. Among all species, exotic trees represent 84% (172 species of which 69 are eucalyptus species), while native species represent 13% (32 species) (RFA, 2021). All plots are regularly managed by the removal of invading vegetation and planting young trees in replacement of dead plants to maintain a constant density of the initial tree species in the plots, except on an undisturbed plot (4 ha) of self-

regenerated mixed native species (Mns). No fertilization, weed control treatments, clear-cut, or fire events have occurred since the establishment of the site.

4.2.2. Soil sampling and analyses

Based on the use and species adaptability in different regions of the country (Iiyama et al., 2018), eight species were selected (Figure 2.1). These included three Eucalyptus species (*Eucalyptus grandis*, *Eucalyptus maidenii*, and *Eucalyptus saligna*), three agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*), two native species (*Entandrophragma excelsum* and *Polyscias fulva*), and a self-regenerated plot of native forest (Mixed native species = Mns). Three plots per species were selected and each plot was divided into two sub-plots (25 × 50 m). One composite soil sample was taken in each sub-plot for two soil layers (0-5 cm and 5-10 cm) by mixing five soil samples (X-shaped sampling) collected by using a 30x30 cm frame and a shovel under the tree's canopy at 1–1.5 m from the tree base (Bini et al., 2013). Soils were sieved fresh on sterilized sieves (4 mm) and stored at 4 °C until analysis. A sub-sample was freeze-dried and stored at -20°C for molecular analyses. Detailed soil characteristics of the studied plots can be found in Rwibasira et al. (2021). For molecular analyses, soil of one subplot only was analysed.

Potential nitrification rates

Potential nitrification rates (PNR) were determined using the shaken soil slurry method (Hart et al., 1994), with and without addition of 80 µM of allylthiourea (ATU), which selectively inhibits the AOB nitrification (Taylor et al., 2010). PNR from soil slurry without ATU reflects the total PNR while ATU-added slurry serves to determine archaeal PNR (AOA–PNR). The bacterial nitrification rate was calculated as the difference between the total and archaeal nitrification rates. The soil slurry,10 g soil added to 100 ml of a buffered solution (1 mM PO_4^{3-}, 1.5 mM NH_4^+, and pH 7.2), was shaken (180 rpm) at 20 ^oC in the dark. Samples (15 ml) of homogeneous soil slurries were taken after 2, 5, 23, and 26 hours of shaking, filtered (Whatmann $595^{1/2}$ filter paper) and kept at –20 °C until analysis. The concentration of extracted nitrate was determined colorimetrically using a flow autoanalyzer (BranLuebbe, SPX Process Equipment, Germany) and PNR was calculated by linear regression of NO_3–N concentrations over time.

Abundance of bacterial and archaeal *amoA* genes

DNA extraction was performed from 0.25 g freeze-dried soil, using the DNeasy PowerSoil Pro kit (QIAGEN) in accordance with the manufacturer's protocols, and the DNA extract was stored at −20°C until use. The concentration and purity of extracted DNA were measured using a Qubit™ fluorometer (Invitrogen). To determine the abundance of nitrifying bacteria and archaea, we used quantitative PCR targeting *amo*A gene, a functional gene encoding for ammonia monooxygenase subunit A. Primer pairs *amo*A_1F (5' -GGGGTTTCTACTGGTGGT -3') and *amo*A_2R (5'- CCCCTCKGSAAAGCCTTCTTC -3') (Rotthauwe et al., 1997) were used for bacterial *amo*A, while primer pairs CrenamoA23f (5'- ATGGTCTGGCTWAGACG -3') and CrenamoA616r (5'- GCCATCCATCTGTATGTCCA -3') (Tourna et al., 2008) were used for archaeal *amo*A. The final reaction mixture (20 µl) contained: 0.5 µM of amoA_1F and 0.5 µM of amoA_2R for AOB or 0.75 µM of CrenamoA616r and 1 µM of CrenamoA23f for AOA, 2% bovine serum albumin (BSA), 1X of QuantiTect SybrGreen PCR Master Mix (Qiagen, Courtaboeuf, France) and 10 ng of soil-extracted DNA. Upon confirmation by melting curve analysis for efficient amplification of AOB-*amo*A and AOA-*amo*A, all samples were run in duplicate on a Lightcycler 480 (Roche Diagnostics, Meylan, France). The thermal cycling conditions for bacterial *amo*A were: 15 min at 95 °C, followed by 45 cycles (denaturation at 95 °C for 30 s, annealing at 54 °C for 45 s, extension at 72 °C for 45 s and 80 °C for 15 s) and 30 s at 40 °C. For archaeal *amo*A, the amplification was run for 15 min at 95 °C, followed by 50 amplification cycles (denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s) and 10 s at 40 °C.

Soil pH, labile N fractions, and net N mineralization

Soil pH was determined in a soil solution with 1 M KCl (1:2.5 w/v), using a pH meter (HI2550, HANNA® Instruments, USA) as described by Allen (1989). Water-extractable N fractions were determined using the method of Ghani et al. (2003). Fresh soil was extracted with distilled water (1:6, *w*/*v*), shaken (120 rpm, 30 min), centrifuged (4000 rpm, 10 min), and filtered (Whatman #42), representing water-soluble N (WSN) fractions (data not presented). Hot water-extractable N (HWN) were subsequently extracted from the remaining wet soil, mixed with distilled water (30 ml), and placed in the oven for 16 h at 80 °C. Total nitrogen in extracts was measured colorimetrically using a continuous

flow autoanalyzer equipped with a UV digestor (Autoanalyser3, BranLuebbe, Germany). Net nitrogen mineralization (Nmin) was determined according to Hart et al. (1994), from 15 g of fresh soil adjusted to 60% water holding capacity and incubated at 20 °C for 28 days. Extraction of inorganic nitrogen ($NH4^+$–N and $NO3^-$–N) was performed on sub-samples at the beginning and the end of the incubation period using 1 M KCl (1:5; w:v), after 1 h agitation at 180 rpm and centrifugation at 4000 rpm (Allen, 1989). The water loss during incubation was monitored gravimetrically and compensated by adding distilled water as necessary. Extracts were analysed colorimetrically using a continuous flow analyser equipped with a UV digestor (AutoAnalyser3, BranLuebbe, Germany). Net nitrogen mineralization rate (Nmin) was calculated as the ratio between the net increase in inorganic nitrogen and the number of incubation days.

4.2.3. Statistical analyses

Effects of Species (9 levels) and soil layer (two levels: 0–5 cm and 5–10 cm) as well as their interactions on measured soil variables were analysed using mixed effects models using the R Statistical language (version 4.3.1; R Core Team, 2023). Species, Layer, and their interactions were included as fixed effects, while the Plot (27 plots) were included as random effects to account for the non-independence of the two soil samples collected within the same plot. We fitted a linear mixed model with lmerTest (version 3.1.3; Kuznetsova et al., 2017), estimated using REML and nloptwrap optimizer, with random intercept:

response ~ 1 + Species + Layer + Species*Layer + (1|Plot)

The assumptions of normality and homoscedasticity of the residuals were assessed by visual inspection of the q-q plots and plots of the normalized residuals against the fitted values (Zuur et al., 2010). Conditional (R^2c; total variance explained by the model), and marginal (R^2m; variance explained by the fixed effects) coefficients of determination were calculated using MuMIn (version 1.47.5; Barton, 2020). F-test analysis of variance for fixed-effect terms used Satterthwaite and Kenward-Roger methods (Kuznetsova et al., 2017). Estimated means were calculated and pairwise comparisons were performed emmeans (version 1.8.7; Lenth et al., 2018), according to recommendations for non-interacting or interacting factors. Plots were performed according to Jeffrey (2018). The data of amoA gene copy numbers, amoA–AOA:amoA–AOB, and specific nitrification (i.e., PNR/amoA) were log–transformed to meet normality and homoscedasticity

assumptions. We performed correlational analysis (Pearson) using *Corrr* package, version 0.4.4 (Kuhn et al. 2020) to determine the relationships between soil variables (i.e., pH, Nmin, and HWNtot) considered to as key drivers of AOA's and AOB's PNR and amoA. We also performed PCA using *FactoMineR*, version 2.8 (Husson et al. 2020) and *ggplot2*, version 3.4.2 (Wickham et al. 2022) packages to explore and visualize relationships between selected soil variables (i.e., pH, HWNtot, and Nmin) and tree species in both soil layers separately.

4.3. Results

4.3.1. Effects of tree species on abundance and activity of AOA and AOB

Abundance and activity of AOA and AOB were significantly influenced by tree species and soil layers (Table 4.1). Bacterial *amoA* gene abundance was significantly higher in the upper (0–5 cm) compared to the lower (5–10 cm) soil layer under all tree species, ranging from 2.51×10^4 to 6.5×10^6 and 3.08×10^3 to 6.9×10^5 gene copies g^{-1}, respectively (Fig. 4.1, Table 4A.1).

Table 4. 1. Results of linear mixed-effects modelling to predict the effects of tree species and soil layer on AOA and AOB nitrification activity and abundance of amoA genes.

Variables	**Tree species (S)**		**Soil layer (L)**		**Interaction of Species and Layer (S * L)**		**R squared**	
	F-statistics	P-value	F-statistics	P-value	F-statistics	P-value	R^2m	R^2c
AOA–PNR (mg $N–NO_3$ kg^{-1} d^{-1})	119.174	5.38e-14 ***	4933.468	< 2.2e-16 ***	68.113	< 2.2e-16 ***	0.983	0.983
AOB–PNR (mg $N–NO_3$ kg^{-1} d^{-1})	52.538	< 2.2e-16 ***	134.971	< 2.2e-16 ***	15.913	2.16e-14 ***	0.786	0.803
PNRtot (mg $N–NO_3$ kg^{-1} d^{-1})	143.113	< 2.2e-16 ***	1829.469	< 2.2e-16 ***	30.321	< 2.2e-16 ***	0.957	0.960
AOA–*amo*A (*amo*A gene copies g^{-1})	1.965	0.111	31.493	3.505e-07 ***	3.541	0.00165 **	0.384	0.812
AOB–*amo*A (*amo*A gene copies g^{-1})	5.562	0.0012 **	970.818	< 2.2e-16 ***	13.367	1.203e-11 ***	0.825	0.939
AOA–PNR:AOB–PNR	37.144	< 2.2e-16 ***	152.242	< 2.2e-16 ***	22.216	< 2.2e-16 ***	0.854	0.854
AOA–*amo*A:AOB–*amo*A	3.554	0.012*	493.805	< 2.2e-16 ***	9.277	1.086e-08 ***	0.744	0.895
AOA–PNR:*amoA*-AOA (specific AOA-PNR)	1.92	0.119	162.84	< 2.2e-16 ***	6.71	1.551e-06 ***	0.514	0.851
AOB–PNR: *amoA*-AOB (specific AOB-PNR)	10.48	2.192e-05 ***	285.16	< 2.2e-16 ***	8.253	7.313e-08 ***	0.780	0.90

AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; AOA–PNR: AOA's potential nitrification rates; AOB–PNR: AOB's potential nitrification rates; PNRtot: total potential nitrification rates; *amo*A: ammonia monooxygenase gene (subunit A); AOA–*amo*A: AOA's gene abundance; AOB–amoA: AOB's gene abundance.

In the upper (0–5 cm) soil layer, we observed significantly higher values of AOB–*amoA* under agroforestry tree species, such as *Grevillea robusta*, *Calliandra calothyrsus* and *Cedrela serrata,* while values were lowest under *Eucalyptus saligna*. In the lower (5–10 cm) soil layer, the number of AOB–*amoA* genes copies was significantly higher under mixed native species and agroforestry species, compared to eucalyptus species (Fig. 4.1).

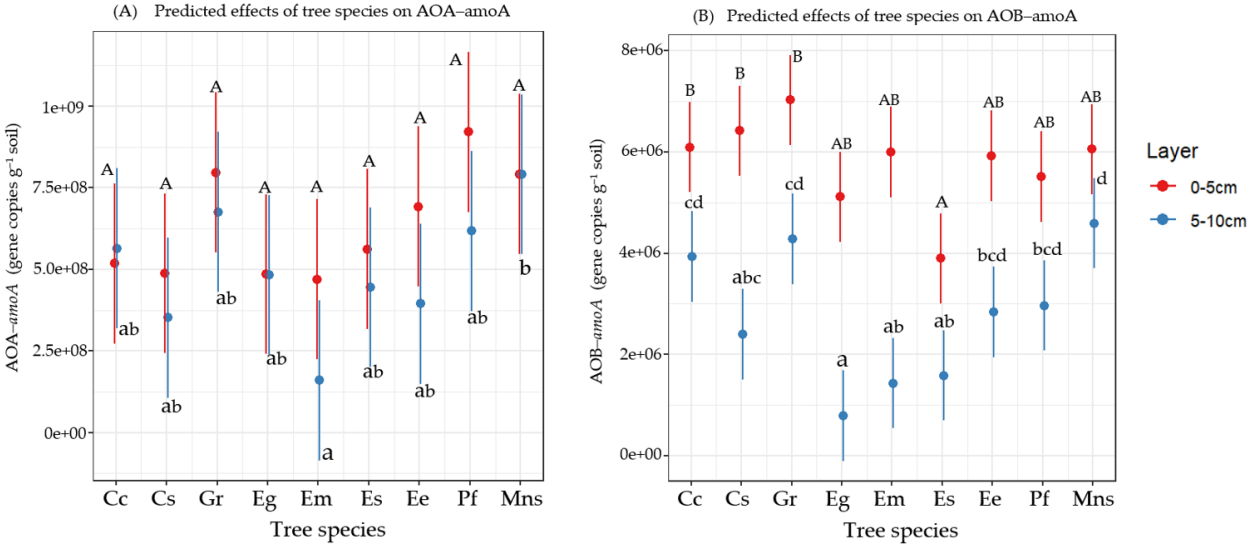

Fig. 4. 1. Predicted effects of tree species and soil layer on amoA gene abundance for AOA and AOB.

Figure 2. Predicted effects of tree species and soil layer on amoA gene abundance for AOA and AOB. The model was: response ~ 1 + Species + Layer + Species*Layer + (1|Plot), where Plot is the Plot ID. Red circles indicate mean values of samples in the 0–5 cm soil layer and blue circles indicate samples in the 5–10 cm layer. Capital letters indicate significant differences (p-value < 0.05, Tukey tests) between tree species in the 0–5 cm layer and small letters indicate significant differences for the 5–10 cm layer. AOA-amoA: amoA gene copies for AOA; AOB-amoA: amoA gene copies for AOB; *Tree species: Cc= Calliandra calothyrsus; Cs= Cedrela serrata; Gr= Grevillea robusta; Eg= Eucalyptus grandis; Em= Eucalyptus maidenii; Es= Eucalyptus saligna; Ee= Entandrophragma excelsum; Pf= Polyscias fulva;* and Mns= mixed native species.

Real time qPCR amplification yielded 5.35×10^7 to 1.24×10^9 and 2.86×10^6 to 1.08×10^9 of AOA–*amoA* gene copies g^{-1} in the 0–5 cm and 5–10 cm soil layers, respectively (Table A4). There were no significant differences of AOA–*amoA* gene copies between tree species in the upper soil layer. In the 5–10 cm soil layer, highest values of AOA–*amoA* were observed under mixed native species (Mns) and lowest AOA–*amoA* values were observed under *Eucalyptus* (Fig. 4.1).

Potential nitrification rates of AOB (AOB–PNR) ranged from 0.1 to 1.3 and 0.01 to 0.6 mg $N–NO_3$ kg^{-1} d^{-1} in the 0–5 cm and 5–10 cm soil layers, respectively (Table 4A.1). In 0–5 cm soil layer AOB–PNR was significantly higher under *Polyscias fulva*, *Eucalyptus grandis*,

and *Eucalyptus saligna*, and lower under *Calliandra calothyrsus*, *Eucalyptus maidenii* and *Entandrophragma excelsum* (Fig. 4.2). In the 5–10 cm soil layer, the highest AOB–PNR values were found under *Calliandra calothyrsus*, *Polyscias fulva*, and *Eucalyptus saligna*; while species including *Grevillea robusta*, *Eucalyptus maidenii*, and *Entandrophragma excelsum* showed lowest AOB–PNR (Fig. 4.2).

Archaeal potential nitrification rate (AOA–PNR) ranged from 0.4 to 2.5 and 0.1 to 0.4 mg $N–NO_3$ kg^{-1} d^{-1} in 0–5 cm and 5–10 cm soil layers, respectively (Table 4A.1, Fig. 4.2). In 0–5 cm soil layer, AOA–PNR was significantly higher under species such as *Polyscias fulva*, *Eucalyptus grandis*, *Grevillea robusta* and mixed native species, and significantly lower under eucalyptus species AOA–PNR (Fig. 4.2-A). In the 5–10 cm soil layer, AOA–PNR was significantly higher under *Eucalyptus grandis* followed by *Calliandra calothyrsus* and *Grevillea robusta*, and lowest under mixed native species and *Cedrela serrata* (Fig. 4.2-B).

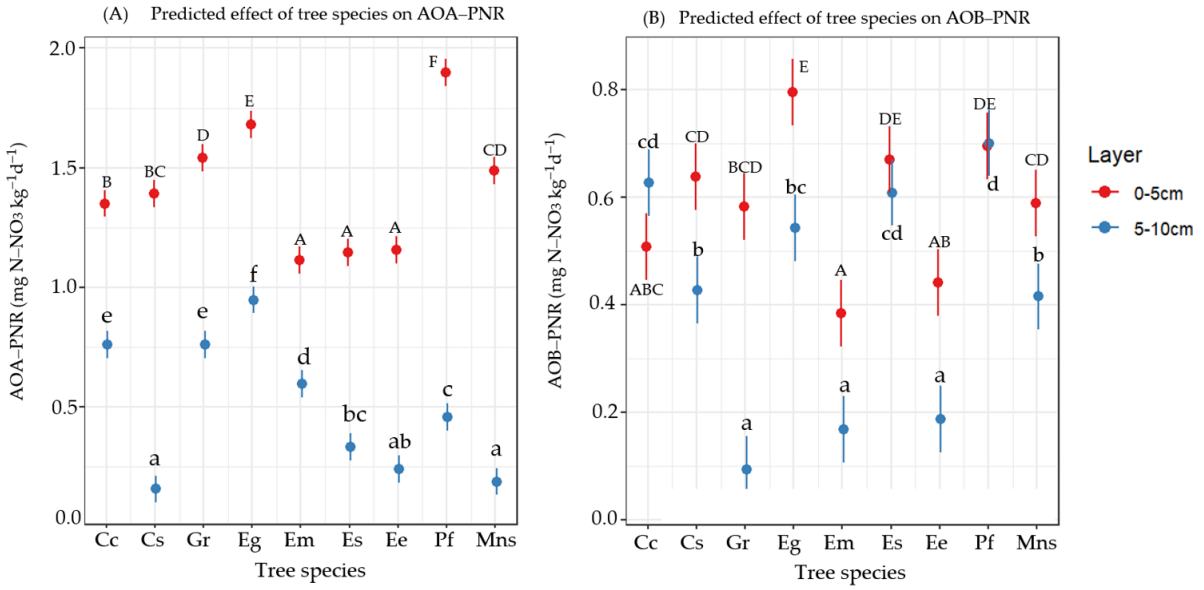

Fig. 4. 2. Predicted effects of tree species and soil layer on AOA and AOB potential nitrification rates.

The model is: response ~ 1 + Species + Layer + Species*Layer + (1|Plot), where Plot is the Plot ID. Red circles indicate mean values of samples at 0–5 cm soil layer and blue circles indicate samples at 5–10 cm. Capital and small letters indicate significant differences (p-value < 0.05, Tukey tests) between tree species at 0–5 cm and 5–10 cm, respectively. AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; AOA–PNR: AOA's potential nitrification rates; AOB–PNR: AOB's potential nitrification rates; Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species.

Table 4. 2. Potential nitrification rates (Means±SEM, n=6) and contribution of AOA and AOB (%) to total potential nitrification rates under different tree species.

Tree species	Potential nitrification rates (PNR; mg $N–NO_3$ kg^{-1} d^{-1})			Contribution to total PNR (%)	
	AOA–PNR	AOB–PNR	Total PNR	AOA	AOB
Potential nitrification rates in 0 – 5 cm soil layer					
Calliandra calothyrsus	0.742±0.01	0.269±0.06	1.01±0.07	73.4	26.6
Cedrela serrata	0.82±0.07	0.488±0.08	1.31±0.12	62.7	37.3
Grevillea robusta	1.11±0.09	0.358±0.04	1.47±0.06	75.6	24.4
Eucalyptus grandis	1.45±0.10	1±0.09	2.45±0.15	59.2	40.8
Eucalyptus maidenii	0.463±0.02	0.15±0.04	0.613±0.04	75.5	24.5
Eucalyptus saligna	0.498±0.03	0.539±0.05	1.037±0.06	48.0	52.0
Entandrophragma excelsum	0.505±0.02	0.187±0.04	0.692±0.03	73.0	27.0
Polyscias fulva	2.22±0.08	0.64±0.10	2.86±0.07	77.6	22.4
Mixed native species	0.979±0.02	0.387±0.07	1.366±0.06	71.7	28.3
Potential nitrification rates in 5 – 10 cm soil layer					
Calliandra calothyrsus	0.229±0.01	0.429±0.03	0.658±0.03	34.8	65.2
Cedrela serrata	0.068±0.00	0.158±0.01	0.226±0.01	30.1	69.9
Grevillea robusta	0.227±0.00	0.031±0.00	0.259±0.00	88.0	12.0
Eucalyptus grandis	0.332±0.01	0.279±0.00	0.61±0.01	54.3	45.7
Eucalyptus maidenii	0.168±0.01	0.043±0.00	0.211±0.02	79.6	20.4
Eucalyptus saligna	0.096±0.00	0.39±0.02	0.486±0.02	19.8	80.2
Entandrophragma excelsum	0.081±0.00	0.051±0.01	0.132±0.01	61.4	38.6
Polyscias fulva	0.125±0.00	0.612±0.01	0.737±0.01	17.0	83.0
Mixed native species	0.072±0.00	0.148±0.01	0.221±0.00	32.7	67.3

AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; AOA–PNR: AOA's potential nitrification rates; AOB–PNR: AOB's potential nitrification rates; PNRtot: total potential nitrification rates.

The total PNR ranged from 0.5 to 3. 1 and 0.1 to 0.8 mg $N–NO_3^-$ kg^{-1} d^{-1} in the 0–5 cm and 5–10 cm soil layers, respectively. Total PNR was significantly higher under species including *Polyscias fulva* and *Eucalyptus grandis*, and lower under species such as *Eucalyptus maidenii* and *Entandrophragma excelsum*, both 0–5 cm and soil 5–10 cm layers (Fig. 4.3). The contribution of AOA and AOB to the total potential nitrification rates only differed between tree species in the 0–5 cm soil layer. On average, AOA and AOB contributed about 71% and 29% of the total potential nitrification rates, respectively (Table 4.2).

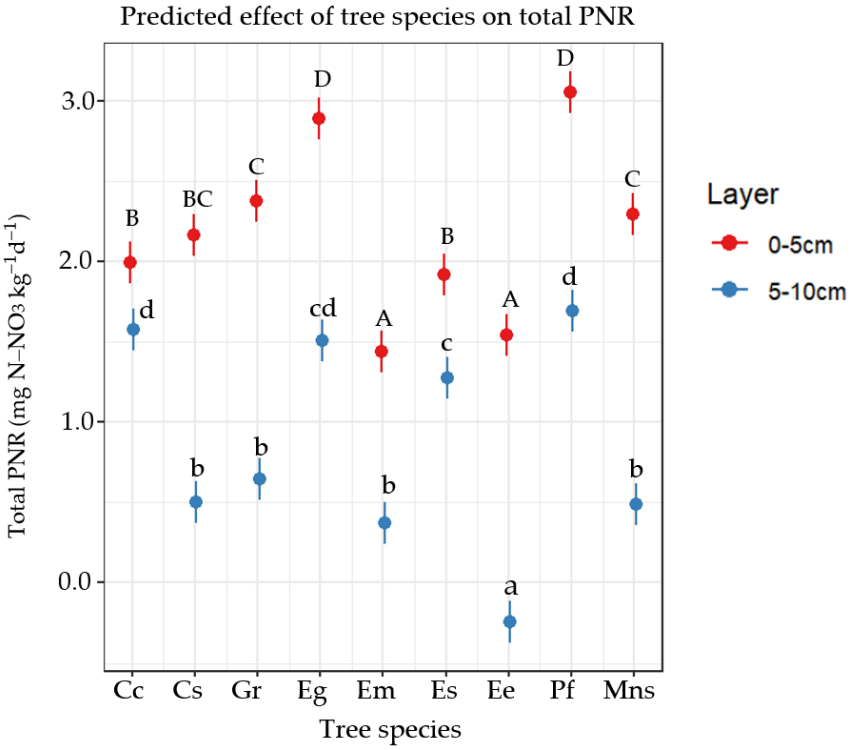

Fig. 4. 3. Predicted effects of tree species and soil layer on total potential nitrification rates. The model is: response ~ 1 + Species + Layer + Species*Layer + (1|Plot), where Plot is the Plot ID. Red circles indicate mean values of samples at 0–5 cm soil layer and blue circles indicate samples at 5–10 cm. Capital and small letters indicate significant differences (p-value < 0.05, Tukey tests) between tree species at 0–5 cm and 5–10 cm, respectively. Total PNR: total potential nitrification rates; Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species.

4.3.2. Relationship between amoA gene abundance and potential nitrification rates

Soil potential nitrification rates were significantly correlated to *amoA* gene abundance of AOA and AOB in the 0–5 cm soil layer only (Fig. 4.4 and 4.5). A strong positive correlation was found for archaea (Fig. 4.4–A), while a negative correlation was found for bacteria (Fig. 4.4–B).

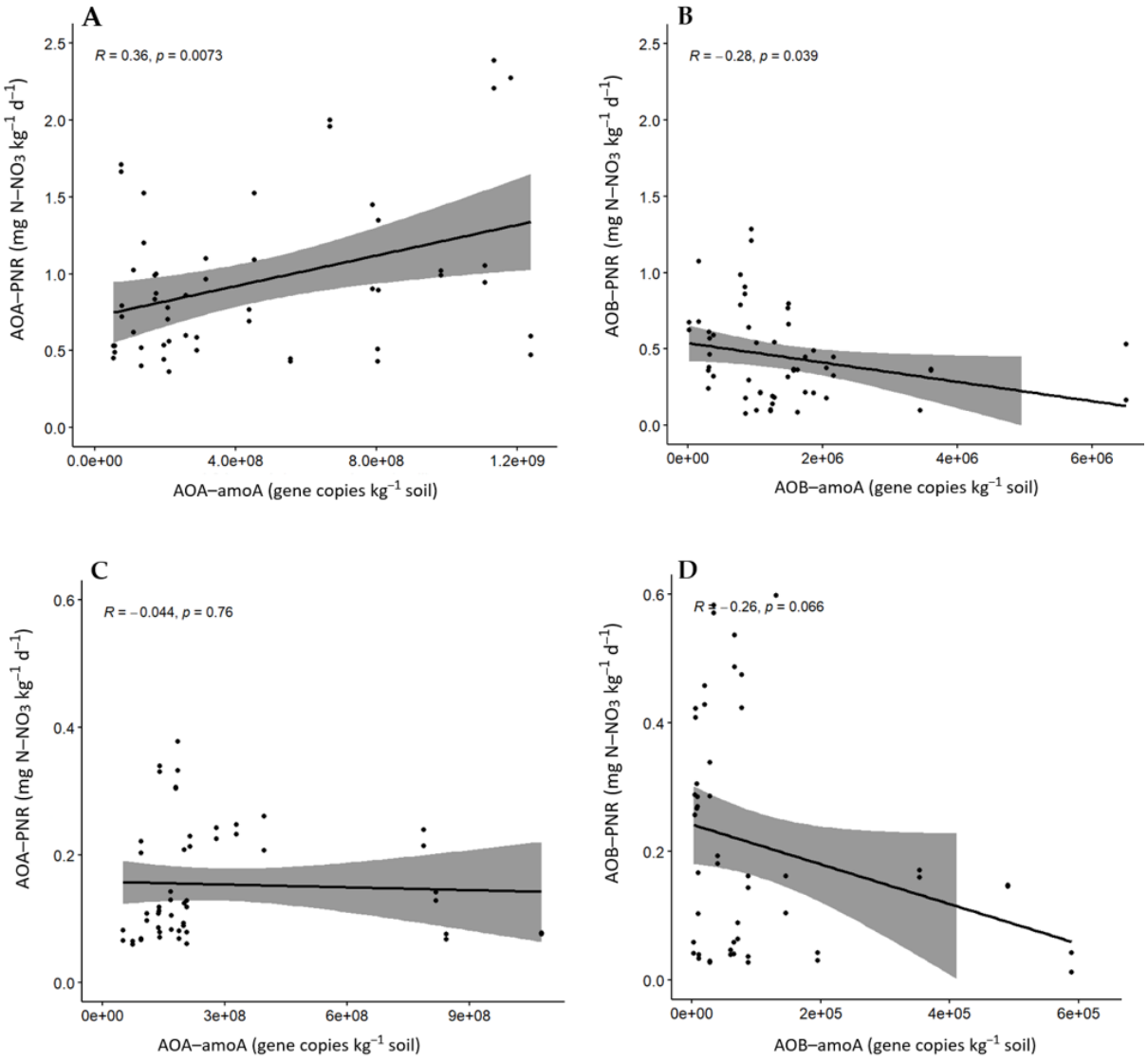

Fig. 4. 4. Relationships between soil potential nitrification rates and AOB and AOA abundance in 0–5 cm and 5–10 cm layers.
AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; AOA–PNR: AOA's potential nitrification rates; AOB–PNR: AOB's potential nitrification rates; amoA: ammonia monooxygenase gene (subunit A); AOA–amoA: amoA gene abundance for AOA; AOB–amoA: amoA gene abundance for AOB.

Specific nitrification rates (potential nitrification rates per unit *amoA* gene copies) were significantly influenced by tree species and soil layers (Fig. 4.5). AOA specific nitrification rates were significantly higher in the upper 0–5 cm compared to the 5–10 cm soil layer, and highest under *Calliandra calothyrsus* and *Eucalyptus grandis* in both soil layers. In the upper layer archaeal specific nitrification rates were lowest under *Eucalyptus maidenii*, while in the lower layer, they were lowest under mixed native species (Fig. 4.5-A). In contrast, AOB specific nitrification rates were lower in upper compared to the lower soil layer. On both layers, they were highest under *Eucalyptus grandis*, *Eucalyptus saligna*, and *Polyscias fulva*, and lowest under *Grevillea robusta*, *Entandrophragma excelsum*, and *Eucalyptus maideni* (Fig. 4.5-B).

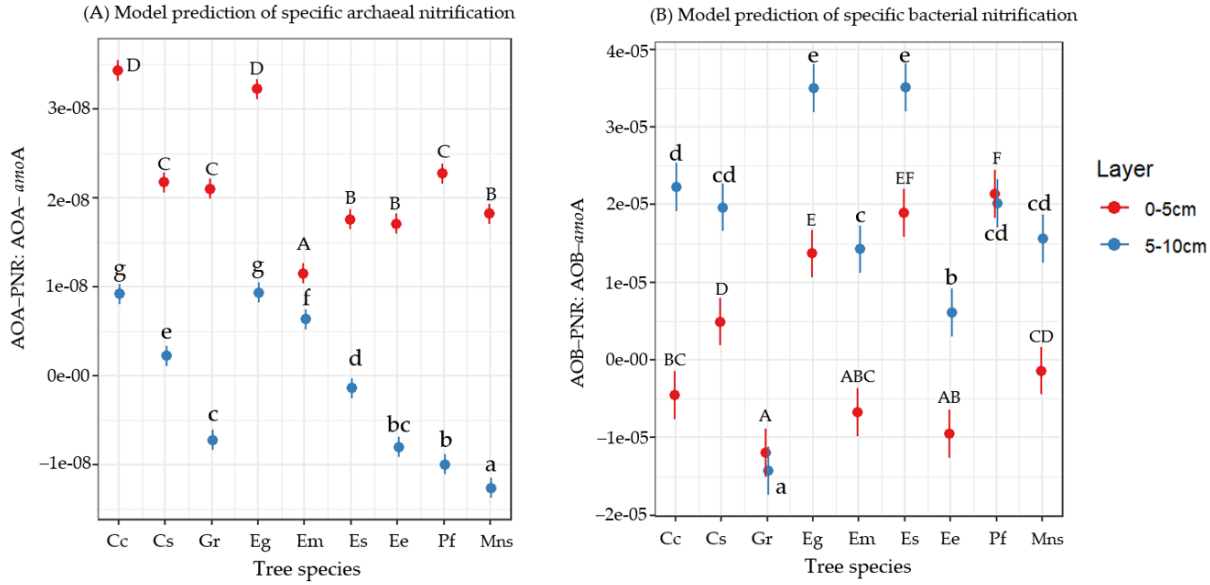

Fig. 4. 5. Differences of AOA and AOB specific potential nitrification rates (PNR per unit of amoA gene copies).

The model is: response ~ 1 + Species + Layer + Species*Layer + (1|Plot), where Plot is the Plot ID. Red circles indicate mean values of samples at 0–5 cm soil layer and blue circles indicate samples at 5–10 cm. Capital and small letters indicate significant differences (p-value < 0.05, Tukey tests) between tree species at 0–5 cm and 5–10 cm, respectively. Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species.

4.3.3. Relationship between AOA and AOB abundance and activity and soil properties

The relationships between abundance and activity of AOA and AOB and soil pH, hot water-extractable total nitrogen (HWNtot) and net N mineralization (Nmin) were different in both soil layers (Fig. 4.6). In the 0–5 cm soil layer, soil pH was positively related to the number of *amoA* gene copies for both AOA and AOB, and to AOA–PNR. Nmin was negatively related to AOA–PNR, AOB–PNR, as well as total potential nitrification rates, while no significant relationship was observed between Nmin and the number of *amoA* gene copies for both AOA and AOB. In this upper layer, HWNtot was significantly negatively related to AOB abundance, and positively to AOB (Fig. 4.6–A).

In the 5–10 cm soil layer, soil pH was also positively related to the number of *amoA* gene copies for both AOA and AOB. pH was negatively related to AOA–PNR and showed no significant correlation with AOB–PNR. As in the upper soil layer, there was no significant relationship between Nmin and the number of amoA gene copies for both AOA and AOB in 5–10 cm soil layer. In contrast, Nmin was positively related to total PNR and AOB–PNR but not with AOA–PNR. Unlike the upper soil layer, HWNtot was positively related to both *amoA*–AOA and *amoA*–AOB but was negatively related to AOA–PNR and had no significant relationship with AOA–PNR (Fig. 4.6–B).

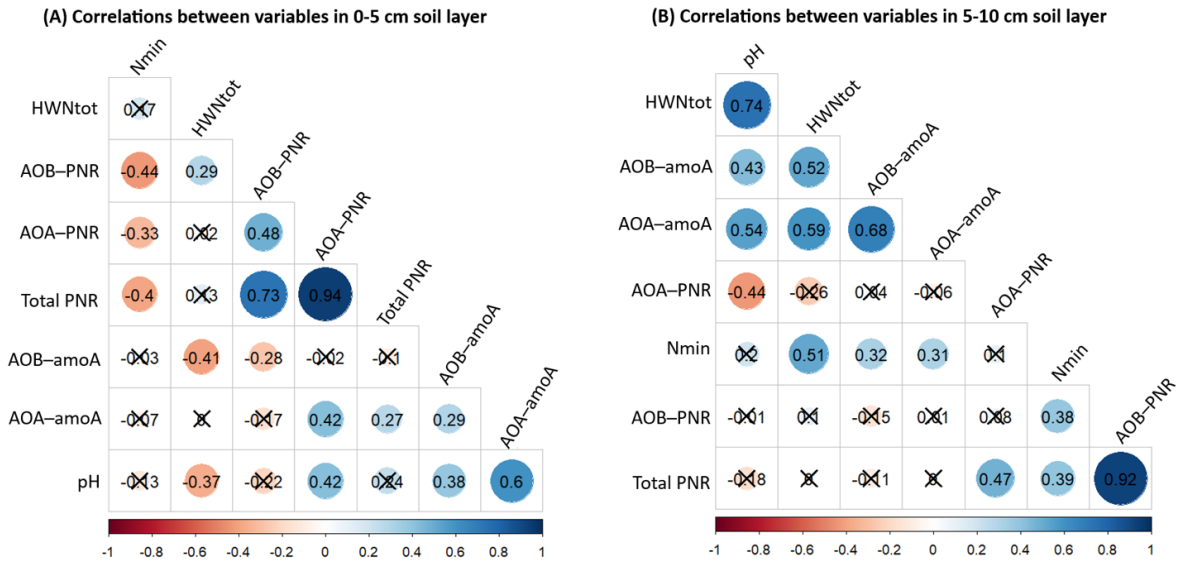

Fig. 4. 6. Relationships between AOA and AOB abundance and activity and soil properties. Correlation coefficients (r) with crosses indicate insignificant relationships.
AOA-PNR: archaeal potential nitrification rates; AOB-PNR: bacterial potential nitrification rates; Total PNR: total potential nitrification rates; AOA-amoA: archaeal amoA gene copies; AOB-amoA: bacterial amoA gene copies; Nmin: net nitrogen mineralization; HWNtot: hot water-extractable total nitrogen.

4.3.4. Multivariate relationships between PNR, amoA, tree species and soil parameters

Relationships between tree species, the activity and abundance of ammonia oxidizers and selected soil variables varied in the two soil layers (Fig. 4.7).

In the 0–5 cm soil layer, the two principal components (PC1 and PC2) represented 64.3% of the total variance (Table 4.3, Fig. 4.7–A). The first component (PC1 =36.1%) had high positive loadings for AOA–PNR, AOB–PNR, Tot–PNR, amoA–AOA, and a negative loading for Nmin. *Polyscias fulva* clustered along the positive side of PC1, while *Eucalyptus maidenii, Calliandra calothyrsus*, and *Entandrophragma excelsum* clustered along the negative side. The second component (PC2 = 28.2%) was positively associated with high loadings for AOB–PNR and HWNtot and clustering of *Eucalyptus grandis* and *Eucalyptus saligna*, while AOB–*amoA*, AOA–*amoA*, and pH showed negative loadings on PC2, with clustering of *Grevillea robusta* and mixed native species.

In the 5–10 cm soil layer, the first two principal components accounted for 67.2% of the total variance (Table 4.3, Fig. 7–B). AOA–*amoA*, AOB–*amoA*, pH, and HWNtot had significantly high loadings on PC1 (38.1%), with clustering of *Calliandra calothyrsus*, and *Entandrophragma excelsum*, while PC2 (29.1%) was associated with high loadings for AOA–PNR, AOB–PNR, Tot–PNR, and Nmin with clustering of *Calliandra calothyrsus* and *Polyscias fulva*. Eucalyptus maidenii, *Entandrophragma excelsum* and *Cedrela serrata* were clustered in the first quadrant of the biplot and negatively associated with all of the analyzed variables.

Table 4. 3. Multivariate PCA results for 8 soil variables assessed in nine treatments (tree species) at two soil layers.

Analysis	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Variable loadings in 0 – 5 cm soil layer								
Eigen value	2.88	2.25	1.15	0.61	0.59	0.27	0.20	0.01
Variance %	36.1	28.2	14.3	7.69	7.40	3.49	2.55	0.16
AOB–PNR	0.382424	0.412801	-0.19775	0.140541	0.233096	-0.14031	0.69017	-0.27228
AOA–PNR	0.548618	-0.031	0.129816	-0.24122	0.174841	0.165716	-0.45924	-0.59505
Total PNR	0.559941	0.122542	0.041759	-0.11791	0.252367	0.087169	-0.11704	0.755525
amoA–AOB	-0.00268	-0.47839	-0.20983	0.60356	0.588349	-0.05574	-0.1143	-0.02243
AamoA–AOA	0.260618	-0.3937	0.464429	0.281986	-0.32374	0.486554	0.374604	-0.00093
pH	0.250996	-0.51133	0.120098	-0.25096	-0.15335	-0.74123	0.158252	0.012369
Nmin	-0.32814	-0.03023	0.565433	-0.37454	0.610205	0.052248	0.236893	-0.01476
HWNtot	0.025107	0.409136	0.590288	0.510981	-0.06768	-0.39148	-0.25399	-0.00942
Variable loadings in 5 – 10 cm soil layer								
Eigen value	3.04	2.32	1.16	0.60	0.39	0.28	0.18	0.001
Variance %	38.1	29.1	14.5	7.5	4.9	3.5	2.2	0.02
AOB–PNR	-0.03429	-0.57389	0.378958	0.268701	0.188489	0.126753	0.036217	-0.63305
AOA–PNR	0.163006	-0.3247	-0.68866	0.042452	-0.5492	-0.0497	-0.07594	-0.28651
Total PNR	0.027715	-0.64038	0.057677	0.240193	-0.05385	0.088114	0.028751	0.718771
AOB–amoA	-0.42765	0.042516	-0.46325	0.108952	0.373598	0.669937	0.021217	0.000144
AOA–amoA	-0.46642	-0.01197	-0.25144	0.401671	0.280398	-0.6919	-0.01993	-0.0001
pH	-0.466	0.137453	0.285228	0.21967	-0.49703	0.151596	-0.60346	0.012455
Nmin	-0.30445	-0.36501	-0.03474	-0.78318	0.169892	-0.15219	-0.3278	-0.00447
HWNtot	-0.51108	-0.03233	0.135775	-0.18295	-0.40725	0.00373	0.720855	-0.01866

AOA-PNR: archaeal potential nitrification rates; AOB-PNR: bacterial potential nitrification rates; Total PNR: total potential nitrification rates; AOA-amoA: archaeal amoA gene copies; AOB-amoA: bacterial amoA gene copies; Nmin: net nitrogen mineralization; HWNtot: hot water-extractable total nitrogen.

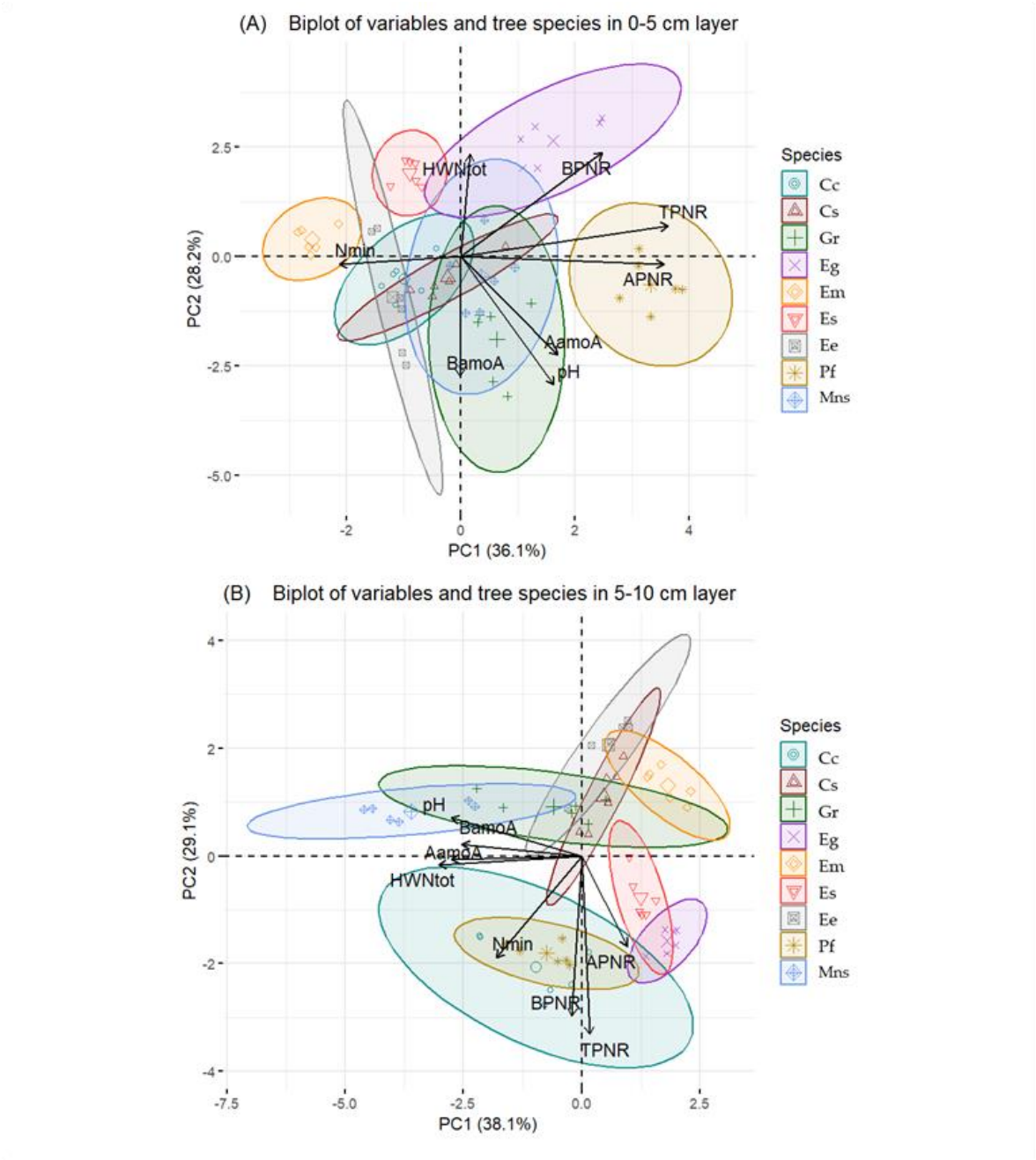

Fig. 4. 7. Principal component analyses performed on activity and abundance of AOA and AOB with selected soil properties and processes as influenced by tree species in upper (A) and lower (B) soil layers.

For each tree species, the 95% confidence ellipses are presented in distinct colours. HWNtot: hot water-extractable total nitrogen; Nmin: net nitrogen mineralization; BPNR: bacterial potential nitrification rates; APNR: archaeal potential nitrification rates; TPNR: total potential nitrification rates; BamoA: bacterial amoA gene copies; AamoA: archaeal amoA gene copies; Tree species: Cc= *Calliandra calothyrsus*; Cs= *Cedrela serrata*; Gr= *Grevillea robusta*; Eg= *Eucalyptus grandis*; Em= *Eucalyptus maidenii*; Es= *Eucalyptus saligna*; Ee= *Entandrophragma excelsum*; Pf= *Polyscias fulva*; Mns= Mixed native species.

4.4. Discussion

The effects of tree species on ammonia-oxidizers and their nitrifying activity may have ecological relevance and environmental implications in the global nitrogen cycle (Laffite et al., 2020; Florio et al., 2021), but they have rarely been assessed especially in acidic tropical forest soils (Singh & Kashyap, 2007; Nelson et al., 2016). This is the first study to evaluate the effects of planted tree species on the abundance of AOA and AOB and their contribution to nitrification in a tropical acidic forest soil in Southern Rwanda. In this study, we reported the significant effects of tree species on AOA and AOB abundance, with significant numerical and functional dominance of AOA over AOB across the tree species studied. We interpreted our findings based on the assumption that present changes in the activity and abundance of ammonia-oxidizers mainly reflected the influence of tree species planted on the same site with similar previous land use, climate, and soil conditions.

4.4.1. Effects of tree species and soil layers on nitrification rates of AOA and AOB

Our study showed that the effects of tree species on potential nitrification rates (PNR) were most pronounced in the thin upper soil layer, and about 5 (AOA) and 2 (AOB) times higher compared to the lower soil layer. High rates of nitrification were expected in the upper soil layer because of continuous litterfall and mineralization that supply ammonium substrate to nitrifiers (Xiao et al., 2017). In accordance with most research, the AOA predominated over the AOB in abundance and activity

Our findings corroborated with previous studies which reported that AOA contributed more than AOB to the nitrification in acidic soils (Li et al., 2018; Liang et al., 2020). In average, AOA contributed about 71% to the total PNR, while there was an equal contribution to the nitrification activity of the two domains in the 5–10 cm. Generally, the rates of AOA and AOB nitrification were within the ranges reported in other studies on forest soils (Nugroho et al., 2007; Coca-Salazar et al., 2021). The response of AOA and AOB potential nitrification rates to tree species partially contradict our hypothesis stating that AOA would dominate over AOB in terms of nitrification rates under soil acidifying tree species (i.e., Eucalyptus species) while we expected to see AOB having higher rates of nitrification compared to AOA under native species with less acidic soils. have

We generally found that species including *Polyscias fulva* (native species), *Eucalyptus grandis*, and *Calliandra calothyrsus* (nitrogen-fixing agroforestry species) had highest AOA and AOB nitrification rates compared to species such as *Eucalyptus maidenii*, *Eucalyptus saligna* and *Entandrophragma excelsum* (native species) that showed the lowest rates of AOA and AOB nitrification. This indicates that we cannot generalize of the effects of Eucalyptus species or native species, as they may differently influence factors such as soil pH and substrate availability that mainly drive soil nitrification process (Ste-Marie and Paré, 1999; Xiao et al., 2017). In the previous study on the same soils (Rwibasira et al., 2021), soil pH was highest under *Polyscias fulva,* while the highest concentrations of HWNtot (used here as a proxy for substrate availability for nitrifiers) were measured under *Eucalyptus grandis*, which could explain the increased potential nitrification rates under these species. The higher nitrification rates under *Calliandra calothyrsus* could be explained by increased substrate availability in nitrogen-fixing trees (Koutika et al., 2005), as observed in the previous study where the highest concentrations of water-soluble organic nitrogen (WSNorg) were measured under this species (Rwibasira et al., 2021). The decrease in nitrification under *Eucalyptus maidenii* and *Eucalyptus saligna* might be explained by the presence of nitrification inhibitory compounds (e.g., terpenoids) released by Eucalyptus species (White, 1986; Sauder et al., 2016).

4.4.2. The contribution of AOA and AOB to potential nitrification rates

For over a century, AOB were thought to be solely responsible for ammonia-oxidation, but the relatively recent discovery of AOA has revolutionized our understanding of the nitrification process (Treusch et al., 2005). Although AOA and AOB coexist in terrestrial environments (Hou et al., 2013), their relative contributions to soil nitrification vary widely and may depend on soil conditions (Taylor et al., 2012).

Based on the assumption of 2 *amoA* gene copies per AOB genome against 1 copy per AOA genome (Norton et al., 2002; Chain et al., 2003), our results of real-time PCR quantification of *amoA* genes indicates that AOA were 15–150 times more abundant than AOB in our soil samples. The results of real-time PCR targeting *amoA* gene showed that AOA were more abundant than AOB, with AOA yielding 5 and 50 times higher *amoA* gene copies in 0–5 cm and 5–10 cm soil layer, respectively.

Considering that AOA:AOB ratios in lower soil layer (5–10 cm) showed a much wider range than in upper soil layer (0–5 cm) for PNR (0.2 to 18.2 versus 0.6 to 8.8 mg $N–NO_3$ kg^{-1} h^{-1}) and *amoA* gene copies (14.4 to 114 versus 0.5 to 24.1), it is likely that AOB were strongly controlled by tree species-induced changes in soil conditions which vary with soil depth (Watanabe et al., 2023), while AOA had a wider ecological and functional range than AOB under acidic and nutrient-poor soil conditions (Erguder et al., 2009). The values of nitrification rates and *amo*A gene abundance were within the ranges reported by other studies for forest topsoils (Kreitinger et al., 1985; Isobe et al., 2011; Watanabe et al., 2023).

Although the abundance of microbial communities may often reflect activity (Baldrian, 2017; Hicks et al., 2018), there is no direct relationship between the abundance of *amoA* genes of AOA and AOB and their nitrifying capacity (Prosser and Nicol, 2008). Furthermore, it was recently reported that *amoA* functional genes might not be fully transcribed or that their resulting enzymes might be inactivated under various soil conditions (Gwak et al., 2020). This difference between species in the number of *amoA* gene copies and nitrification rates in relation to soil layers could be due to the difference in substrate availability and the community of active nitrifiers dominating in different soil layers (Hanan et al., 2016; Trivedi et al., 2019).

In this study, relationship between the number of *amoA* gene copies and potential nitrification was only significant at 0–5 cm soil layer. The observed positive correlation between AOA–*amoA* gene copies and AOA–PNR and the negative correlation between AOB–*amoA* gene copies and AOB–PNR indicate that AOA was the main contributor to soil nitrification in these tropical acidic soils. The negative correlation between bacterial *amoA* gene copies and the corresponding AOB–PNR might indicate that AOB mainly relied on produced nitrates to support their growth, while the positive relationship between AOA and AOA–amoA might be explained by high affinity of AOA for ammonium substrate and very low rates of AOB nitrification(Hink et al., 2018). It was reported by Walker et al. (2010) that AOA, but not AOB, can have a mixotrophic lifestyle allowing them to thrive under substrate scarcity and growth-limiting conditions.

High abundance of *amoA* genes may not always reflect the high rates of nitrification (Leininger et al., 2006), thus assessing the potential nitrification rate per *amoA* gene

(specific PNR) can reflect the specific contribution of AOA and AOB to nitrification (Prosser & Nicol, 2012). The results of specific PNR showed that AOA had higher specific activity in the upper soil layer, while AOB specific PNR was lower in the upper soil layer across tree species. Highest specific AOB-PNR under *Eucalyptus grandis* and *Eucalyptus saligna*, might suggest acidic conditions under these soil acidifying species (Rwibasira et al., 2021) might have caused reduction of AOB microorganisms. The difference in AOA and AOB specific PNR between two soil layers may reflect the niche differentiation related to ammonium substate and oxygen availability (Ginestet et al., 1998; Trivedi et al., 2019).

4.4.3. Relationships between nitrification potential, amoA gene abundance and soil properties

In the present study, the total and AOA nitrification rates correlated positively and negatively with soil pH in the upper and lower soil layers, respectively. These results suggest that soil pH might have an important influence on the availability of N substrate for nitrifiers in these acidic soils, as evidenced with significant relationships between soil pH and HWNtot. In general, potential nitrification rates were negatively correlated with Nmin in the 0–5 cm soil layer and positively correlated with Nmin the 5–10 cm layer. These results may indicate the competitive advantage of AOA over AOB for substrate availability which favours AOA under low ammonia availability (Prosser and Nicol, 2012; Trivedi et al., 2019). Also, abundance of amoA genes of AOA and AOB were positively correlated with soil pH in both soil layers. Considering the acidity levels and narrow pH range of studied soils (pH 3.7–4.9), this positive correlation in abundance of both AOA and AOB with soil pH might indicate not only the importance of soil pH as key factor for nitrifying microbial community, but also the adaptation of nitrifiers to acidic conditions in these tropical soils (Gieseke et al., 2006; Watanabe et al., 2023).

Tree species may influence soil nitrification and ammonia-oxidizing communities directly or indirectly via supply of nitrogen substrates or production of activity and growth inhibiting compounds (Lehtovirta-Morley et al., 2016; Thion et al., 2016). Our results of multivariate analysis (PCA) showed evidence of tree species effects and soil layer on ammonia-oxidizers and nitrification activity in relation to soil pH and nitrogen transformation proxies (i.e., Nmin and HWNtot). In the 0–5 cm soil layer, AOB-PNR was associated with Eucalyptus species while increased AOA-PNR and AOA *amoA* gene

abundance were associated with *Polyscias fulva* (native species). These results contradicted our hypothesis about AOA dominance under Eucalyptus species (lower pH) and AOB functional dominance under native species (higher pH). *Grevillea robusta* were associated with an increase in bacterial *amoA* gene abundance while *Eucalyptus saligna* and *Eucalyptus grandis* led to higher rates of AOB nitrification. These results highlights the importance of both soil pH and its consequences for substrate availability for influencing the abundance and nitrification activity (Sun et al., 2019). In the 5–10 cm soil layer, tree species including *Grevillea robusta*, and mixed native had high soil pH and HWNtot which were positively related the abundance of both archaeal and bacterial *amoA* genes. In this layer, potential nitrification rates in both AOA and AOB were positively associated with *Calliandra calothyrsus* (N_2–fixing) and *Polyscias fulva* (native) which showed higher rates of N mineralization. The negative relationships of PNR and *amoA* gene copies with Eucalyptus species in this layer might be related to possible inhibitors of microbial processes produced by these species (Sauder et al., 2016). This may explain their observed negative relationship with Nmin, likely leading to the decrease in ammonia substrate under Eucalyptus species.

4.5. Conclusion

To evaluate the abundance and contribution of AOA and AOB to the nitrifying activity under different tree species in tropical acidic and nutrient-poor forest soils, this study investigated the potential nitrification rates and abundance of functional *amoA* genes under planted monospecific stands (eight species) and a self-regenerated mixed native species in Southern Rwanda. This study demonstrated that tree species significantly influenced the abundance and activity of AOA and AOB both in 0–5 cm and 5–10 cm soil layer, with far high number of *amo*A gene copies and rates of nitrification in the upper layer (0–5 cm). We demonstrated the numerical and functional dominance of AOA over AOB in terms of amoA gene copies and potential soil nitrification rates across tree species. The results showed that planting species such as *Polyscias fulva*, mixed natives, *Eucalyptus grandis*, *Grevillea robusta*, and *Cedrela serrata* significantly increased potential nitrification rates both by AOA and AOB. These results are consistent with other studies indicating higher abundance and activity of AOA under low pH and limited substrate availability. Further, soil pH and labile nitrogen were found to influence the differences in abundance and activity of nitrifiers between tree species.

Author Contributions:

Peter Rwibasira: Conceptualization (equal); investigation (lead); formal analysis (lead); writing–original draft (lead), writing–review and editing (equal), visualization (lead). Donat Nsabimana: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Francois Xavier Naramabuye: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Monique Carnol: Conceptualization (equal); formal analysis (supporting); writing–original draft (equal); writing–review and editing (equal); resources (lead); funding acquisition (equal); project administration (lead); supervision (lead).

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CHAPTER 5 – Hillslope positions drive the effects of land terracing on soil properties and processes in steep croplands of Southern Rwanda

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Abstract

The understanding of the effects of land terracing, a widespread technique to limit erosion and improve agricultural productivity in mountainous regions, is essential for the sustainable use of soils and to preserve soil quality. This study evaluated the effects of land terracing on selected soil physical, chemical, and microbial properties and processes in the Central Plateau, agroecological zone, of Southern Rwanda. Soil samples were collected from three sites at upper, middle, and lower positions in over ten-year-old terraced and unterraced adjacent fields. Most soil physico-chemical and microbial properties differed between terraced and unterraced fields and between hillslope positions. Terracing slightly increased sand particle proportions and decreased clay content, though the overall textural class (i.e., sandy clay loam) remained unchanged. Soil organic matter slightly decreased in terraced fields, but water-extractable labile soil carbon and nitrogen fractions increased at lower and middle hillslope positions in terraced soils. The total exchangeable acidity was slightly higher in terraced than unterraced fields. Soil microbial parameters including microbial biomass (MBC, MBN, MBP), respiration potential, net N mineralization were higher in terraced than unterraced fields. Hillslope position rather than terracing affected soil aggregate stability, where more stable soil aggregates were found at lower than middle and upper hillslope positions. Total exchangeable acidity decreased, while soil microbial parameters increased from upper to lower hillslope positions, respectively. Overall, we cannot explicitly conclude that land terracing improvement soil quality compared to unterraced land, despite having higher values in soil microbial parameters and labile C and N pools in terraced than unterraced land. Although the effect of hillslope positions on most soil variables was less pronounced in terraced than in unterraced land, the persistently higher values in lower positions could either indicate a legacy of sediment accumulation in lower hillslope before terracing or the continuous soil erosion in both agricultural practices.

Keywords: *agricultural terraces; soil quality indicators; tropical ferralsols; microbial properties; central plateau, Rwanda.*

5.1. Introduction

Land terracing has been practiced since the beginning of agriculture in many parts of the world to reduce soil loss by water erosion and to sustain agricultural activities in mountainous regions (Tarolli et al., 2014). This practice provides several benefits, including increased surface of cultivable land, higher productivity, reduced surface runoff, improved soil moisture content through increased infiltration (Chen et al., 2020), reduced soil loss and sedimentation by water erosion, and formation of cultural aesthetic landscapes (Liu et al., 2011). The effects of terracing on soils, depend strongly on the terracing technique used (e.g., bench terraces, parallel terraces, broad-base terraces, and level ditches), in addition to local climate, topography, and soil characteristics (Wei et al., 2016). For example, it was reported that high levels of soil loss and risk of landslides are frequent when terraces are constructed on very steep slope with heavy and prolonged rainfalls (Deng et al., 2021).

Compared to unterraced slopes, agricultural terraces may reduce runoff and soil loss by more than 42% and 52%, respectively (Deng et al., 2021). However, a decline of as much as 45% and 50% in water-holding capacity and organic matter content, respectively, have been reported for terraced systems (Ramos et al., 2007). These effects may be linked to the construction works of terraces that significantly disrupt soil structure and the distribution of carbon stocks and fluxes (Liu et al., 2011), which may further change the dynamics of soil properties and processes linked to soil organic matter. Furthermore, the effects of terracing on soil properties and processes may depend on environmental characteristics such as climate, topography, land use, and initial soil types, which may result in either improvement or degradation of soil quality (Arnáez et al., 2015).

Soil is considered to be degraded when it loses its intrinsic physical, chemical, or biological qualities, which may lead to either a decline or a complete loss of essential ecosystem functions (Nunes et al., 2020). These functions include soil fertility and productivity, storage and supply of mineral elements, filtration and cycling of pollutants, biodiversity habitat, biogeochemical cycling, climate regulation, and supporting living organisms and infrastructure (Blum, 2005; Vogel et al., 2019). The capacity of a particular soil to perform these functions within a specific ecosystem is referred to as “soil quality” and is usually determined by assessing the physical (e.g., texture, structure, porosity,

temperature, aggregate stability, etc.), chemical (e.g., pH, SOM and nutrient content, CEC, salinity, etc.), and biological (e.g., macro-, and microbial community, microbial biomass, respiration, N mineralization, nitrification, enzyme activity, etc.) properties of the soil (Karlen et al., 2003; Bai et al., 2018; Juhos et al., 2019). Changes in these soil properties and processes are often used to assess the extent of degradation and/or restoration of soils as a response to natural events or anthropogenic activities (Muscolo et al., 2015). Soil microorganisms and their activity drive important processes in agricultural systems which are closely associated with soil structural integrity. Management practices such as land terracing significantly disturb soil structure, which may decrease SOM content and its associated benefits (Liu et al., 2011). Soil organic matter is certainly the most critical component of soils, as it influences nearly all soil parameters (Wander, 2004). Soil with higher organic matter content stores more nutrients and moisture, facilitating plant rooting and soil workability, thus contributing to greater crop production. SOM also enhances the strength of soil particle aggregation, which limits soil loss by water erosion, and creates a better soil structure allowing air and water to circulate through the soil (Krull et al., 2009). Furthermore, SOM serves as a substrate for heterotroph soil microorganisms, which play an important part in the stabilization of soil aggregates by cementing mineral particles with organic and inorganic microbial metabolites (Dexter, 2004). When soil structure is disrupted, large and stable soil aggregates are broken down into small and structurally unstable aggregates that can be easily transported by rainfall or winds, resulting in substantial soil losses (Mehra et al., 2018). In their comprehensive review including studies of varying climates and soil types, Deng et al. (2021) demonstrated how land terracing may both positively and negatively affect soil quality and ecological functions. These authors highlighted that significant terracing-induced changes are most reflected in microbially-mediated soil properties and processes, such as C and N cycling, soil respiration, N mineralization, and enzyme activities, which can be accelerated by environmental and climate conditions like those found in tropical regions (Barantal, 2011; Kidinda et al., 2020).

In tropical regions with predominately hilly topography, like Rwanda, the risk of soil degradation with cultivating sloped farmlands is high, primarily due to rainfall-induced soil erosion (Karamage et al., 2016). Factors including mountainous landscapes, heavy and frequent rainfall, high population density, decreasing size of cultivable lands, and

overexploitation of nutrient-poor soils exacerbate the risk of soil degradation in Rwandan agricultural systems (Rutebuka, 2021). Since the 1970s, land terracing has been adopted in Rwanda as the main soil conservation approach to mitigate land degradation by water erosion (Kagabo et al., 2013; Karamage et al., 2016). The recent evaluation of the Rwandan Fourth Strategic Plan for Agriculture Transformation (*Plan Stratégique pour la Transformation de l'Agriculture*; PSTA4) reported an increased area of bench terraces by 28.4% between 2018-2021 (MINAGRI, 2022), and the practice is expected to continue with the establishment new terraces and the renewal of the old ones. Through the national land consolidation and crop intensification programs, land terracing is being implemented in Rwanda's steep agricultural terrains to minimize soil erosion, reverse soil degradation, and improve crop production (Del Prete et al., 2019). Although some basic soil analyses are undertaken before implementing these agricultural practices, they are mostly performed on soils from non-terraced lands, which may differ from terraced lands in terms of soil characteristics (Fashaho et al., 2020). Previous studies on the effects of terraces in Rwanda have mainly focused on hydrological and runoff analyses (Bugenimana et al., 2019; Rutebuka et al., 2021), technical and socio-economic aspects (Bizoza, 2014), and a few attempted to analyze soil physico-chemical properties in terraced systems (Fashaho et al., 2020). Given that alteration of soil's physical and chemical conditions also has significant effects on the structure, activity, and biomass of soil microorganisms (Graham et al., 2021), there is a need to assess the implications of land terracing for selected microbial soil properties and processes that reflect important ecosystem functions. Understanding the long-term dynamics of soil characteristics and functioning in terraced systems under local environmental conditions is essential for ensuring the sustainable management of soils in hillside farmlands.

The aim of this study was to assess the effects of land terracing on selected physico-chemical and microbial properties at upper, middle, and lower hillslope positions in terraced and unterraced sloping farmlands in Southern Rwanda. We tested the hypotheses suggesting that (1) soil physico-chemical and microbial properties differ between terraced and unterraced land as a result of terracing-induced disturbance of soil structure, (2) soil quality increases with slope gradient from upper to lower position due to sediment transportation and accumulation of fertile topsoil downhill by water erosion,

(3) labile C and N concentrations, as well as microbial properties and processes are more sensitive to changes in management practices and hillslope positions than commonly analyzed soil physico-chemical properties.

5.2. Materials and Methods

5.2.1. Study site

The study was conducted at three sites (Karubanda, Tonga, and Save) in the Southern Province of Rwanda (Fig. 1.5). Sites were located at 1–2.5 km from the center of Huye city (2°34′15.0″S; 29°45′47.8″E; 1700m asl) and characterized by a typical traditional organic cropping system with beans (*Phaseolus vulgaris*), maize (*Zea mays*), and casava (*Manihot esculenta*). Each site comprised both terraced and adjacent unterraced fields of minimum 100 m x 100 m size. Bench terraces had been established at least 10 years prior to sampling on the hills with slopes ranging between 25%– 45% (Fashaho et al., 2020). The region has a humid tropical climate characterized by a mean annual temperature of 20 °C, and approximately 1230 mm rainfall per year, with a bimodal distribution pattern of two rainy seasons (heavy rains: March-May; and irregular rains: September–December) and two dry seasons (short: January–February; and long: June–August) (Meteorwanda, 2021). The soils are former Haplic Ferralsols which changed to Anthropic Ferralsols due to deep bench terracing (Mukangango et al., 2020). They are characterized by a brown-red colour with a sandy loam texture and diffuse horizons, classified as Ferralsols or Oxisols according to FAO and USDA Soil Taxonomy, respectively (Verdoodt et al., 2006).

5.2.2. Soil sampling and analyses

Soil samples were collected from both terraced (on the bench surface approximately 4m wide) and adjacent unterraced fields. Samples were taken at three slope positions (upper, middle, and lower), separated by approximately 30 m. At each slope position, five plots (10 m x 10 m each) horizontally distant by 10 m were established, and a composite sample was collected in each plot. The composite sample consisted of five sub-samples taken with an auger at 20 cm depth by following the X-shaped method (4 corners and 1 middle point). Soil samples were sieved fresh (4 mm mesh) and stored at 4 °C before laboratory analyses.

5.2.2.1. Soil physico-chemical properties

Soil texture

The Bouyoucos-hydrometer method was used to determine soil texture (Okalebo et al., 2002a). Fifty grams of air-dried and sieved (<2mm) soil were dispersed in 150 ml distilled water, and aliquots of hydrogen peroxide (30% H_2O_2) were added (~5 ml) to oxidize organic matter until effervescent bubbles disappeared. The solution was heated for two hours in a water bath at 90 ^oC and allowed to cool before adding 50 ml of sodium hexametaphosphate [(10% $NaPO_3$)6] as a soil particle (sand, silt, clay) dispersing agent (Mwendwa, 2022). The solution was thoroughly mixed using a high-speed stirrer, transferred to a sedimentation glass cylinder, and mixed after the total volume was brought to 1 L with distilled water. After covering the cylinder with a tight-fitting rubber stopper, the solution was gently mixed by inverting ten times, placed on a flat surface and two drops of amyl alcohol ($C_5H_{11}OH$) were quickly added to remove the froth. The hydrometer and thermometer were directly introduced in the cylinder and the first hydrometer and temperature readings were performed after 40 seconds (H40s and T40s). The soil suspension was again mixed by gently inverting the cylinder ten times and allowed to stand undisturbed before the second reading after three hours (H3h and T3h). Temperature readings served to adjust the hydrometer records (Table A1) because the hydrometer had been calibrated at 20 °C (Okalebo et al., 2002). The soil particle distribution was calculated as follows:

$$\%\ Sand = \frac{(W - (H1 + hc)}{W} \times 100$$

$$\%\ Clay = \frac{(H2 + hc)}{W} \times 100$$

$$\%\ Silt = 100 - (\%\ Sand + \%\ Clay)$$

Where: $H1$= the first hydrometer reading (i.e., clay + silt); $H2$= the second hydrometer reading (i.e., clay); hc= the hydrometer reading correction factor; and W= the weight of soil sample (i.e., 50 g).

Wet aggregate stability

Wet aggregate stability (WAS) was determined by a wet sieving method (Okalebo et al., 2002). Four grams of air-dried soil were placed in the wet sieving apparatus (Eijkelkamp model 08.13, NL), which constantly moves the soil-containing cans in and out of water for three minutes to break up soil aggregates. After wet sieving, the soil was dried to constant weight in an oven at 110 ^{o}C. Another sieving was performed using sodium hexametaphosphate [$(NaPO_3)6$], a soil aggregate dispersion solution agent used for acid soils, with the sieving period extended to 8 minutes. Soil wet aggregate stability (%) was calculated as follows:

$$WAS = \frac{(Ps - 0.2)}{(Ps - 0.2) + Pw}$$

Where: *Ps*= weight (g) of stable aggregates (i.e., sieving with dispersing solutions); *Pw*= weight (g) of unstable/weak aggregates (i.e., sieving with water); 0.2= weight (g) of the dispersing solute (i.e., $(NaPO_3)6$).

Soil pH and total exchangeable acidity (TEA)

Soil pH was determined in distilled water (pH_{H2O}) and 0.1M KCl (pH_{KCL}) (1:2.5 w:v) using a combined glass electrode pH meter. Exchangeable acidity and exchangeable Al^{3+} were measured through titration (Okalebo et al., 2002a). Briefly, five grams of air-dried and ground (2mm) soil were drained through the filter using ten series of 10 ml 1N KCl with an interval of 15 minutes between drainages, and the filtrate (work solution) was brought to 100 ml with 1N KCl. To determine the exchangeable acidity, five drops of phenolphthalein indicator were added to the aliquot (25 ml) from the work solution and titrated with NaOH (0.01N) until the appearance of a persistent pink colour. Exchangeable acidity (EA; expressed as $cmol_c\ kg^{-1}$ of soil) was then calculated as follows:

$$EA = \frac{(T - Bl) \times N \times V \times 100)}{(W \times v)}$$

Where: *T* = volume of NaOH (ml) used for sample titration; *Bl* = volume of NaOH (ml) used for blank titration; *N* = Normality of NaOH (i.e., 0.01); *V* = total end volume of solution (ml); *W* = weight of soil sample; *v* = volume of aliquot analysed.

Exchangeable Al^{3+} was also determined in 25 ml from the same work solution by adding 10 ml of sodium fluoride (4% NaF) which turns the solution to pink. The solution was then titrated with 0.01N HCl until total discoloration to the colourless state of the solution. The exchangeable Al^{3+} ($cmol_c\ kg^{-1}$ of soil) was then calculated as follow:

$$Al^{3+} = \frac{(T - Bl) \times N \times V \times 100)}{(W \times v)}$$

Where: T = volume of HCl (ml) used for sample titration; Bl = volume of NaOH (ml) used for blank titration; N = Normality of HCl (i.e., 0.01); V = total end volume of solution (ml); W = weight of soil sample; v = volume of aliquot analysed. The total exchangeable acidity was computed as a sum of exchangeable acidity and exchangeable Al^{3+} (TEA= $H^+ + Al^{3+}$).

Soil organic carbon (SOC)

Soil organic carbon was determined using the Walkley and Black chromic acid wet oxidation method (Okalebo et al., 2002b). Briefly, 0.5 g of air-dried and ground (0.5mm) soil were dispersed in 10 ml potassium dichromate solution (1N $K_2Cr_2O_7$), digested with 20 ml concentrated sulphuric acid (98% H_2SO_4) for 30 min at 125 ^oC. Distilled water (100 ml) and 10ml phosphoric acid (85% H_3PO_4) were added after the solution had cooled to eliminate potential interferences from the ferric (Fe^{3+}) ions that may be present in the sample. Two ml barium diphenylamine sulphonate (0.16% $C_{24}H_{20}BaN_2O_6S_2$) were added to serve as a carbon indicator. Ferrous ammonium sulphate (1M $(NH_4)_2Fe(SO_4)_2$) was used to back-titrate unreduced dichromate, while mixing with a magnetic stirrer until the initial brown colour changes sharply to the end point green. The amount of $Cr_2O_7^{2-}$ reduced during the reaction is proportional to the quantity of oxidizable organic carbon in the soil, while the blank analysis determines the extract strength of $FeSO_4$ solution. Soil organic carbon was calculated as follows:

$$N_{FeSO4} = \frac{(V_{K2Cr2O7} \times N_{K2Cr2O7})}{(V_{FeSO4.7H2O})}$$

$$\%\ SOC = \frac{(V_{Blank} - V_{Sample}) \times N_{FeSO4} \times 0.003 \times 1.3 \times 100}{W}$$

Where: N_{FeSO4} = normality of $FeSO_4$; V_{Blank} = volume of titrant in blank (ml); V_{Sample} = volume of titrant in sample (ml); N_{FeSO4} = concentration of $FeSO_4.7H_2O$ used in titration

(normality); 1.3 = correction factor to account for unrecovered organic carbon; W =weight of soil sample (g); 0.003 = correction factor for oxidized carbon according to titration reaction.

Total soil nitrogen (TN)

Total soil nitrogen was assessed using the micro-Kjeldahl digestion method in the presence of a catalyst and colorimetric determination with ultraviolet light (Okalebo et al., 2002). Five grams of air-dried and ground (0.5 mm) soil were mixed with 1.5 g of digestion catalyst mixture (100g K_2SO_4 + 5g $FeSO_4$ + 10g $CuSO_4$ +1g Se) and digested with 10 ml of concentrated sulfuric acid (98% H_2SO_4). The solution was brought to 50 ml with distilled water and allowed to settle for 2h before diluting the digest (1:9) with distilled water. An aliquot (0.2 ml) of digest was mixed with 5 ml of each of the two prepared work solutions: N1(34g sodium salicylate + 25 g sodium citrate + 25g sodium tartrate + 0.12 g sodium nitroprusside in 1 litre of distilled water) and N2 (30g sodium hydroxide + 10 ml sodium hypochlorite solution in 1litre of distilled water), and the mixture was allowed to settle for 2h before measuring the absorbance at 650 nm. The concentration of nitrogen (% N) in soil sample was calculated as follow:

$$\% N = \frac{(\mathrm{N_{Sample}} - \mathrm{N_{Blank}}) \times \mathrm{V} \times D \times 100}{W \times v \times N1 \times N2}$$

Where: N_{Sample} = concentration of N in the sample; N_{Blank} = concentration of N in blank; V= total volume at the end of analysis procedure (i.e., 0.2 ml of digest + 5 ml of N1 + 5 ml of N2); *D*= final volume of digest (i.e., 50 ml); *W*= weight of the dried soil sample (i.e., 5 g); *v*= volume of analysed aliquot (i.e., 0.2 ml); *N*1= final volume of N1 solution (i.e., 1000 ml); *N*2= final volume of N2 solution (i.e., 1000 ml). The percentage of nitrogen could also be converted in g kg^{-1} by multiplying the % TN with ten.

Soil organic matter and water-extractable C and N fractions

Soil organic matter (SOM) content was calculated as weight loss from oven-dry soil after overnight ignition at 550 °C in a muffle furnace as described by Allen (1989). Water-extractable C and N were determined using the method of Ghani et al. (2003). Fresh soil was extracted with distilled water (1:6, *w*/*v*), shaken (120 rpm, 30 min), centrifuged (4000 rpm, 10 min), and filtered (Whatman #42), representing water-soluble C and N

(WSC, WSN) fractions. Hot water-extractable C and N (HWC, HWN) were subsequently extracted from the remaining wet soil, mixed with distilled water (30 mL), and placed in the oven for 16 h at 80 ^{o}C. Organic C in the cold (WSC) and the hot water (HWC) extracts was measured using a Total Organic Carbon analyzer (LabToc, Pollution and Process Monitoring, UK). Water soluble and hot water-extractable nitrogen forms were measured colorimetrically using a continuous flow autoanalyzer equipped with a UV digestor (Autoanalyser3, BranLuebbe, Germany). Organic nitrogen in the extracts (WSNorg, HWN org) was calculated as the difference between total nitrogen and mineral nitrogen.

Available phosphorus (AvP)

Available phosphorus in soil was determined using the Bray II method which is best suited to acid soils (Okalebo et al., 2002d; FAO, 2021). Available phosphorus is extracted using the combination of hydrochloric acid to recover easily acid-soluble forms of P and ammonium fluoride which dissolves Ca, Al, and Fe phosphates by its complex formation with these metal ions in acid solution. Briefly, 2.50 g of air-dried and ground (2 mm) soil were mixed with 50 ml of the Bray II P-extracting solution (mixture of 0.1N HCl and 0.03N NH_4F), shaken for 5 minutes, and filtered (Whatman # 42). An aliquot (10 ml) from the extract was mixed with 20 ml distilled water, 5 ml of boric acid (0.8M H_3BO_3; to eliminate any fluoride interference from the extractant), and 10 ml of reducing agent solution (i.e., 1.054 g ascorbic acid in 200 ml of ammonium molybdate/antimony potassium tartrate solution) were added before the solution was brough to the final volume of 50 ml with distilled water. The mixture was shaken for 1h and the measure of intensity of molybdenum blue complex was performed at 880 nm using a spectrophotometer. Available phosphorus (mg kg^{-1}) estimation was calculated as follow:

$$P = \frac{(\mathrm{P_{Sample}} - \mathrm{P_{Blank}}) \times v \times f \times 1000}{W \times 1000}$$

Where: P_{Sample}= concentration of P (mg L^{-1}) in soil extract; P_{Blank}= concentration of P (mg L^{-1}) in blank; *v*= extract volume; *f*= dilution factor for standard series; *W*= weight of soil sample.

5.2.2.2. Soil microbial properties

Soil microbial biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined with the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Fumigation of 10 g fresh soil was carried out for 3 days in a vacuum desiccator with alcohol-free chloroform. Fumigated and non-fumigated samples were extracted with 50 ml 0.5 M K_2SO_4 (1 h shaking at 180 rpm and filtration through Whatman filter #42). Organic carbon in the extracts was measured with a Total Organic Carbon analyser (Lab Toc, Pollution, and Process Monitoring, UK), and total nitrogen were analysed colorimetrically using a continuous flow analyser equipped with a UV digestor (AutoAnalyzer 3, BranLuebbe, Germany). Soil microbial biomass C and N were calculated as the difference between fumigated and non-fumigated samples with a conversion factor of 0.45 for biomass C (Joergensen and Mueller, 1996), and 0.54 for biomass N (Brookes et al., 1985). Soluble phosphorus was measured using ICP-AES in fumigated (3 days with chloroform without ethanol) and unfumigated soil samples (10 g) extracted using a 0.5 M $NaHCO_3$ solution at pH 8.5. A conversion factor of 0.4 (Brookes et al., 1982) was applied to the difference between the fumigated and unfumigated P extractable content to convert into microbial biomass P (MBP).

Soil respiration potential

The soil respiration potential (RP) was measured at 20 °C as CO_2–C accumulation in the headspace (125 ml) of an amber bottle (Supelco, USA) from 20 g of fresh soil adjusted to 60% water holding capacity (see below), after an overnight pre-incubation at 20 °C in the dark (Robertson et al., 1999). Gas samples (4 ml) were taken at 0, 120, 150, 180, and 210 minutes with an airtight syringe (Hamilton Model 1005) and analysed with an infrared absorption gas analyser (EGM-4, Ppsystem, UK). The respiration potential was estimated by linear regression of CO_2–C against time (μg CO_2–C g^{-1} h^{-1}).

Microbial and Metabolic quotients

The microbial quotient (qmic) is an indicator of the availability of soil C for microorganisms (Anderson and Domsch, 1990). It was calculated by dividing soil microbial biomass carbon by the total soil organic carbon content, estimated as 58% of soil organic matter (Allen, 1989). The metabolic quotient (qCO_2) was calculated by

dividing the respiration potential by the microbial biomass carbon (Anderson, 2003). It is an indicator of microbial maintenance energy requirement (Dilly and Munch, 1998).

Nitrogen transformation rates

Net nitrogen mineralization (Nmin) was determined from 15 g of fresh soil adjusted to 60% water holding capacity and incubated at 20 °C for 28 days (Hart et al., 1994). Extraction of inorganic nitrogen (NH_4^+–N and NO_3^-–N) was performed on sub-samples at the beginning and the end of the incubation period using 1 M KCl (1:5; w:v), after 1 h agitation at 180 rpm and centrifugation at 4000 rpm (Allen, 1989). The water loss during incubation was monitored gravimetrically and compensated by adding distilled water as necessary. Extracts were analysed colorimetrically using a continuous flow analyser equipped with a UV digestor (AutoAnalyser3, BranLuebbe, Germany). Net nitrogen mineralization (Nmin) and relative nitrification (Nit_{rel}) rates were calculated as the ratio between the net increase in inorganic nitrogen (NH_4^+–N and NO_3^-–N), and the number of incubation days, and as the percentage of nitrate produced (NO_3^-– N) of the total N produced, respectively.

5.2.3. Statistical analyses

Effects of management practices (Management: Terraced, Unterraced) and hillslope position (Position: Lower, Middle, Upper), as well as their interactions were analysed using mixed effects models using the R Statistical language (version 4.3.1; R Core Team, 2023). Management, Position and their interactions were included as fixed effects, while the sites (Site: Karubanda, Tonga, Save) were included as random effects. We fitted a linear mixed model with lmerTest (version 3.1.3; Kuznetsova et al., 2017), estimated using REML and nloptwrap optimizer with random intercept:

response ~ 1 + Management + Position + Management*Position + (1|Site)

or random intercept and slope:

response ~ 1 + Management + Position + Management*Position + (1+Management|Site).

The model with lowest AIC was selected. The assumptions of normality and homoscedasticity of the residuals were assessed by visual inspection of the q-q plots and plots of the normalized residuals against the fitted values (Zuur et al., 2010). Conditional ($R\_GLMM^2=R^2c$, variance explained by the entire model,) and marginal ($R\_GLMM^2=R^2m$; variance explained by the fixed effects) coefficients of determination were calculated

using MuMIn (version 1.47.5; Barton, 2020). F-tests analysis of variance for fixed-effect terms used Satterthwaite and Kenward-Roger methods (Kuznetsova et al., 2017). Estimated means were calculated and pairwise comparisons were performed using emmeans (version 1.8.7; Lenth et al., 2018), according to recommendations for non-interacting or interacting factors. Plots were performed according to Jeffrey (2018). Correlations among variables were examined using the correlation-based network analysis ("*Corrr*", version 0.4.4 (Kuhn et al. 2020)), computing pairwise correlation coefficients (Pearson) between any two pairs of analysed variables, and generating a correlation matrix according to the strength of correlation. Additionally, a Principal Component Analysis (PCA) was performed using packages "*FactoMineR*", version 2.8 (Husson et al. 2020) and "*ggplot2*", version 3.4.2 (Wickham et al. 2022) to explore relationships between selected soil variables as a response to management practice and hillslope positions.

5.3. Results

5.3.1. Effects of terracing and hillslope position on physico-chemical soil properties

Most soil physico-chemical properties differed in two management practices with relatively higher values in terraced than unterraced lands, except SOM, C/N, and Clay (Table 5.1). According to the results of mixed effects models, data variance in most soil physico-chemical properties were significantly explained by the model (conditional R^2 >50%), except SOM, WSC, HWC, and Silt for which the model's total explanatory power was between 40% and 49%. Overall model fit was relatively good, explaining between 40 and 94% of total variance. Management and hillslope position explained between 10% and 58% of data variance, but explanatory power was low for soil C/N (2%), Silt (6%), SOC (7%), and pH_{KCL} (8%).

Table 5. 1. Summary statistics of the linear mixed effect model for the effects of management (Management) and hillslope position (Position) on soil physico-chemical properties.

Parameter	Model	Goodness of fit		Pr(>F) statistics analysis of variance		
		R^2m	R^2c	Management (M)	Position (P)	M*P
SOM (g kg^{-1})	B	0.21	0.47	0.3359	0.0026	0.0634
SOC (g kg^{-1})	B	0.07	0.76	0.5809	0.8953	0.0002
TN (g kg^{-1})	B	0.10	0.53	0.3491	0.3233	0.6743
HWC (mg C kg^{-1})	A	0.39	0.40	< 0.0001	0.0003	0.0031
HWNtot (mg N kg^{-1})	A	0.58	0.63	< 0.0001	< 0.0001	0.1368
C/N	B	0.02	0.76	0.7620	0.6246	0.3342
pH_{KCL}	B	0.08	0.93	0.6808	< 0.0001	0.0035
pH_{H2O}	B	0.20	0.90	0.2468	< 0.0001	0.0692
AvP (mg P kg^{-1})	B	0.32	0.94	0.1854	< 0.0001	0.0061
TEA (cmolc kg^{-1})	B	0.47	0.74	0.1616	< 0.0001	0.8434
Sand (%)	B	0.12	0.56	0.4181	0.0686	0.0049
Silt (%)	A	0.06	0.49	0.0020	0.8555	0.7354
Clay (%)	B	0.11	0.64	0.2663	0.1972	0.0076
WAS (%)	B	0.30	0.54	0.7051	< 0.0001	0.9648

We fitted a linear mixed model (estimated using REML and nloptwrap optimizer) with random intercept (Model A: response ~ 1 + Management + Position + Management*Position+(1|Site) or random intercept and slope (Model B: response ~ 1 + Management + Position + Management*Position+ (1+Management|Site)). The model with the lowest AIC was selected. R^2c: conditional R^2 (model's total explanatory power); R^2m: marginal R2 (explanatory power of fixed effects). Pr(>F) statistics analysis of variance indicates the decomposition of fixed-effects contributions (Bates et al., 2015). Model A was used for WSC, HWC, HWNtot, and Silt, as model B resulted in a singular model (some dimensions of the variance-covariance matrix have been estimated as exactly zero). Values in bold indicate statistically significant effects of model terms on soil parameters. (SOM: soil organic matter, SOC: soil organic carbon, TN: total nitrogen, WSC: water soluble carbon, HWC: hot water-extractable carbon, WSNtot: water soluble total nitrogen, HWNtot: hot water-extractable total nitrogen, C/N: soil carbon to nitrogen ratio, AvP: soil available phosphorus, TEA: total exchangeable acidity, WAS: wet aggregate stability).

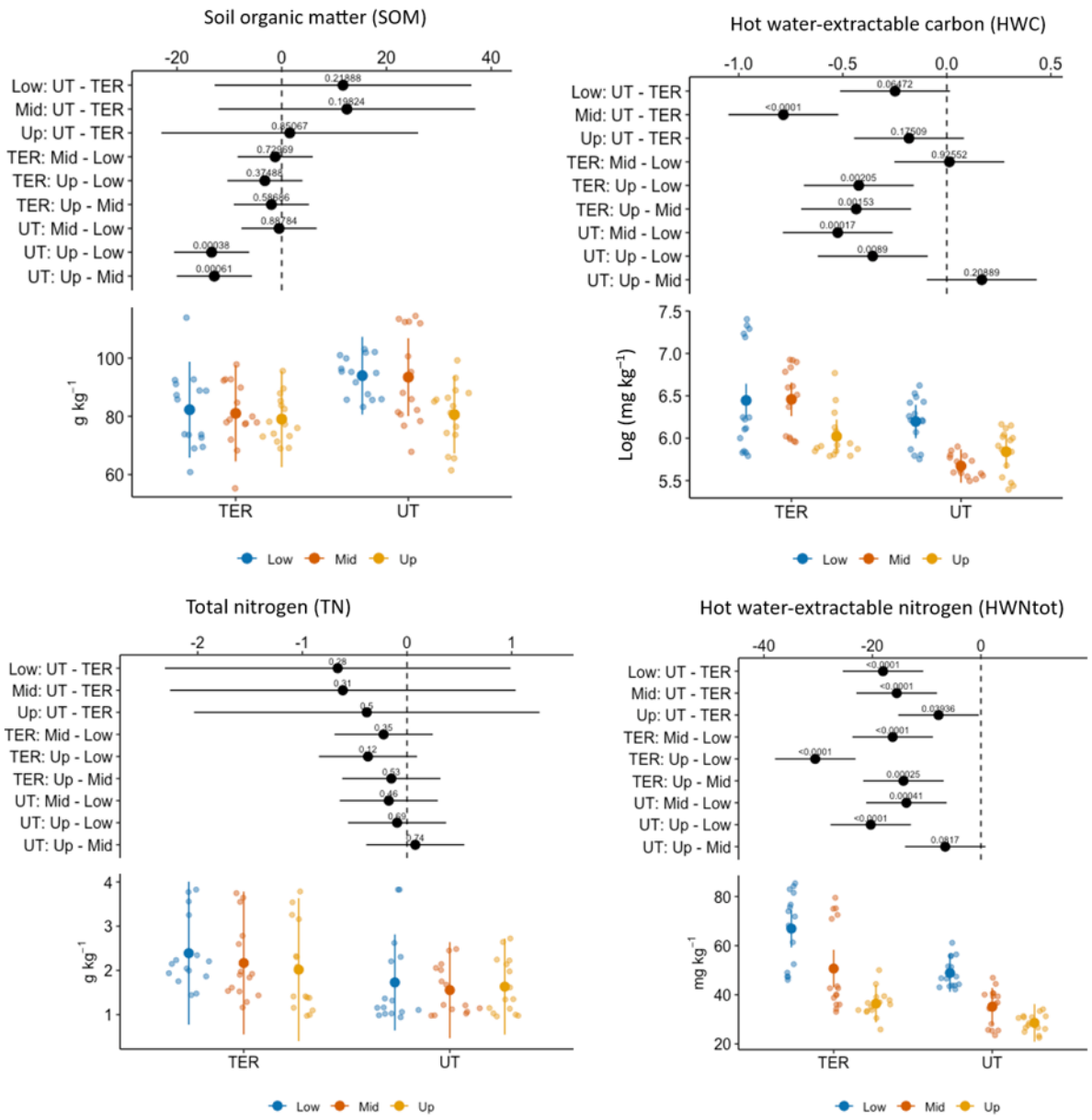

Fig. 5. 1. Effects of land terracing on soil organic matter (SOM), total soil nitrogen (Total N), and hot water-extractable C and N content (HWC, HWNtot).
Effect of management (TER: terraced, UT: unterraced) and hillslope position (Low: lower, Mid: middle, Up: upper) on soil parameters. Top: effects plot of 2 X 2 simple effects (difference in means). Bars are 95% confidence intervals of the effects. Unadjusted p-values from the mixed linear model are given. Bottom: response plot of the estimated means (large circles), modelled 95% confidence interval of each mean (bars) and model-adjusted individual response values (small, coloured dots).

Terracing did not significantly influence SOM (Table 5.1, Fig. 5.1), but SOM was significantly higher in the middle and lower positions in the unterraced fields. Labile carbon (HWC) was significantly higher in the middle position of the terraced fields compared to the unterraced fields. Within the terraced fields, HWC was higher in the mid and low position, while it was significantly higher in the low position of the unterraced

field (Fig. 5.1). Total soil nitrogen was not influenced by management and hillslope position, HWNtot was significantly lower in the unterraced soils and increased significantly downhill both in terraced and unterraced sites.

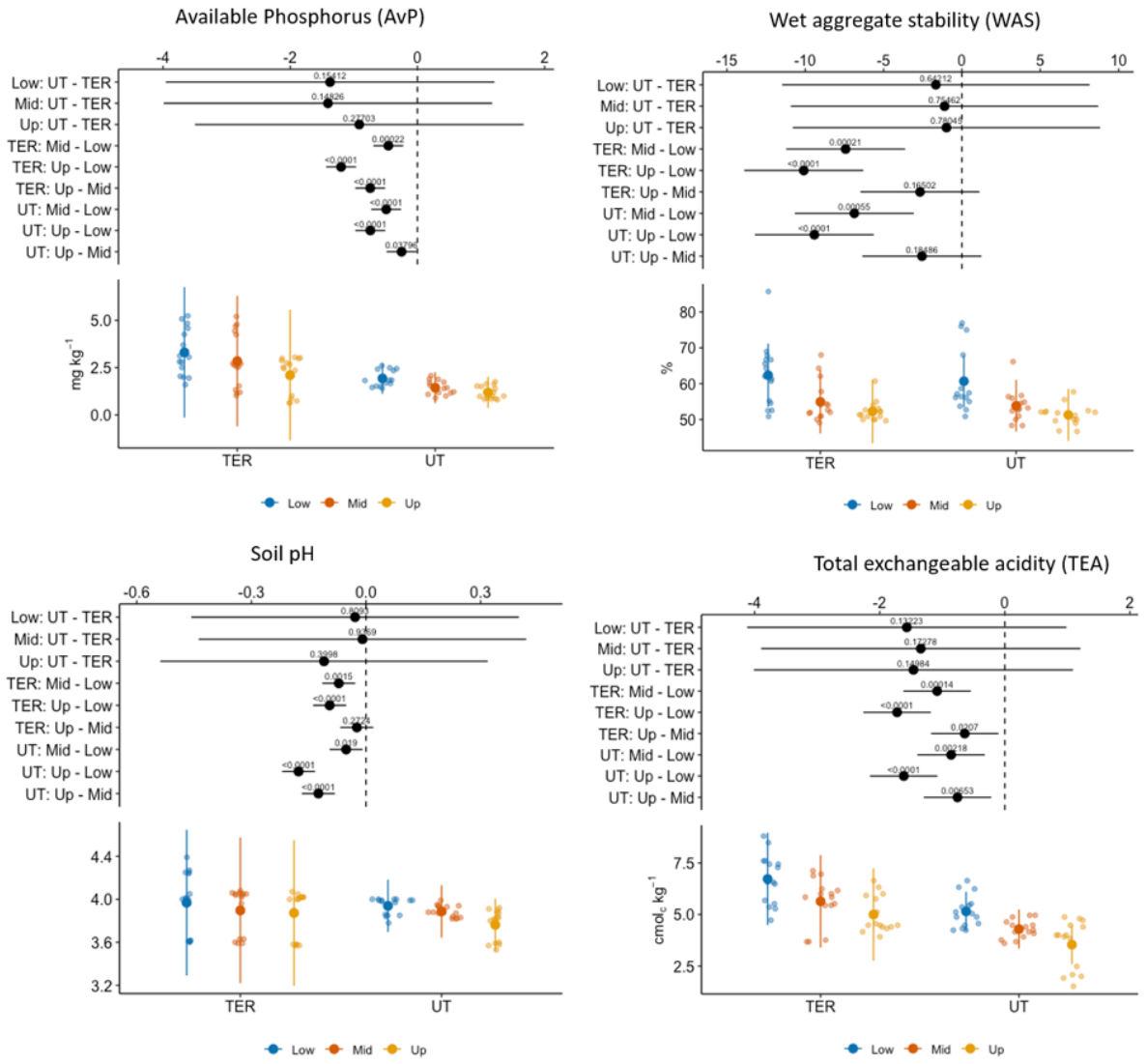

Fig. 5. 2. Effects of land terracing on soil available phosphorus, wet aggregate stability, and soil acidity.
Effect of management (TER: terraced, UT: unterraced) and hillslope position (Low: lower, Mid: middle, Up: upper) on soil parameters. Top: effects plot of 2 X 2 simple effects (difference in means). Bars are 95% confidence intervals of the effects. Unadjusted p-values from the mixed linear model are given. Bottom: response plot of the estimated means (large circles), modelled 95% confidence interval of each mean (bars) and model-adjusted individual response values (small coloured dots).

Soil properties including available phosphorus (AvP), wet aggregate stability (WAS), pH, and total exchangeable acidity (TEA) were significantly influenced by hillslope positions rather than land terracing (Table 5.1, Fig. 5.2). AvP and WAS increased significantly

downslope both in terraced and unterraced fields with higher WAS values in terraced compared to soil unterraced fields. Soil pH showed a relatively greater range in terraced than unterraced land with higher pH in lower than middle and upper hillslope positions both in terraced and unterraced fields. TEA was significantly lower in unterraced fields and significantly increased downhill both in terraced and unterraced soils. The proportion of sand particles was significantly higher in the upper position of terraced compared to unterraced fields, while the proportion of clay particles was significantly higher in the upper position of unterraced fields compared to terraced fields (Fig. 5.3).

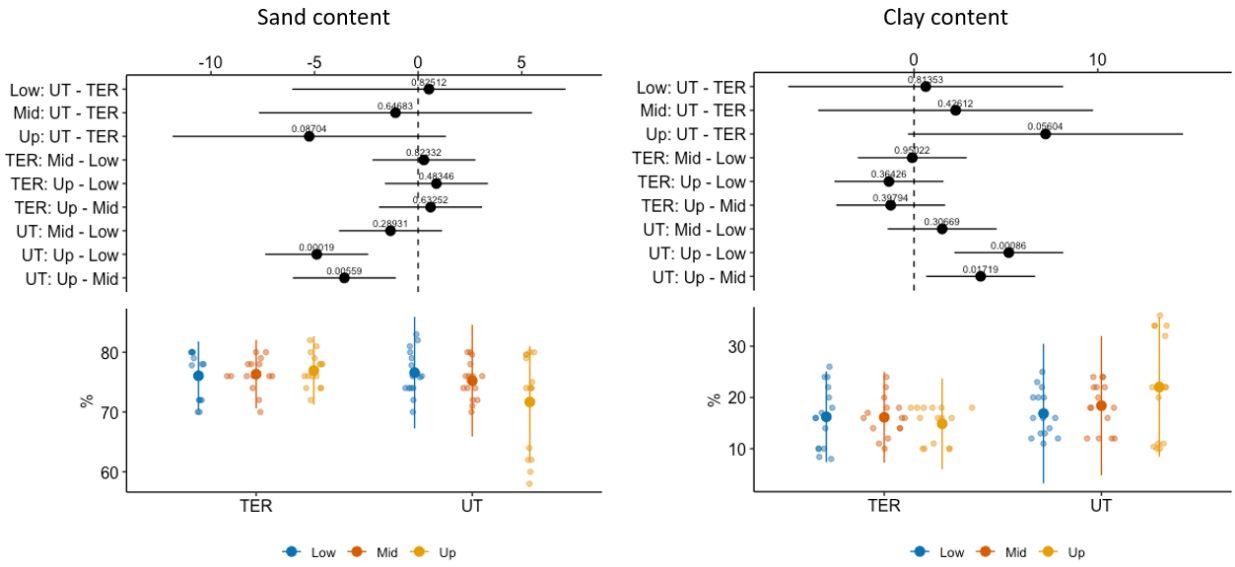

Fig. 5. 3. Effects of land terracing on oil sand and clay particle size distribution.
Effect of management (TER: terraced, UT: unterraced) and hillslope position (Low: lower, Mid: middle, Up: upper) on soil parameters. Top: effects plot of 2 X 2 simple effects (difference in means). Bars are 95% confidence intervals of the effects. Unadjusted p-values from the mixed linear model are given. Bottom: response plot of the estimated means (large circles), modelled 95% confidence interval of each mean (bars) and model-adjusted individual response values (small colored dots).

5.3.2. Effects of terracing and slope position on microbial soil properties and processes

Land terracing and hillslope positions significantly affected soil microbial biomass and activity (Table 5.2; Fig. 5.4). The model's explanatory power (conditional R^2) was substantial, above 60% for most parameters, except MBP, and qCO2 for which it was around 40%. Fixed factors, management, and hillslope position explained between 15% and 58% of data variance (except for qCO2: 7%). For the parameters without a significant management*position interaction effect, RP showed no management effect and increased significantly downhill both in terraced and unterraced sites (Fig. 4), while qCO2 was

significantly altered by management practices (Fig. 6). For MBP and Nmin, there was both a significant management and hillslope position effect (Table 3). MBP significantly decreased in the unterraced soil for the lower hillslope position and increased significantly downhill (Fig 4). Nmin was significantly lower in the unterraced soils and was highest at the mid position in the terraced soils, and lowest in the Up position for the unterraced soils (Fig. 5.4). qmic was significantly lower in the unterraced soils at the lower hillslope position, and lower at the upper hillslope position for the terraced and unterraced soils, with a smaller effect size for the unterraced soils (Fig. 5.5) .

Table 5. 2. Summary statistics of the linear mixed effect model for the effects of management (Management) and hillslope position (Position) on soil microbial properties and processes

Parameter	Model	Goodness of fit		Pr(>F) statistics analysis of variance		
		R^2m	R^2c	Management (M)	Position (P)	M*P
RP (mg CO_2–C kg^{-1} h^{-1})	B	0.32	0.63	0.4113	< 0.0001	0.5666
MBC (mg C kg^{-1})	B	0.56	0.78	0.1138	< 0.0001	< 0.0001
MBN (mg N kg^{-1})	A	0.42	0.62	0.0002	< 0.0001	0.0008
MBP (mg P kg^{-1})	A	0.22	0.38	0.0576	< 0.0001	0.1056
Nmin (mg N kg^{-1} d^{-1})	A	0.14	0.62	< 0.0001	0.0078	0.6377
Nitrel (%)	B	0.49	0.81	0.2407	< 0.0001	< 0.0001
qCO2 (µg CO_2–C mg^{-1} MBC h^{-1})	B	0.07	0.44	0.5403	0.0679	0.5059
qmic (mg MBC g^{-1} Ctot)	B	0.51	0.78	0.1385	< 0.0001	< 0.0001

We fitted a linear mixed model (estimated using REML and nloptwrap optimizer) to with random intercept (**Model A**: response ~ 1 + Management + Position + Management*Position + (1|Site)) or random intercept and slope (**Model B**: response ~ 1 + Management + Position + Management*Position + (1+Management|Site)). The model with the lowest AIC was selected. R^2c: conditional R^2 (model's total explanatory power); R^2m: marginal R^2 (explanatory power of fixed effects). Pr(>F) statistics analysis of variance indicates the decomposition of fixed-effects contributions (Bates et al., 2015). Data for RP, Nmin, and qCO2 were log-transformed; model A was used for MBN, MBP and Nmin, as model B resulted in a singular model (some dimensions of the variance-covariance matrix have been estimated as exactly zero). Values in bold indicate statistically significant effects of model terms on soil parameters. (RP: soil respiration potential, MBC: microbial biomass carbon, MBN: soil microbial biomass nitrogen, MBP: microbial biomass phosphorus, Nmin: net nitrogen mineralization, Nitrel: relative nitrification, qCO2: metabolic quotient, qmic: microbial quotient).

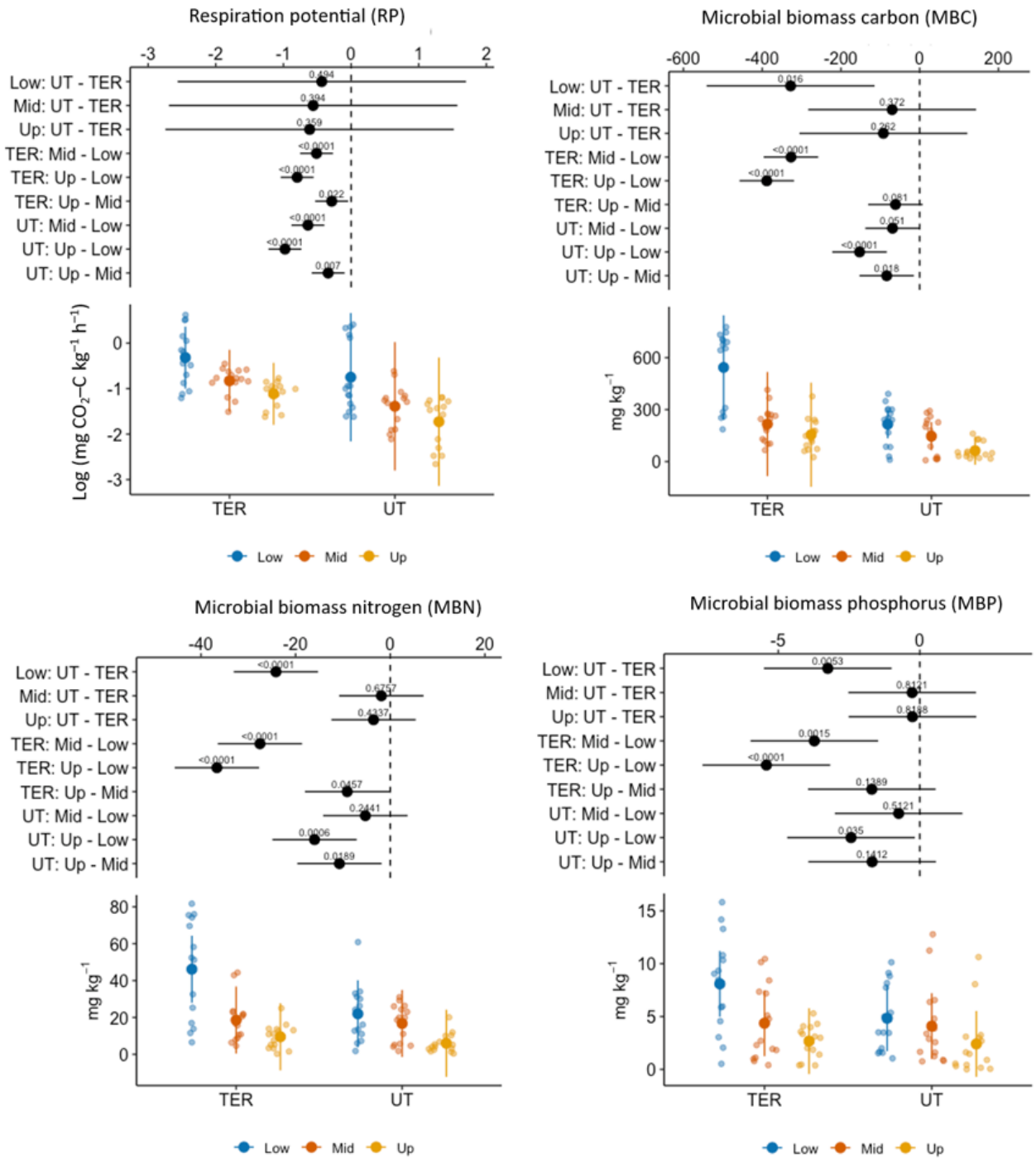

Fig. 5. 4. Effects of land terracing on soil respiration and microbial biomass carbon, nitrogen, and phosphorus.
Effect of management (TER: terraced, UT: unterraced) and hillslope position (Low: lower, Mid: middle, Up: upper) on soil parameters. Top: effects plot of 2 X 2 simple effects (difference in means). Bars are 95% confidence intervals of the effects. Unadjusted p-values from the mixed linear model are given. Bottom: response plot of the estimated means (large circles), modelled 95% confidence interval of each mean (bars) and model-adjusted individual response values (small, coloured dots).

The interaction effect management*Position was significant for MBC, MBN, Nitrel, and qmic (Table 5.2). MBC and MBN were significantly lower in the unterraced soils at the lower hillslope position and showed a clear significant increase from the upper to lower hillslope position, although the size of the effect was less marked in the unterraced soils (Fig. 5.4). Nitrel was near 100% for the terraced soils but showed high variability in the unterraced soils with significantly higher values in the lower hillslope position (Fig. 5.5).

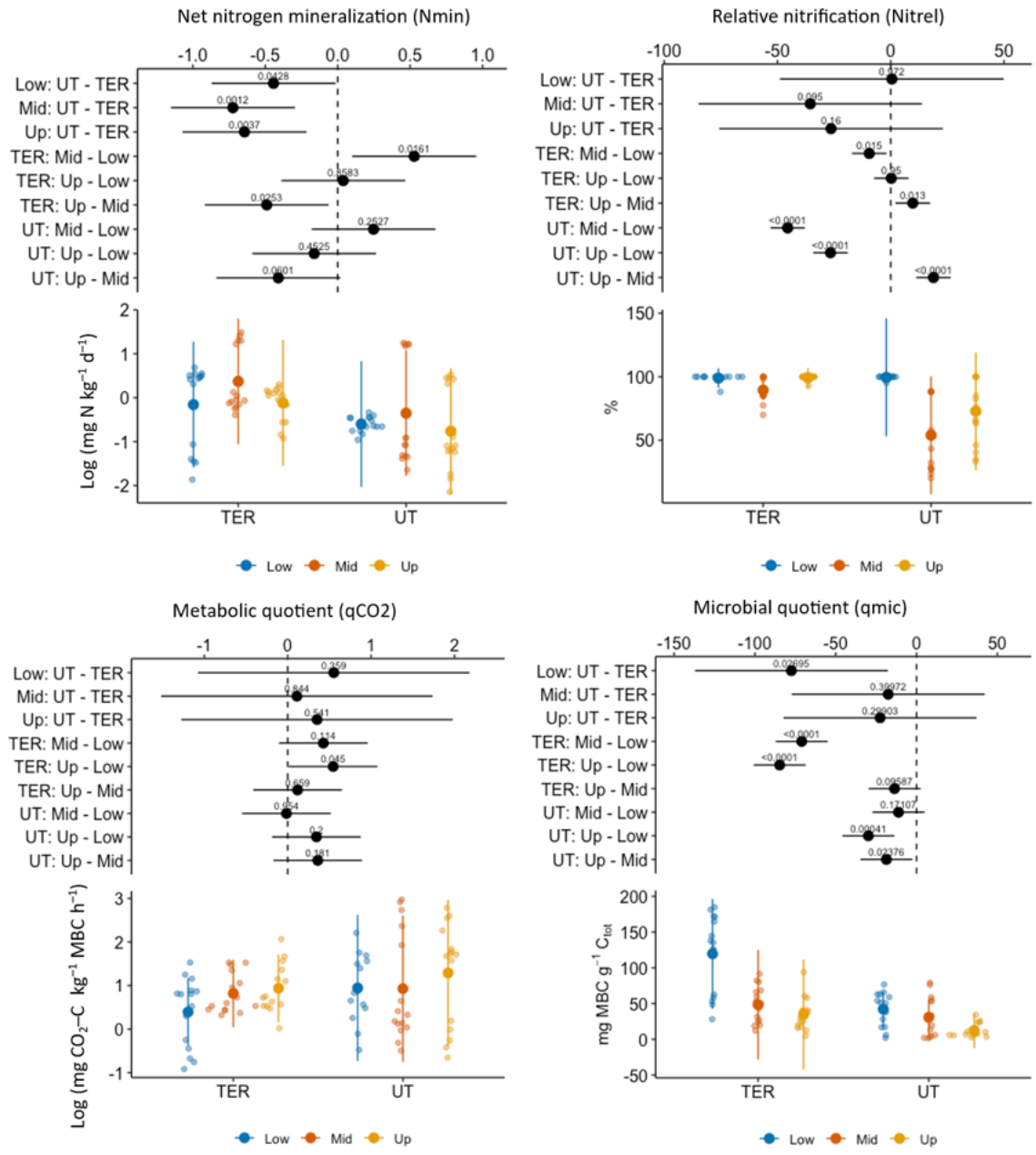

Fig. 5. 5. Effects of land terracing on Nmin, relative nitrification, and microbial ecophysiological indices.
Effect of management (TER: terraced, UT: unterraced) and hillslope position (Low: lower, Mid: middle, Up: upper) on soil parameters. Top: effects plot of 2 X 2 simple effects (difference in

means). Bars are 95% confidence intervals of the effects. Unadjusted p-values from the mixed linear model are given. Bottom: response plot of the estimated means (large circles), modelled 95% confidence interval of each mean (bars) and model-adjusted individual response values (small colored dots).

5.3.3. Relations between soil physico-chemical and microbial properties and processes

In terraced fields (Fig. 5.6-A), WAS had significant positive correlations with microbial properties including RP (r=0.63), Nitnet (r=0.45), Nmin (r=0.38), and MBC (r=0.31). HWC was positively correlated with RP (r=0.70) and MBC (0.43). TEA was positively correlated to RP and MBC (r=0.54 and r=0.74, respectively), while MBC was positively correlated to AvP and Sand (r=0.59 and r= 0.34, respectively). A significant negative correlation was only found between clay content and MBC (r= −0.38)

In unterraced fields (Fig. 5.6-B), the proportion of clay positively correlated to Nitnet (r=0.31). SOM correlated positively with Nmin, Nitnet and MBC (r=0.71, r=0.72, and r=0.36, respectively). WAS correlated positively to MBC (r=0.42), while HWC had a positive correlation only with RP (r=0.80). TEA also had significant positive correlations with RP and MBC (r=0.44 and r=0.42, respectively). The significant negative correlation between soil physico-chemical and microbial parameters was only found between HWC and Nmin (r= −0.36).

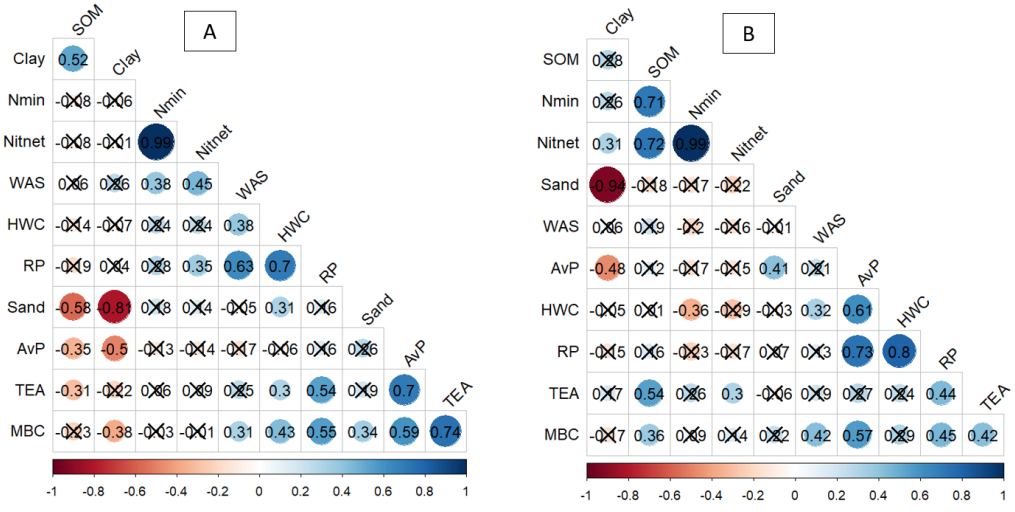

Fig. 5. 6. Pearson's correlation between physico-chemical and microbial soil properties in terraced (A) and unterraced (B) agricultural practices.
Coefficients of correlation between parameters are indicated by the values at the intersection of parameters as interpreted by the contrast in circle size and colour gradient in the legends.

5.3.4. Relations between soil variables, land management, and hillslope positions

Land terracing altered the distribution of soil variables across hillslope positions. The first two principal components accounted for 58.7% and 60% of variance in terraced and unterraced lands, respectively (Fig. 5.7). Furthermore, the statistical ellipses grouping observations (at 95% confidence level) in response to hillslope positions showed different patterns between terraced and unterraced land.

In the terraced land, there was no discrimination of soil parameters according to hillslope position. Only Clay and SOM were positively related to the first axis, explaining 34.3 % of the total variation. Although the clusters of soil parameters overlapped in the PCA of terraced land, they showed different loadings onto the first two PC axes (Table 4). All parameters contributed between 28% and 42% on the first two PC axes.

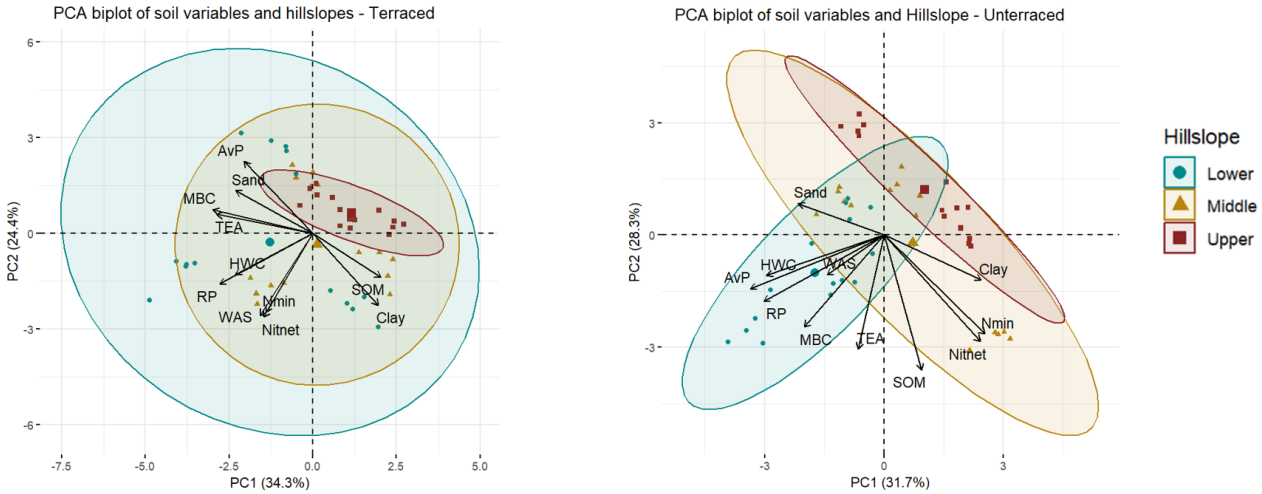

Fig. 5. 7. Relationships between soil variables for two management practices (Terraced, Unterraced) at different hillslope positions Lower, Middle, Upper).
Statistical ellipses at 95% confidence level group hillslope positions (represented by different symbols and colours) based on soil variables depicted by vectors (SOM = soil organic matter; TEA = total exchangeable acidity; Sand = proportion of sand particles, Clay = proportion of clay particles; HWC = hot water-extractable carbon; WAS = wet aggregate stability; MBC = microbial biomass carbon; RP = soil respiration potential; Nmin = net nitrogen mineralization; and Nitnet = net nitrification).

Soil parameters from unterraced land showed distinct clustering of the lower hillslope position compared to the overlapping upper and middle hillslope positions (Fig. 5.7). Significant positive loadings of variables onto the PCs in unterraced land involved Clay, Nmin, Nitnet. Most of the variables were clustered on the left quadrants of the first PC (31.7%, variance) with high loadings of MBC, WAS, AvP, HWC, and RP (Table 5.3). Soil variables showed between 32% and 49% contribution to the first two components, except WAS which contributed 18% and 14% to PC1 and PC2, respectively. The clustering of soil parameters according to hillslope positions showed an obvious discrimination. The lower hillslope position was related with increased scores of HWC, AvP, RP, MBC, and WAS, while the middle hillslope position was characterized by high scores of Nmin, Nitnet, Sand, and the upper hillslope showed increased Clay contents.

Table 5. 3. Principal component analysis (PCA) of 11 selected soil variables measured in 90 samples under two treatments (terraced and unterraced) at three hillslope positions.

PCA analyses	Terraced				Unterraced			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigenvalues	3.78	2.69	1.79	1.04	3.49	3.11	1.81	1.03
% variance	34.33	24.42	16.24	9.49	31.70	28.28	16.47	9.36
Cumulative % of total variance	34.33	58.75	74.99	84.48	31.70	59.97	76.44	85.80
Soil variables	Loadings (relationship) of soil variables on PCs							
SOM (g kg^{-1})	**0.276**	-0.223	-0.275	-0.107	0.123	**-0.494**	0.088	0.078
HWC (mg C kg^{-1})	**-0.314**	-0.209	-0.102	0.550	**-0.383**	-0.149	-0.331	-0.231
AvP (mg P kg^{-1})	-0.279	**0.360**	-0.153	-0.467	**-0.436**	-0.196	0.127	-0.160
TEA (cmolc kg^{-1})	**-0.389**	0.095	-0.309	-0.335	-0.084	**-0.418**	-0.059	-0.039
WAS (%)	-0.214	**-0.417**	-0.186	0.043	-0.186	-0.145	-0.218	0.792
Sand (%)	**-0.313**	0.214	0.401	0.325	**-0.280**	0.116	0.584	0.097
Clay (%)	0.266	**-0.363**	-0.367	-0.062	**0.317**	-0.165	-0.540	0.004
MBC (mg C kg^{-1})	**-0.405**	0.118	-0.302	-0.029	-0.260	**-0.337**	0.117	0.326
RP (mg CO_2–C kg^{-1} h^{-1})	**-0.377**	-0.256	-0.250	0.183	**-0.392**	-0.243	-0.175	-0.387
Nmin (mg N kg^{-1} d^{-1})	-0.190	**-0.398**	0.417	-0.322	0.329	**-0.364**	0.287	-0.099
Nitnet (mg NO_3–N $kg^{-1}d^{-1}$)	-0.198	**-0.423**	0.372	-0.328	0.313	**-0.391**	0.237	-0.104

The first four principal components explaining about 85% of the cumulative total variance are presented. Variable loadings express the strength of relationship (loading weight) between soil variables and PCs, while the sign on variable loadings indicates the direction of the variable on PC axes.

5.4. Discussion

Terraces are commonly constructed in mountainous regions for many functions, including slowing runoff velocity, reducing the risks of soil erosion, promoting water infiltration, facilitating the cultivation of hillsides, and increasing crop productivity (Liu et al., 2011). However, soil properties may be altered by terracing operations that disrupt the inherent soil structure and affect soil quality (Deng et al., 2021). Commonly reported soil characteristics altered by terracing include soil texture, bulk density, porosity, water and nutrients retention ability, organic and inorganic C pools, pH, and biological activity (Ramos et al., 2007; Rutebuka et al., 2021). The physico-chemical soil properties measured in our study were in ranges with previous studies conducted around the same region (Van der Zaag et al., 1984; Mbonigaba et al., 2009; Fashaho et al., 2020).

5.4.1. Effects of land terracing and hillslope positions on physico-chemical soil quality

Although the proportions of soil particle size (i.e., Sand and Clay) were slightly altered from upper to lower hillslope positions, the overall textural class was unchanged. According to FAO classification, soils in both terraced and unterraced fields were sandy clay loam. It was also previously suggested by Mesfin et al. (2018), who also reported no significant differences in proportions of soil particle size between terraced and unterraced in hilly croplands of Ethiopia, that changes in textural composition of soils are mainly driven by the soil's parental material. However, the significant effects of hillslope position on assessed soil parameters, especially in unterraced land, may suggest that terracing practice has reduced the downhill movement of easily erodible fine textured soil particles. The soil in studied sites were acid, likely due to intrinsic characteristics of the soil's parental material in combination with intense and frequent rainfall that might have leached base cations on these steep terrains (Mbonigaba et al., 2009). While land terracing did not significantly affect soil pH, soil at lower hillslope had higher pH values than in the middle and upper slopes, which was likely due to accumulation of base cations at lower hillslope level. The soil total nitrogen of both terraced and unterraced lands was very low as it is the characteristic of these highly weathered tropical agricultural soils (Ferralsols/Oxisols) with very limited N supply from the soil's parental material (Weil and Brady, 2017).

The lack of hillslope positions effects on soil organic matter in terraced land is likely due to homogenization of soil layers by deep excavation (>100 cm depth) during terrace construction (Kagabo et al., 2013; Rutebuka et al., 2021) and reduced soil erosion downward (Blanco-Canqui & Ruis, 2018). The highest values of SOM and associated soil properties at the lower hillslope may be caused by long-term deposition of sediments by water erosion (Van Dijk and Bruijnzeel, 2003). Liu et al. (2006) highlighted the interconnections and negative effects of agricultural practices on soil quality in different climates and soil types mainly due to disturbance of soil structure that has a significant effect on the distribution of C and N, as well as the rates of organic matter decomposition and N mineralization.

The results in this study contradicted our hypothesis which predicted a significant decline of soil organic matter content in terraced compared to non-terraced, due to accelerated microbial decomposition of SOM exposed from deeper soil layers during construction of terraces (Zhao et al., 2021). We observed, significant effects of management and position on hot water-extractable carbon and nitrogen in both terraced and unterraced practices, suggesting that these labile C and N fractions are better indicator to changes in soil conditions compared to total SOM and total nitrogen. Our results showing no effect of terracing on SOM, which contradict some recent studies (Chen et al., 2020; Deng et al., 2021) which reported significant decline of SOM contents in terraced compared to adjacent unterraced lands. This lack of terracing effects is likely due to very low levels of SOM contents studied sites, characterizing the highly weathered acidic tropical ferralsols (Nyssen et al., 2009; Tenywa et al., 2015). As expected, the highest values and more stable soil aggregates were measured lower hillslope position which is likely associated with accumulation of stabilizing organic matter and clay content (Kumar et al., 2014; Yao et al., 2022). The soil in terraced land also had more stable aggregates compared to soil unterraced land, which may indicate soil stabilization and reduced risk of erodibility in bench terraces as previously reported by Kagabo et al. (2013). An increase in available phosphorus at middle and lower hillslope position was also reported by (Fashaho et al., 2020) in terraces of eastern plateau of Rwanda.

5.4.2. Effects of land terracing and hillslope positions on microbial soil quality

Although we generally found relatively greater values for most soil microbial parameters in terraced than unterraced fields, the significant differences were mostly observed at

lower hillslope position, which might be a result of downward soil redistribution during the construction of terraces (Rutebuka et al., 2021). For example, the significant increase of soil microbial biomass in the lower hillslope position of terraced compared to unterraced soils was not sufficient evidence to support our hypothesis of improved soil quality by terracing. It was proposed that higher soil microbial biomass may indicate the increased soil's capacity to cycle and store more nutrients (Ghani et al., 2003; Joergensen, 2010). In this study, higher N mineralization in terraced soils might indicate higher microbial activity. High qCO_2 at upper hillslope position in both terraced and unterraced might indicate a microbial metabolic stress linked to low SOM contents and low soil pH associated with a legacy of downhill transportation of fertile topsoil by erosion. Previous studies conducted in the same agroecological zone (Kagabo et al., 2013; Karamage et al., 2016; Fashaho et al., 2020) have reported the positive effect of land terracing in improving soil quality through minimizing soil erosion. For most analysed parameters in this study, the relatively pronounced effect size and reduced differences between hillslope positions in terraced compared to unterraced soils may indicate, at some extent, soil redistribution and stabilization rather than a direct improvement of soil quality by terracing.

5.4.3. Relationships between physico-chemical and microbial soil properties

The correlation and multivariate analyses showed that physical chemical properties such as wet aggregate stability, percentage of clay particles, hot water-extractable carbon, and were significantly related to soil microbial parameters. For example, the positive relationship between soil microbial soil variables (i.e., MBC, RP and Nmin) and WAS and HWC in terraced land may indicate a stable soil ecosystem with labile C that serves as microbial substrate for activity. It was reported that soil aggregation is stabilized by microbial binding agents and organic matter (Blanco-Canqui & Ruis, 2018). In PCA analysis, the lack of distinct hillslope clusters in terraced compared to unterraced land may indicate that soil has been stabilized to limit the continuous water erosion and downhill deposition or more likely that the soil was so much disturbed that differences were eliminated. In addition, the effects of terracing and hillslope positions that were undetectable with physico-chemical properties such as total N, SOM, texture, pH, WAS, and C/N were sensitively detected by using labile C and N fractions and microbial soil properties. Thus, as previously suggested by many studies, microbial biomass and

activity may be a valuable indicator of soil quality a soil component that is sensitive to management approaches (Dexter, 2004; Singh et al., 2013). Soil microbial biomass and labile C and N fractions often have a very high turnover rate and can respond quickly to changes in management practices (Ghani et al., 2003; Gregorich et al., 1998).

5.5. Conclusion

In this study, we evaluated the effects of land terracing and hillslope position on soil properties and processes which differed between terraced and unterraced lands and varied with hillslope positions. Results suggest the effect of hillslope position rather than land terracing on physico-chemical soil properties such as SOM, total nitrogen, soil pH, available phosphorus, aggregate stability, total exchangeable acidity, and soil textural class. Most of these soil properties increased significantly downslope both in terraced and unterraced fields, where the values of SOM contents, soil pH, proportion of clay particles, available phosphorus, aggregate stability, and total exchangeable acidity were significantly higher in the lower hillslope position of terraced compared to unterraced fields. In contrast, terracing significantly increased the labile C and N fractions (i.e., HWC and HWNtot) and soil microbial parameters (i.e., MBC, MBN, MBP, qmic, and Nmin), which increased downhill with significantly higher values in lower hillslope position than middle and upper hillslope positions both in terraced and unterraced soils. In assessing the influence of management practice and hillslope positions on relationships between soil variables, we found that HWC, MBC, RP, Nmin, and Nitnet were highly sensitive to changes in soil conditions. Overall, the results from this study contradict our hypothesis about the effects of land terracing on physico-chemical soil properties, except for labile C and N contents. The results however agreed with our hypothesis suggesting the soil quality increases with slope gradient as result of the long-term erosional transportation and accumulation of fertile topsoil downhill. Further, the results supported our hypothesis about the labile C and N fractions and microbial parameters being more sensitive than physico-chemical properties in detecting changes in soil conditions caused by management practice and hillslope position. Although some soil variables significantly increased in terraced compared to unterraced fields, the overall findings from this study were not sufficient to explicitly conclude on soil quality improvement by land terracing in the studied sites.

Author Contributions:

Peter Rwibasira: Conceptualization (equal); investigation (lead); formal analysis (lead); writing–original draft (lead), writing–review and editing (equal), visualization (lead). Donat Nsabimana: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Francois Xavier Naramabuye: Conceptualization (equal); writing – review and editing (supporting), funding acquisition (equal); supervision (supporting). Monique Carnol: Conceptualization (equal); formal analysis (supporting); writing–original draft (equal); writing–review and editing (equal); resources (lead); funding acquisition (equal); project administration (lead); supervision (lead).

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CHAPTER 6 – GENERAL DISCUSSION AND CONCLUSIONS

Chapter 6 - General Discussion and Conclusions

The ability of the soil to provide crucial ecosystem services and functions can be adversely affect by human actions, associated with changes in land use and inappropriate management practices (Fentie et al., 2020). Changes in land use and management practices can have contrasting effects on soil quality depending on the climate and soil conditions of the site (Paz-Ferreiro and Fu, 2016). This thesis investigated the long-term effects of tree species and land terracing on soil properties and microbiological processes, with the aim to understand how these land restoration practices have affected soil quality. We expected (i) an improvement of soil quality under native and agroforestry tree species compared to Eucalyptus species, due to soil acidification and litter recalcitrance under eucalyptus species; and (ii) differences in aggregate stability and soil quality along hillslope positions between terraced than unterraced lands, due to the disturbance of soil structure by terracing and downslope soil losses by water erosion. To test these hypotheses, soil variables (e.g., soil pH, exchangeable cations, SOM contents, microbial properties and processes) were assessed in two topsoil layers (i.e., 0–5 cm and 5–10 cm depth) under replicated planted monoculture plots of eight tree species and in a self-regenerated mixed species plot, within the arboretum of Ruhande in southern Rwanda. The effects terracing on selected physico-chemical and microbial properties were assessed at three hillslope positions in three paired terraced-unterraced fields. Main results show the soil acidification and high SOM accumulation under eucalyptus species, whereas native species alleviate soil acidity and improve the concentrations of base cations. In assessing the effectiveness of land terracing for improving soil quality, the results show that hillslope position rather than terracing practice influenced most physico-chemical properties, while most microbial soil parameters are influenced by both land terracing and hillslope positions. The high sensitivity of soil microbial properties and labile fractions of C and N to changes in tree species and terracing practice suggests the use of these parameters as indicators to monitors management-induced changes in soil quality.

6.1. Long-term effects of planted tree species on soil quality

Because various biotic and abiotic characteristics often co-vary when assessing changes in soil quality at different sites (van Leeuwen et al., 2019), we studied the long-term effects of forest tree species planted on one site, the arboretum of Ruhande, Rwanda, to exclude the effects of confounding factors. Overall, our findings of tree species effects on soil properties and processes showed higher values, more significant differences between tree species, and stronger relationships between soil variables in the upper (0–5 cm) compared to the lower (5–10 cm) soil layer. These results highlight the importance of the thin (0–5 cm) topsoil layer in nutrient cycling and the quality of tropical soils. It was previously reported that the fertility of tropical forest soil mainly depends on the efficient internal nutrient cycling resulting from the optimal conditions for microbial decomposition of plant litter that mainly takes place in the thin upper soil layer (Pabst et al.2013; Sayer & Banin, 2016). Therefore, unless otherwise specified, the discussion about tree species effects on soil variables mainly focussed on the 0–5 cm soil layer. Globally, the effects of tree species on soil quality as assessed by soil chemistry and microbial processes indicated that native tree species improved soils through increasing both chemical and microbial indicators of soil quality. On the other hand, eucalyptus significantly caused soil acidification, but increased SOM contents and did not adversely affect microbial biomass and activity as expected. However, that the natural acidity of soils in the study site and many parts of the country, the issues of soil fertility and Al toxicity to plants under acidic conditions should be cautiously taken into consideration before planting eucalyptus species.

6.1.1. Effects of tree species on chemical soil quality

In tropical soils with inherently poor chemical properties (Celentano et al., 2011), planting trees is one of the key strategies for improving degraded soils. Overall, significant changes in soil chemical properties (e.g., pH, SOM, labile C and N fractions, and exchangeable base cations) were mostly pronounced in the thin upper soil layer (0–5 cm) across tree species, and the labile C and N fractions were more sensitive to changes in tree species than total soil organic matter (Chapter 2). The fertility of tropical forest soil is highly dependent on the continuous supply of organic materials produced by growing plants (Lynch, 1995; Bauhus & Khanna, 1999) and the rapid decomposition of the litter facilitated by optimum temperature, moisture conditions throughout the year (Krishna

and Mohan, 2017). The above conditions may lead to a rapid internal cycling of nutrients by high microbial activity, generally constrained to a thin upper soil layer (Bauters et al., 2017b). Eucalyptus species led to soil acidification and accumulation of soil organic matter while native species increased soil pH and nutrient concentrations. Consistent with our hypothesis, Eucalyptus species significantly acidified the soil by a decrease in 0.6 pH unit compared to exotic agroforestry species (*Calliandra calothyrsus*, *Cedrela serrata*, and *Grevillea robusta*) and 1.7 pH unit compared to native species (*Entandrophragma excelsum*, *Polyscias fulva*, and self-regenerated mixed natives).

The soil acidifying effects of eucalyptus species were reported in previous studies conducted at this site and nearby forest plantations (Nsabimana et al., 2008; Mugunga et al., 2015), in other tropical (Behera and Sahani, 2003; J.-P. Laclau et al., 2010) as well as non-tropical regions (Rhoades and Binkley, 1996). Previous studies also reported high sensitivity labile C and N fractions compared to the total SOM for detecting changes in soil conditions, and thus suggested the use of water soluble and hot water-extractable C and N fractions as proxies for soil microbial biomass and activity (Curtin et al., 2021; Ghani et al., 2003). High accumulation of soil organic matter under Eucalyptus species might be explained by reduced activity of soil microorganisms under acidic conditions. As expected, we found high concentrations of exchangeable Al^{3+} and Fe^{2+} under eucalyptus species compared to other species. As fast-growing species, eucalyptus exhibit high uptake and storage of base cations, and release of protons by roots to maintain ionic balance of soil (Augusto et al., 2002; Bauters et al., 2017b). Furthermore, acidic conditions (pH<5.0) increase the solubility of reactive Al^{3+} which can interfere with microbial processes and lead to toxic effects on soil microorganisms and plants (Fujii, 2014; Singh et al., 2017). Considering that eucalyptus species now account for around 89% of forest plantations in Rwanda (RFA, 2021), planting more of these species poses a high risk for further soil acidification of these inherently acid and low fertile tropical soils, which may profoundly affect soil quality and plant productivity. We demonstrated in this study the benefits of planting native species including *Entandrophragma excelsum* and *Polyscias fulva* for improving chemical soil quality through increased soil pH and concentration of base cations, in addition to their long-known valuable timber and many uses in local traditional medicine (Ndoli et al., 2021). Although farmers prefer eucalyptus due to their economic benefits within a relatively short period of time (approximately six years from

planting to potential harvest), afforestation programs should provide planting materials and advocate for planting native species to improve soil quality and diversify plantations for the sustainability of forest resources.

6.1.2. Effects of tree species on soil microbial properties and processes

Soil microbial parameters are often more responsive to changes in land use than physicochemical properties (Temesgen et al., 2019), thus evaluating microbial properties and processes can help in timely detection of the changes in soil quality. We assessed soil microbial parameters including microbial biomass, respiration, N mineralization, nitrification, and ecophysiological indices, which were generally influenced by tree species both in the 0 – 5 cm and 0 – 5 cm soil layers (Chapter 3).

The long-term effects of tree species on soil microorganisms and their activity have rarely been studied in experimental forest plantations, particularly with introduced tree species in the tropics (Veldkamp et al., 2020). Soil microorganisms play a determinant role in biogeochemical cycling of elements and availability of nutrients to plants (Aponte et al., 2013; Lathwell and Grove, 2011). Thus, assessing the response of soil microbial properties and processes is essential for understanding the long-term effects of afforestation tree species on soil quality and functioning.

The findings of this study were partially consistent with our hypothesis (Chapter 3), suggesting highest values (i.e., 2–12 times higher) and pronounced tree species effects on soil microbial properties and processes (e. g., microbial biomass, soil respiration, and N mineralization) in the 0–5 cm compared to the 5–10 cm soil layer. In contrast to our expectations, we found higher microbial biomass under Eucalyptus species compared to most of other species. These results were unexpected because of relatively higher acidity found under these eucalyptus species (Nsabimana et al., 2009; Rwibasira et al., 2021), and considering the reported significant decrease in soil microbial biomass due to increased soil acidity under eucalyptus plantations (Temesgen et al., 2016). Although high levels of soil microbial biomass are often considered as an indicator for good soil quality (Babur et al., 2021a; Bardgett, 2005), the higher soil microbial biomass under Eucalyptus measured this study, might not necessarily reflect improved soil quality. It is likely that the extent of soil acidification by eucalyptus species was not deleterious to the growth of microorganisms, which might be adapted to acidic soils in this tropical region

(Nelson and Su, 2010). Furthermore, the observed high accumulation of SOM under eucalyptus could serve as the main source for energy and C substrate for activity, and thus promoting the growth and maintenance of soil microorganisms in these nutrient-poor soils (Malý et al., 2014; Taylor et al., 2021). This is supported by a significant positive relation between SOM and labile carbon with microbial biomass. The observed differences between eucalyptus species in terms of soil respiration and nitrogen mineralization indicate that we cannot generalize the effects of Eucalyptus species on soil microbial properties. In this study, *Eucalyptus grandis* significantly increased soil respiration, while *Eucalyptus maidenii* increased Nmin but reduced RP and *Eucalyptus saligna* significantly reduced Nmin. The reported high lignin content in *Eucalyptus grandis* (Bini et al., 2013) as well as the high litter C/N ratios in *Eucalyptus maidenii* ratios (Demessie et al., 2012; Cizungu et al., 2014) might have influenced the differences between these species in soil organic matter mineralization (Luo & Zhou, 2006; Pietri et al., 2008).

The highest levels of microbial activity observed in the soil under *Calliandra calothyrsus* (an exotic nitrogen-fixing species) may indicate enhanced soil quality, which support the use of this species in agroforestry systems (Kisaka et al., 2023). However, this species had significantly lower MBC and high specific respiration (qCO_2), which may indicate a physiological stress of soil microorganisms (Anderson and Domsch, 2010), possibly caused by litter recalcitrance resulting from high concentrations of condensed tannins in the leaves of this species (Temesgen et al., 2019). Although greater MBC are often related with enhanced soil respiration (Babur et al., 2021a), negative relation between MBC and RP, as shown for *Calliandra calothyrsus*, were also reported in a previous study conducted in a tropical forest plantation (Temesgen et al., 2019).

Effects of planting native tree species (*Entandrophragma excelsum* and *Polyscias fulva*) showed significantly improved soil quality as indicated by high microbial biomass, Nmin, qmic, and low qCO_2. The results from these monospecific stands of native species were approximately similar to the values measured in self-regenerated native forest, which is mainly dominated by mature *Polyscias fulva* trees (Mugunga et al., 2022). Compared to monoculture plots of exotic species, there was more understorey vegetation under both monospecific and self-regenerated native species, which in part may indicate the

availability of quality substrate (Xu et al., 2007), reflecting the ecological importance and soil quality improvement by native species (Bauhus et al., 1998; Chen et al., 2018).

6.1.3. The contribution of ammonia-oxidising archaea (AOA) and bacteria (AOB) to soil nitrification under different tree species

Nitrification is a critical process in nitrogen cycling, which may contribute to substantial losses of nitrogen from terrestrial ecosystems via nitrate leaching and losses of nitrogen in gaseous forms due to denitrification (Gineyts and Niboyet, 2023). Ammonia-oxidation, the rate-limiting step of nitrification is driven by ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Tao et al., 2021). The use of a selective inhibitor of AOB nitrification (ATU) allowed us to quantify the relative contributions of AOA and AOB to soil nitrification (Taylor et al., 2010), while the quantitative analysis of *amoA* gene reflected the abundance of these nitrifiers (Prosser and Nicol, 2008). To the best of our knowledge, this is the first study to evaluate the effects of planted tree species on the abundance of AOA and AOB and their nitrification activity in a forest plantation in Rwanda.

In agreement with our hypothesis (Chapter 4), AOA were the main nitrifiers under the different tree species, with AOA and AOB contributing approximately 71% and 29%, respectively, to the total potential nitrification rates. We demonstrated the significant effects of tree species on the abundance and activity of nitrifiers, with significant functional dominance of AOA over AOB across the tree species studied. As expected, we found more pronounced effects of tree species on potential nitrate production (PNR) in the thin upper (0–5 cm) than in the lower (5–10 cm) soil layer. About 5- and 2-times higher nitrification rates of AOA and AOB, respectively, were measured in (0–5 cm) compared to (5–10 cm) soil layer soil layer, possibly due to continuous litterfall and mineralization supplying ammonia substrate to nitrifiers (Xiao et al., 2017).

The relationship between the abundance of ammonia-oxidizers, nitrification rates, and their main controlling factors (i.e., pH, Nmin, and HWNtot) depended on soil layers, suggesting that soil conditions are very important in determining the presence and contribution of AOA and AOB to nitrification in acidic tropical soils (Ste-Marie and Paré, 1999; Watanabe et al., 2023). Low soil pH measured under most species (pH <4.9) might have reduced the availability of substrate, giving a competitive advantage to AOA as the dominant ammonia oxidizer while increasing maintenance and physiological stress for

AOB (Trivedi et al., 2019). Tree species that had highest soil nitrification rates for both AOA and AOB include *Polyscias fulva* (native species), *Calliandra calothyrsus* (nitrogen-fixing agroforestry species), and *Eucalyptus grandis,* and were associated with either less acid soil or high concentration of N substrate. For example, *Polyscias fulva* significantly increased both soil pH and concentration of labile nitrogen (HWNtot), while *Calliandra calothyrsus* and *Eucalyptus grandis* were associated with high concentration of HWNtot soils (Rwibasira et al., 2021). In contrast to our expectations, *Entandrophragma excelsum* (native species) significantly increased both soil pH and HWNtot but showed the lowest rates of AOA and AOB nitrification at similar levels of soil acidifying eucalyptus spp. (i.e., *Eucalyptus maidenii* and *Eucalyptus saligna*). We suspected the presence of chemical compounds (e.g., terpenoids) released from these species which could inhibit nitrification (White, 1986; Sauder et al., 2016).

The quantitative PCR analyses targeting *amoA* marker gene showed that tree species and soil layer significantly influenced the abundance of AOA and AOB. We observed the numerical dominance of AOA over AOB with a wider range in 5 – 10 cm (50 times higher) compared to (5 times higher) in 0–5 cm soil layer. While AOA have been reported to be adapted to greater ecological and functional ranges compared to AOB under acidic and nutrient-poor soil conditions (Erguder et al., 2009), it is likely that AOB were substantially influenced by tree species-induced changes in soil pH and N substrate availability, often varying between soil layers (Watanabe et al., 2023).

The observed negative correlation between AOB abundance and their corresponding PNR might be explained by very low rates of AOB nitrification, while the positive relationship between AOA–PNR and AOA–amoA might be explained by their high affinity for ammonium substrate (Hink et al., 2018). Given that the abundance of nitrifiers may not always reflect their nitrification activity (Leininger et al., 2006), assessing the specific nitrification rate (PNR per *amoA* gene copy) can give indication about the nitrification efficiency (Prosser & Nicol, 2012). Furthermore, difference in AOA and AOB specific PNR between two soil layers may reflect the niche differentiation related to ammonium substate and oxygen availability (Ginestet et al., 1998; Trivedi et al., 2019).

Our results of correlations and multivariate analysis demonstrated the tree species effects and soil layer on ammonia-oxidizers and nitrification activity through changes in

soil pH and nitrogen transformation proxies (i.e., Nmin and HWNtot). Overall, the observed opposite relationships between PNR and Nmin in two soil layers might explain the importance of Nmin in providing substrate for nitrification, as well as the niche differentiation giving a competitive advantage to AOA due to their high substrate affinity (Prosser and Nicol, 2012; Trivedi et al., 2019). The PCA analysis distinctively associated tree species that had less soil acidifying effects and high N substrate concentrations (i.e., *Polyscias fulva*, *Grevillea robusta*, and *Calliandra calothyrsus*) with high loadings of AOA and AOB abundance and activity.

6.2. Effects of land terracing on soil properties and processes

Although terracing practices are applied for the protection of soils against water erosion and for increasing arable land surfaces in mountainous landscapes (Karamage et al., 2017), the mechanical disruption of soil structure, decline of organic matter stocks and associated consequences on soil quality may be some disadvantages of terraced lands (Deng et al., 2021). The evaluation of terracing effects on soil properties and processes from three agricultural sites in southern Rwanda revealed that not only terracing, but also hillslope position (upper, middle, and lower) influenced soil quality.

The observed differences in proportions of soil particle size between terraced and unterraced fields might be explained by the reduced downhill movement of easily erodible fine textured soil particles in terraced land (Chapter 5). This was evidenced by significant differences between upper and lower hillslope positions in proportions of sand and clay particles. The higher values of pH and SOM contents at lower hillslope position both in terraced and unterraced soils were likely due to the legacy of erosional downward transportation of sediments and accumulation of SOM and the leaching of base cations downhill (J. Mbonigaba et al., 2009). Although there was no hillslope positions effect on SOM in terraced soils, the significant effects of management and position on hot water-extractable carbon and nitrogen in both terraced and unterraced practices might indicate that these labile C and N fractions are better indicators to changes in soil conditions compared to total SOM and total nitrogen. Consistent with our hypothesis, higher values and more stable soil aggregates in terraced land may indicate soil stabilization and reduced risk of erodibility in bench terraces as previously reported by Kagabo et al. (2013). Our results of increased available phosphorus downhill was also reported by (Fashaho et al., 2020) in terraces of eastern plateau of Rwanda.

Overall, this study demonstrated that hillslope position rather than management practice influenced differences in soil quality indicators between terraced and unterraced soils. The observed greater effect size and reduced differences between hillslope positions in terraced compared to unterraced land could be explained by soil redistribution and reduced soil loss downhill (Li and Lindstrom, 2001; Ni and Zhang, 2007). Our analyses of results from this study did not corroborate the previous studies (Kagabo et al., 2013; Karamage et al., 2016; Fashaho et al., 2020), which reported effectiveness of land terracing in restoring soil quality of Rwandan steeped croplands.

6.3. Future perspectives

The effectiveness of soil restorative measures (e.g., afforestation and land terracing) should be experimentally explored and confirmed through research before implementation, in order to avoid worsening the land degradation processes (Ghosh et al., 2021; Baradwal et al., 2022). Yet, soil conservation measures are often applied without in-depth knowledge of potential consequences, especially in developing countries (Melaku et al., 2018). Given that soil restoration processes generally tend to be too slow, in comparison to current global rates of soil degradation (Baradwal et al., 2022), it is essential to carefully choose appropriate land management practices for sustainably improving soil quality (Sileshi et al., 2019).

In Rwanda, like in most African countries, the lack of baseline values and soil data from long-term monitoring in relation to the effectiveness of restorative measures implemented over past decades can be the challenge for planning and implementing future land use policy. We were confronted with the scarcity of baseline data to evaluate the changes in soil characteristics since the establishment of studied forest stands or agricultural terraces, especially for soil microbial parameters. To the best of our knowledge, this is the first study to integrate a relatively substantial number of physico-chemical and microbial indicators of soil quality to evaluate the effectiveness of the two common practices in Rwanda (agricultural terraces and forest plantation) for restoration of degraded soils. The findings of this study can have broad applications for assessing the effects of the choice of land management practices in the context of highly degraded soils and increasing demand for agricultural land and forest resources.

The results from tree species comparison raised novel insights for not only their varied effects on different soil quality indicators, but also for the thin upper (0 – 5 cm) soil layer at which significant differences between tree species were mostly observed compared to the lower (5 – 10 cm) layer. For example, the significant differences between species in some chemical soil properties found in this study were not found in previous studies under the same stands (Nsabimana et al., 2008; Nsengumuremyi et al., 2022), likely because they sampled relatively thicker (0 – 10 cm) soil layer which might have diluted the effects of tree species. Further, we demonstrated that the studied three eucalyptus species differed in their effects on most soil quality indicators. Except for their common effects on soil acidification (Chapter2; Rwibasira et al., 2021), their lack of detrimental effects on soil microbial parameters contradicted the hypothesis of this study (Chapter 3). The current environmental policy in Rwanda prohibits planting eucalyptus species in agricultural fields and in proximity to water bodies as they are believed to excessively reduce soil fertility, water resources and soil biodiversity (Mugunga, 2016; Ndoli et al., 2021). However, the results from this study suggested that we should not generalize over the adverse effects of eucalyptus species, which opens new research perspectives to explore the effects of more eucalyptus species on soil quality. The study site (Arboretum of Ruhande) contains 69 species of eucalyptus, among which only about 10 species are commonly planted countrywide. Therefore, this study could trigger curiosity to explore more eucalyptus species that remained hidden in this site and provide research-based data on their effects on soil quality and ecosystem functioning.

Improvement of soil quality in steep cropland were recently reported in Rwanda (Kagabo et al., 2013; Karamage et al., 2016; Fashaho et al., 2020). In contrast, our results that simultaneously evaluated both the effects of management practice and hillslope position on soil properties and microbial processes revealed that hillslope position rather than land terracing significantly influenced changes in soil quality (Chapter 5). Long-term monitoring of newly terraced fields based on baseline values before terracing, by considering different elevation gradients and agro-ecological zones.

6.4. General conclusions

Afforestation and land terracing are the major land conservation strategies widely adopted in Rwanda to address environmental and socio-economic consequences associated with land degradation and decline of soil quality. We investigate the effects of planted tree species (i.e., eight monospecific stands and a plot of self-regenerated mixed native species) and land terracing (three sites) on soil properties and processes which may reflect changes in soil quality in the Central Plateau agro-ecological zone, Southern Rwanda.

Our results demonstrated that the effects of tree species on soil parameters were generally pronounced in the 0–5 cm soil layer which had significantly higher values than 5–10 cm soil layer for most analysed soil properties and processes. We reported soil acidifying and SOM accumulation effects of eucalyptus species, while native species were found to alleviate soil acidity and increase the concentrations of base cations. Labile fractions of C and N, particularly those extracted with hot water were better indicators of tree species-induced changes in soil conditions compared to the total SOM. The analyses of tree species effects on soil microbial properties and processes revealed that microbial biomass and activity were not adversely affected by soil acidifying eucalyptus species, likely because of high concentrations of labile C and N in soils under these species, fractions found to be the main drivers of the measured soil processes. Soil quality was high under studied native trees (i.e., *Entandrophragma excelsum*, *Polyscias fulva*, and mixed native species), possibly due to availability of quality substrate under these species as evidenced by high microbial biomass, nitrogen mineralization, microbial quotient, and low metabolic quotient. Further, results from tree species effects on abundance and activity of ammonia-oxidizing archaea (AOA) and bacteria (AOB) indicate the dominance of AOA over AOB in terms of abundance and rates of nitrification across tree species planted on acidic and nutrient-poor soil. This study showed that planting native species (e.g., *Polyscias fulva*), Eucalyptus grandis, and agroforestry species (e.g., *Grevillea robusta* and *Cedrela serrata*) significantly increased both AOA and AOB nitrification rates. Tree species influenced the abundance and activity of nitrifiers via changes in soil pH and N substrate availability. The assessment of land terracing effects on soil properties and processes indicated that hillslope position rather than terracing practice influenced most physico-chemical properties, while most microbial soil parameters were influenced by both land terracing and hillslope positions. Generally, we observed highest values in

lower hillslope position for most soil quality indicators assessed, which likely imply the long-term erosional transportation and accumulation of sediments downhill. In contrast to our expectation, land terracing did not significantly reduce soil organic matter contents, possibly due to very low levels of SOM contents naturally characterizing the studied sites.

Appendices

Table 2.A 1. Selected study plots in the Arboretum of Ruhande, Rwanda.

Plot ID	Species	Native/ Exotic	Latitude	Longitude	Elevation	Age in 2016 (Years)
Plot273	*Calliandra calothyrsus*	Exotic	02°36′69′′ S	29°45′30′′ E	1722 m	31
Plot265	*Calliandra calothyrsus*	Exotic	02°36′71′′ S	29°45′18′′ E	1713 m	31
Plot267	*Calliandra calothyrsus*	Exotic	02°36′72′′ S	29°45′21′′ E	1714 m	31
Plot56	*Cedrela serrata*	Exotic	02°36′94′′ S	29°44′79′′ E	1713 m	70
Plot111	*Cedrela serrata*	Exotic	02°36′75′′ S	29°45′60′′ E	1709 m	79
Plot36	*Cedrela serrata*	Exotic	02°36′83′′ S	29°45′30′′ E	1730 m	73
Plot150	*Grevillea robusta*	Exotic	02°36′97′′ S	29°44′96′′ E	1713 m	75
Plot322	*Grevillea robusta*	Exotic	02°36′94′′ S	29°45′19′′ E	1709 m	69
Plot104	*Grevillea robusta*	Exotic	02º36′84′′ S	29º45′51′′ E	1720 m	35
Plot218	*Eucalyptus grandis*	Exotic	02°37′03′′ S	29°44′83′′ E	1707 m	70
Plot220	*Eucalyptus grandis*	Exotic	02°37′05′′ S	29°44′86′′ E	1706 m	65
Plot181	*Eucalyptus grandis*	Exotic	02°36′65′′ S	29°45′64′′ E	1680 m	65
Plot179	*Eucalyptus maidenii*	Exotic	02°36′66′′ S	29°45′61′′ E	1685 m	70
Plot377	*Eucalyptus maidenii*	Exotic	02°36′59′′ S	29°45′32′′ E	1695 m	82
Plot1	*Eucalyptus maidenii*	Exotic	02°36′89′′ S	29°44′78′′ E	1732 m	67
Plot472	*Eucalyptus saligna*	Exotic	02°37′01′′ S	29°45′12′′ E	1710 m	82
Plot259	*Eucalyptus saligna*	Exotic	02°36′93′′ S	29°45′38′′ E	1709 m	36
Plot20	*Eucalyptus saligna*	Exotic	02°36′89′′ S	29°45′06′′ E	1729 m	59
Plot78	*Entandrophragma excelsum*	Native	02°36′90′′ S	29°45′12′′ E	1727 m	67
Plot44	*Entandrophragma excelsum*	Native	02°36′81′′ S	29°45′42′′ E	1727 m	64
Plot54	*Entandrophragma excelsum*	Native	02°36′78′′ S	29°45′57′′ E	1718 m	45
Plot240	*Polyscias fulva*	Native	02°36′96′′ S	29°45′15′′ E	1714 m	80
Plot262	*Polyscias fulva*	Native	02°36′91′′ S	29°45′46′′ E	1695 m	80
Plot268	*Polyscias fulva*	Native	02°36′88′′ S	29°45′54′′ E	1693 m	80
MNS1	Mix native species	Native	02°36′65′′ S	29°44′65′′ E	1700 m	83
MNS1	Mix native species	Native	02°36′68′′ S	29°45′51′′ E	1692 m	83
MNS3	Mix native species	Native	02°36′59′′ S	29°45′63′′ E	1680 m	83

Table 2.A 2. Summary description of measured soil properties for two soil layers (0–5 cm and 5–10 cm) across all samples: two samples per plot, eight tree species, one mixed natives plot.

Soil Parameters	Layer	N	Mean	SD	Median	Min	Max	Skew	Kurtosis	SE
pH_{KCL}	0–5 cm	54	4.89	0.71	4.96	3.71	5.89	−0.33	−1.38	0.1
	5–10 cm	54	4.21	0.36	4.24	3.71	4.83	0.04	−1.25	0.05
SOM (%)	0–5 cm	54	22.49	4.71	20.55	15.53	31.32	0.63	−1.03	0.64
	5–10 cm	54	9.6	1.4	9.87	5.93	11.56	−0.95	0.21	0.19
SOC (gkg^{-1})	0–5 cm	54	130.4	27.31	119.2	90.1	181.66	0.63	−1.04	3.72
	5–10 cm	54	55.67	8.13	57.22	34.41	67.06	−0.94	0.2	1.11
WSC ($mgkg^{-1}$)	0–5 cm	54	340.8	115.1	323	175.2	683.5	1.1	1.05	15.6
	5–10 cm	54	56.31	9.41	55.9	39.77	78.74	0.36	−0.61	1.28
$WSNO_3$ ($mgkg^{-1}$)	0–5 cm	54	67.7	28.82	58.13	32.77	145.64	1.34	0.93	3.92
	5–10 cm	54	12.31	4.68	12.3	3.93	20.82	0.18	−1.07	0.64
$WSNH_4$ ($mgkg^{-1}$)	0–5 cm	54	12.85	2.97	12.97	7.62	18.34	−0.12	−1.28	0.4
	5–10 cm	54	1.23	0.67	0.95	0.47	2.9	0.96	−0.22	0.09
WSN_{org} ($mgkg^{-1}$)	0–5 cm	54	34.73	9.74	34.41	15.84	57.02	0.22	−0.51	1.32
	5–10 cm	54	6.85	1.74	6.56	2.99	10.42	0.03	−0.48	0.24
WSNmin ($mgkg^{-1}$)	0–5 cm	54	80.54	29.07	70.65	45.01	162.93	1.47	1.38	3.96
	5–10 cm	54	13.54	4.34	13.4	5.53	21.33	0.22	−1.2	0.59
WSNtot ($mgkg^{-1}$)	0–5 cm	54	115.2	29.68	111.2	65.1	190.1	0.97	0.63	4.04
	5–10 cm	54	20.39	4.86	20.38	12.86	28.78	0.1	−1.48	0.66
WSC/WSN_{org}	0–5 cm	54	10.07	2.86	9.28	5.89	18.79	1.13	1.33	0.39
	5–10 cm	54	8.66	2.21	8.43	4.61	15.5	0.96	1.55	0.3
WSC/WSNtot	0–5 cm	54	3.11	1.2	2.71	1.34	6.39	0.86	0.19	0.16
	5–10 cm	54	2.88	0.66	2.87	1.5	4.43	0.02	−0.63	0.09
HWC ($mgkg^{-1}$)	0–5 cm	54	3994.6	1201.2	3904.7	2203.4	5893.4	0.11	−1.51	163.4
	5–10 cm	54	603.43	119.8	601.8	382.16	888.07	0.48	−0.33	16.31
$HWNO_3$ ($mgkg^{-1}$)	0–5 cm	54	14.13	4.86	14.11	4.57	23.36	-0.03	−0.79	0.66
	5–10 cm	54	2.22	0.85	2.01	0.83	3.92	0.32	−0.94	0.12
$HWNH_4$ ($mgkg^{-1}$)	0–5 cm	54	71.7	18.6	71.05	37.07	104.15	-0.01	−0.95	2.53
	5–10 cm	54	8.35	3.15	7.97	4.18	14.97	0.5	−0.91	0.43
HWN_{org} ($mgkg^{-1}$)	0–5 cm	54	301.64	62.12	294.93	194.96	440.04	0.18	−1.2	8.45
	5–10 cm	54	55.44	16.45	53.03	31.94	95.43	0.9	−0.04	2.24
HWNtot ($mgkg^{-1}$)	0–5 cm	54	387.47	73.9	388.71	242.65	536.49	−0.1	−1	10.06
	5–10 cm	54	66.01	19.36	61.47	40.02	111.71	0.9	−0.01	2.63
HWC/HWN_{org}	0–5 cm	54	13.08	2.12	12.64	10.08	17.6	0.37	−1.14	0.29
	5–10 cm	54	11.3	2.15	10.93	8.52	17.22	0.86	0.03	0.29
HWC/HWNtot	0–5 cm	54	10.2	1.91	9.85	7.45	13.77	0.34	−1.26	0.26
	5–10 cm	54	9.48	1.84	9.16	7.01	14.21	0.89	0.09	0.25
Al^{3+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.06	0.05	0.04	0.00	0.16	0.93	−0.31	0.01
	5–10 cm	54	1.22	0.9	0.94	0.01	2.82	0.38	−1.37	0.12
Ca^{2+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	28.5	7.89	27.27	17.39	52.9	1.42	1.74	1.07
	5–10 cm	54	5.97	3.55	5.97	1.54	16.6	1.02	0.81	0.48
Fe^{2+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.00	0.00	0.00	0.00	0.01	1.22	0.89	0.00
	5–10 cm	54	0.01	0.03	0.00	0.00	0.25	6.93	46.94	0.00
K^{+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.61	0.17	0.57	0.31	0.96	0.29	−0.94	0.02
	5–10 cm	54	0.21	0.14	0.15	0.08	0.56	1.47	0.81	0.02
Mg^{2+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	6.9	2.87	5.67	4.45	15.17	1.74	1.94	0.39
	5–10 cm	54	1.51	0.55	1.49	0.67	2.34	0.05	−1.42	0.07
Mn^{2+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.09	0.07	0.06	0.02	0.29	1.22	0.68	0.01
	5–10 cm	54	0.01	0.01	0.01	0.00	0.03	0.35	−0.88	0.00
Na^{+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.15	0.06	0.14	0.07	0.28	0.32	−1.16	0.01
	5–10 cm	54	0.18	0.03	0.19	0.09	0.24	-0.74	0.86	0.00
Zn^{2+} ($cmol_c\ kg^{-1}$)	0–5 cm	54	0.00	0.00	0.00	0.00	0.00	1.08	0.57	0.00
	5–10 cm	54	0.00	0.00	0.00	0.00	0.00	0.96	−0.28	0.00
EBC ($cmol_c\ kg^{-1}$)	0–5 cm	54	36.16	10.46	33.96	23.98	69.18	1.75	2.39	1.42
	5–10 cm	54	7.87	4.1	7.75	2.71	19.27	0.88	0.39	0.56
∑cations ($cmol_c\ kg^{-1}$)	0–5 cm	54	36.31	10.41	34.07	24.32	69.24	1.77	2.42	1.42
	5–10 cm	54	9.11	3.31	8.62	5.08	19.31	1.29	1.33	0.45

Table 2.A 3. Analyses of soil chemical soil properties under different tree species in the Arboretum of Ruhande (means ± SEM, n=6).

Soil Parameters	Layer (cm)	*Calliandra calothyrsus*	*Cedrela serrata*	*Grevillea robusta*	*Eucalyptus grandis*	*Eucalyptus maidenii*	*Eucalyptus saligna*	*Entandrophragma excelsum*	*Polyscias fulva*	Mixed Native species
pH_{KCl}	0–5	4.9 ± 0.01 ^{fg}	4.9 ± 0.01 ^{fg}	5.5 ± 0.02 ^{h}	4.0 ± 0.01 ^{b}	4.2 ± 0.01 ^{c}	3.7 ± 0.01 ^{a}	5.5 ± 0.02 ^{h}	5.8 ± 0.01 ^{i}	5.4 ± 0.04 ^{h}
	5–10	4.2 ± 0.0 ^{c}	4.2 ± 0.0 ^{c}	4.4 ± 0.0 ^{d}	3.8 ± 0.0 ^{a}	3.8 ± 0.0 ^{a}	3.7 ± 0.0 ^{a}	4.4 ± 0.0 ^{d}	4.6 ± 0.0 ^{e}	4.8 ± 0.0 ^{f}
SOM (%)	0–5	19.1 ± 0.52 ^{bc}	18.1 ± 0.81 ^{b}	19.2 ± 0.26 ^{bc}	21.6 ± 0.59 ^{bcd}	27.6 ± 0.92 ^{d}	25 ± 1.33 ^{cd}	25.8 ± 2.24 ^{d}	18.5 ± 0.46 ^{b}	27.5 ± 2.2 ^{d}
	5–10	10.2 ± 0.1 ^{a}	9.19 ± 1.0 ^{a}	8.93 0.7 ^{a}	10.3 ± 0.5 ^{a}	9.58 ± 0.08 ^{a}	9.27 ± 0.4 ^{a}	9.5 ± 0.5 ^{a}	10.3 ± 0.2 ^{a}	9.06 ± 0.7 ^{a}
SOC (g kg^{-1})	0–5	110 ± 3 ^{bc}	100 ± 4.7 ^{b}	110 ± 1.6 ^{bc}	160 ± 5.4 ^{d}	140 ± 7.7 ^{cd}	150 ± 13 ^{d}	160 ± 13 ^{d}	110 ± 2.7 ^{b}	130 ± 3.4 ^{bcd}
	5–10	59 ± 0.57 ^{a}	53 ± 6 ^{a}	52 ± 4.3 ^{a}	56 ± 0.52 ^{a}	54 ± 2.8 ^{a}	55 ± 3.2 ^{a}	53 ± 4.1 ^{a}	60 ± 1.3 ^{a}	60 ± 3 ^{a}
Al^{3+}	0–5	0.079 ± 0.00 ^{a}	0.085 ± 0.01 ^{a}	0.026 ± 0.00 ^{a}	0.006 ± 0.00 ^{a}	0.067 ± 0.01 ^{a}	0.04 ± 0.00 ^{a}	0.15 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}
($cmol_c$ kg^{-1})	5–10	0.93 ± 0.035 ^{d}	1.2 ± 0.036 ^{e}	0.77 ± 0.04 ^{d}	0.027 ± 0.00 ^{a}	2.6 ± 0.083 ^{h}	2.2 ± 0.05 ^{f}	2.4 ± 0.059 ^{g}	0.34 ± 0.01 ^{b}	0.53 ± 0.01 ^{c}
Ca^{2+} ($cmol_c kg^{-1}$)	0–5	19 ± 0.77 ^{e}	24 ± 1.3 ^{fg}	28 ± 0.33 ^{hi}	27 ± 0.45 ^{gh}	29 ± 0.59 ^{hi}	31 ± 1.6 ^{i}	23 ± 0.85 ^{f}	28 ± 0.25 ^{hi}	48 ± 1.2 ^{j}
	5–10	6 ± 0.06 ^{bc}	4.8 ± 0.067 ^{abc}	7 ± 0.47 ^{bc}	14 ± 0.64 ^{d}	1.6 ± 0.026 ^{a}	3.8 ± 0.097 ^{ab}	2.2 ± 0.081 ^{a}	7.8 ± 0.15 ^{c}	6.7 ± 0.18 ^{bc}
Fe^{2+} ($cmol_c kg^{-1}$)	0–5	0.004 ± 0.00 ^{ef}	0.001 ± 0.00 ^{ab}	0.001 ± 0.00 ^{a}	0.0008 ± 0.00 ^{a}	0.002 ± 0.00 ^{abcd}	0.002 ± 0.00 ^{abc}	0.002 ± 0.00 ^{abcd}	0.001 ± 0.00 ^{ab}	0.001 ± 0.00 ^{a}
	5–10	0.003 ± 0.0 ^{bcdef}	0.003 ± 0.00 ^{abcdef}	0.004 ± 0.00 ^{def}	0.0028 ± 0.00 ^{abcdef}	0.005 ± 0.00 ^{f}	0.045 ± 0.04 ^{cdef}	0.004 ± 0.00 ^{def}	0.003 ± 0.00 ^{abcde}	0.003 ± 0.00 ^{abcde}
K^{+}	0–5	0.63 ± 0.023 ^{efg}	0.52 ± 0.016 ^{cde}	0.7 ± 0.045 ^{gh}	0.81 ± 0.018 ^{hi}	0.41 ± 0.014 ^{bc}	0.47 ± 0.012 ^{cd}	0.43 ± 0.042 ^{bcd}	0.64 ± 0.01 ^{fg}	0.85 ± 0.04 ^{i}
($cmol_c kg^{-1}$)	5–10	0.12 ± 0.01 ^{a}	0.12 ± 0.0091 ^{a}	0.13 ± 0.018 ^{a}	0.53 ± 0.009 ^{def}	0.14 ± 0.0044 ^{a}	0.15 ± 0.0083 ^{a}	0.17 ± 0.016 ^{a}	0.17 ± 0.00 ^{a}	0.35 ± 0.01 ^{b}
Mg^{2+} ($cmol_c kg^{-1}$)	0–5	6.3 ± 0.14 ^{h}	4.9 ± 0.08 ^{ef}	5.6 ± 0.036 ^{g}	5.1 ± 0.047 ^{fg}	8.2 ± 0.18 ^{i}	4.6 ± 0.053 ^{e}	5.5 ± 0.099 ^{g}	7.7 ± 0.09 ^{i}	14 ± 0.22 ^{j}
	5–10	1.5 ± 0.042 ^{c}	1.5 ± 0.035 ^{c}	1.4 ± 0.089 ^{bc}	2.2 ± 0.054 ^{d}	0.82 ± 0.015 ^{a}	0.91 ± 0.098 ^{a}	0.92 ± 0.048 ^{ab}	2.1 ± 0.04 ^{d}	2.2 ± 0.03 ^{d}
Mn^{2+} ($cmol_c kg^{-1}$)	0–5	0.26 ± 0.01 ^{g}	0.061 ± 0.00 ^{d}	0.056 ± 0.00 ^{cd}	0.019 ± 0.00 ^{ab}	0.1 ± 0.00 ^{e}	0.13 ± 0.00 ^{f}	0.12 ± 0.01 ^{ef}	0.02 ± 0.00 ^{ab}	0.033 ± 0.00 ^{bc}
	5–10	0.024 ± 0.00 ^{ab}	0.02 ± 0.00 ^{ab}	0.009 ± 0.00 ^{ab}	0.004 ± 0.00 ^{a}	0.017 ± 0.00 ^{ab}	0.013 ± 0.00 ^{ab}	0.022 ± 0.00 ^{ab}	0.006 ± 0.00 ^{a}	0.013 ± 0.00 ^{ab}
Na^{+} ($cmol_c kg^{-1}$)	0–5	0.091 ± 0.01 ^{a}	0.1 ± 0.00 ^{a}	0.11 ± 0.00 ^{a}	0.12 ± 0.017 ^{ab}	0.19 ± 0.01 ^{cde}	0.21 ± 0.0045 ^{e}	0.21 ± 0.01 ^{e}	0.13 ± 0.02 ^{abc}	0.18 ± 0.02 ^{bcde}
	5–10	0.19 ± 0.01 ^{cde}	0.18 ± 0.00 ^{bcde}	0.2 ± 0.00 ^{de}	0.17 ± 0.0077 ^{bcde}	0.2 ± 0.00 ^{de}	0.18 ± 0.015 ^{bcde}	0.19 ± 0.00 ^{cde}	0.17 ± 0.01 ^{bcde}	0.14 ± 0.013 ^{abcd}
Zn^{2+} ($cmol_c kg^{-1}$)	0–5	0.002 ± 0.00 ^{e}	0.001 ± 0.00 ^{abc}	0.001 ± 0.00 ^{bc}	0.0009 ± 0.00 ^{abc}	0.0007 ± 0.00 ^{abc}	0.0008 ± 0.00 ^{abc}	0.0012 ± 0.00 ^{cd}	0.0006 ± 0.00 ^{abc}	0.0017 ± 0.00 ^{de}
	5–10	0.001 ± 0.00 ^{bcd}	0.0006 ± 0.00 ^{abc}	0.0005 ± 0.00 ^{a}	0.0007 ± 0.00 ^{abc}	0.0006 ± 0.00 ^{abc}	0.0006 ± 0.00 ^{abc}	0.0006 ± 0.00 ^{ab}	0.0006 ± 0.00 ^{abc}	0.0011 ± 0.00 ^{bc}
EBC ($cmol_c kg^{-1}$)	0–5	26 ± 0.89 ^{f}	30 ± 1.2 ^{fg}	34 ± 0.36 ^{h}	33 ± 0.48 ^{gh}	38 ± 0.75 ^{i}	36 ± 1.6 ^{hi}	29 ± 0.9 ^{f}	36 ± 0.31 ^{hi}	63 ± 1.4 ^{j}
	5–10	7.9 ± 0.05 ^{cd}	6.6 ±0.08 ^{bcd}	8.7 ± 0.57 ^{d}	17 ± 0.6 ^{e}	2.8 ± 0.02 ^{a}	5 ± ±0.12 ^{abc}	3.4 ± 0.1 ^{ab}	10 ± 0.19 ^{d}	9.3 ± 0.2 ^{d}
Cations	0–5	27 ± 0.9 ^{e}	30 ± 1.2 ^{ef}	34 ± 0.36 ^{g}	33 ± 0.47 ^{fg}	38 ± 0.74 ^{h}	36 ± 1.6 ^{gh}	29 ± 0.91 ^{e}	36 ± 0.31 ^{gh}	63 ± 1.4 ^{i}
($cmol_c kg^{-1}$)	5–10	8.8 ± 0.06 ^{abc}	7.8 ± 0.11 ^{abc}	9.5 ± 0.55 ^{bc}	17 ± 0.6 ^{d}	5.4 ± 0.07 ^{a}	7.2 ± 0.09 ^{abc}	5.9 ± 0.07 ^{ab}	11 ± 0.19 ^{c}	9.9 ± 0.2 ^{c}

Different letters within one parameter denote significant differences between tree species and soil depths (linear mixed-effects models, Tukey's HSD, $p < 0.05$) .

Table 2.A 4. Proportions of water-soluble and hot water-extractable nitrogen forms under different tree species at two soil layers (0–5 cm and 5–10 cm)

Soil layer	Labile Fractions	*Calliandra Calothyrsus*	*Cedrela Serrata*	*Grevillea Robusta*	*Eucalyptus Grandis*	*Eucalyptus Maidenii*	*Eucalyptus Saligna*	*Entandrophragma Excelsum*	*Polyscias Fulva*	Mixed native species
Water-soluble N fractions										
0 – 5 cm	$WSNO_3$ (%)	43	55	73	52	54	46	12	68	64
	$WSNH_4$ (%)	13	18	9	10	13	16	27	6	9
	WSNorg (%)	44	27	18	38	33	38	61	26	27
5 – 10 cm	$WSNO_3$ (%)	60	45	76	58	65	55	41	62	64
	$WSNH_4$ (%)	7	12	2	5	4	8	15	4	3
	WSNorg (%)	33	43	22	37	31	37	44	34	33
Hot water-extractable N fractions										
0 – 5 cm	$WSNO_3$ (%)	4	3	5	3	5	3	3	3	3
	$WSNH_4$ (%)	17	16	22	16	15	15	19	25	23
	WSNorg (%)	79	81	73	81	80	82	78	72	74
5 – 10 cm	$WSNO_3$ (%)	4	3	4	4	4	4	2	3	3
	$WSNH_4$ (%)	15	15	10	11	10	11	17	11	13
	WSNorg (%)	81	82	86	85	86	85	81	86	84

Table 3.A 1. Analyses of soil microbial soil properties under different tree species in the Arboretum of Ruhande (means ± SEM, n=6).

Soil properties	*Calliandra calothyrsus*	*Cedrela serrata*	*Grevillea robusta*	*Eucalyptus grandis*	*Eucalyptus maidenii*	*Eucalyptus saligna*	*Entandrophragma excelsum*	*Polyscias fulva*	Mixed Native species
	Upper soil layer (0 – 5 cm)								
MBC (mg C kg^{-1})	1200±33^{c}	1300±56^{cd}	1100±20^{c}	2000±50^{h}	1900±75^{gh}	1600±26^{ef}	1500±71de	1600±35^{efg}	1800±46^{fgh}
MBN (mg N kg^{-1})	82±3.1^{e}	160±5.7^{f}	250±4.9^{h}	350±6.2^{i}	230±5^{gh}	170±7.3^{f}	230±4^{g}	340±4.3^{i}	150±4.3^{f}
RP (µg C–CO_2 kg^{-1} h^{-1})	12±0.15^{k}	6±0.03^{g}	8.1±0.07^{i}	10±0.05^{j}	5.6±0.03^{f}	4.4±0.03^{e}	5.6±0.08^{f}	7±0.04^{h}	8.4±0.06^{i}
qCO2 (RP MBC^{-1})	0.009±0.0^{h}	0.004±0.0^{ef}	0.007±0.0^{g}	0.005±0.0^{f}	0.003±0.0^{c}	0.002±0.0^{c}	0.003±0.0^{cde}	0.004±0.0^{def}	0.004±0.0^{ef}
qmic (MBC SOC^{-1})	0.011±0.0^{d}	0.013±0.0^{de}	0.010±0.0^{d}	0.016±0.0^{e}	0.012±0.0^{d}	0.011±0.0^{d}	0.010±0.0^{cd}	0.015±0.0^{e}	0.012±0.0^{d}
Nmin (mg N–NO_3 kg^{-1} h^{-1})	3.7±0.09^{de}	2.7±0.17^{bc}	2.6±0.14^{bc}	3.1±0.091^{cd}	5.7±0.17^{f}	2.3±0.085^{b}	4.1±0.16^{e}	3±0.23^{bcd}	3.1±0.13^{cd}
	Lower soil layer (5 – 10 cm)								
MBC (mg C kg^{-1})	280±24^{a}	220±12^{a}	370±14^{ab}	590±8.2^{b}	260±11^{a}	230±13^{a}	360±21^{ab}	310±15^{a}	140±10^{a}
MBN (mg N kg^{-1})	32±0.77^{abc}	23±0.99^{ab}	36±0.95^{abc}	57±0.76^{d}	40±1.6^{bcd}	18±0.57^{a}	30±1.5^{abc}	46±1.5^{cd}	35±1.6^{d}
RP (µg C–CO_2 kg^{-1} h^{-1})	0.73±0.0^{bcd}	0.76±0.0^{cd}	0.4±0.0^{ab}	0.91±0.0^{d}	0.28±0.0^{a}	0.26±0.0^{a}	0.48±0.0^{abc}	0.82±0.01^{cd}	0.88±0.0^{d}
qCO2 (RP MBC^{-1})	0.002±0.0^{bc}	0.003±0.0^{cd}	0.001±0.0^{a}	0.001±0.0^{ab}	0.001±0.0^{a}	0.001±0.0^{a}	0.001±0.0^{a}	0.003±0.0^{bc}	0.006±0.0^{g}
qmic (MBC SOC^{-1})	0.004±0.0^{ab}	0.004±0.0^{ab}	0.007±0.0^{bc}	0.01±0.0^{cd}	0.004±0.0^{ab}	0.004±0.0^{ab}	0.006±0.0^{b}	0.005±0.0^{ab}	0.003±0.0^{a}
Nmin (mg N–NO_3 kg^{-1} h^{-1})	0.62±0.0^{a}	0.32±0.1^{a}	0.3±0.0^{a}	0.38±0.01^{a}	0.14±±0.0^{a}	0.41±0.0^{a}	0.17±0.1^{a}	0.34±0.0^{a}	0.57±0.0^{a}

Different letters within one parameter denote significant differences between tree species and soil depths (linear mixed-effects models, Tukey's HSD, $p < 0.05$) .

Table 4.A 1. Analyses of potential nitrification rates(PNR) and abundance of amoA genes under different tree species in the 0-5 cm and 5-10 cm soil layers (Means ± SEM, n=6 and 3 for PNR and amoA analyses, respectively).

Soil parameters	Layers (cm)	*Calliandra calothyrsus*	*Cedrela serrata*	*Grevillea robusta*	*Eucalyptus grandis*	*Eucalyptus maidenii*	*Eucalyptus saligna*	*Entandrophragma excelsum*	*Polyscias fulva*	Mixed Native species
Potential nitrification rates and N mineralization										
AOB–PNR (mg N–NO_3 kg^{-1} d^{-1})	0-5	0.269±0.06	0.488±0.08	0.358±0.04	1±0.09	0.15±0.04	0.539±0.05	0.187±0.04	0.64±0.10	0.387±0.07
	5-10	0.429±0.03	0.158±0.01	0.031±0.00	0.279±0.00	0.043±0.00	0.39±0.02	0.051±0.01	0.612±0.01	0.148±0.01
AOA–PNR (mg N–NO_3 kg^{-1} d^{-1})	0-5	0.742±0.01	0.82±0.07	1.11±0.09	1.45±0.10	0.463±0.02	0.498±0.03	0.505±0.02	2.22±0.08	0.979±0.02
	5-10	0.229±0.01	0.068±0.00	0.227±0.00	0.332±0.01	0.168±0.01	0.096±0.00	0.081±0.00	0.125±0.00	0.072±0.00
Total PNR (mg N–NO_3 kg^{-1} d^{-1})	0-5	1.01±0.07	1.21±0.12	1.47±0.06	2.45±0.15	0.577±0.04	0.936±0.06	0.639±0.03	2.86±0.07	1.35±0.06
	5-10	0.658±0.03	0.226±0.01	0.259±0.00	0.61±0.01	0.201±0.02	0.487±0.02	0.109±0.01	0.736±0.01	0.221±0.00
AOA–PNR: AOB–PNR	0-5	3.63 ± 0.8	2.06 ± 0.5	3.73 ± 1.0	1.52 ± 0.1	4.27 ± 0.8	0.997 ± 0.1	3.39 ± 0.7	4.17 ± 0.8	3.19 ± 0.7
	5-10	0.55 ± 0.0	0.44 ± 0.03	8.58 ± 1.9	1.2 ± 0.05	3.88 ± 0.26	0.25 ± 0.01	1.88 ± 0.33	0.20 ± 0.01	0.50 ±0.05
Nmin (mg N kg^{-1} d^{-1})	0-5	3.7±0.09	2.7±0.17	2.6±0.14	3.1±0.09	5.7±0.17	2.3±0.08	4.1±0.16	3±0.23	3.1±0.13
	5-10	0.62±0.02	0.32±0.09	0.3±0.02	0.38±0.01	0.14±0.01	0.41±0.03	0.17±0.11	0.34±0.01	0.57±0.01
Archaeal and bacterial *amoA* gene abundance										
AOB–amoA (amoA copies kg^{-1})	0-5	1.39e+06 ±2.1e+05	1.84e+06 ±1.2e+05	3.9e+06 ±9.0e+05	6.3e+05 ±1.5e+05	1.24e+06 ±1.4e+05	2.4e+05 ±7.0e+04	1.67e+06 ±5.8e+05	8.8e+05 ±2.1e+05	1.3e+06 ±1.5e+05
	5-10	2.7e+05 ±1.3e+05	4.6e+04 ±1.4e+04	2.9e+05 ±9.6e+04	6.7e+03 ±6.9e+02	2.4e+04 ±1.12e+04	1.7e+04 ±4.1e+03	5.5e+04 ±8.5e+03	6.9e+04 ±1.9e+04	3.3e+05 ±6.3e+04
AOA–*amo*A (*amo*A copies kg^{-1})	0-5	2.41e+08 ±6.6e+07	1.8e+08 ±2.7e+07	6.37e+08 ±1.02e+08	2.23e+08 ±7.4e+07	2.47e+08 ±9.8e+07	2.32e+08 ±1.8e+07	7e+08 ±2.18e+08	9.95e+08 ±1.03e+08	7.56e+08 ±1.85e+08
	5-10	2.73e+08 ±5.7e+07	1.02e+08 ±1.2e+07	4.27e+08 ±1.15e+08	1.68e+08 ±8.8 e+07	1.23e+08 ±3.8 e+07	1.48e+08 ±1.7e+07	1.36e+08 ±2.7e+07	3.88e+08 ±1.37e+08	7.09e+08 ±1.65e+08
AOA–amoA: AOB–amoA	0-5	162 ± 28.6	101 ± 16.5	240 ± 86.1	507 ± 145	258 ± 124	3260±1620	394 ± 83.9	1760±622	539 ± 123
	5-10	2240 ± 859	3930 ±1140	1870 ± 417	25600±1570	6650±2890	11100±2540	2330±173	9520±4520	2000±188

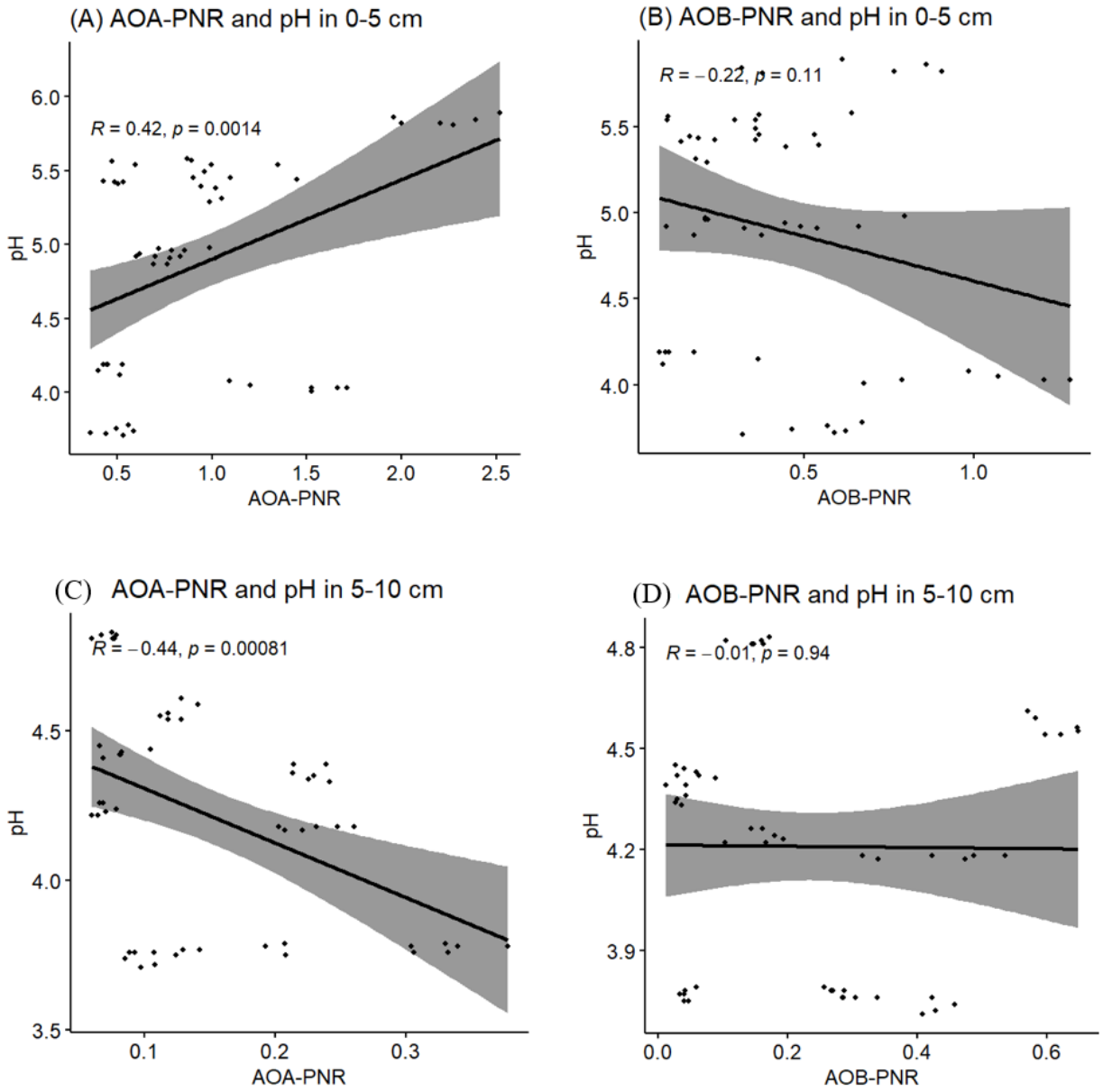

Fig. 4. A 1. Relationship between ammonia-oxidizers' potential nitrification rates and soil pH

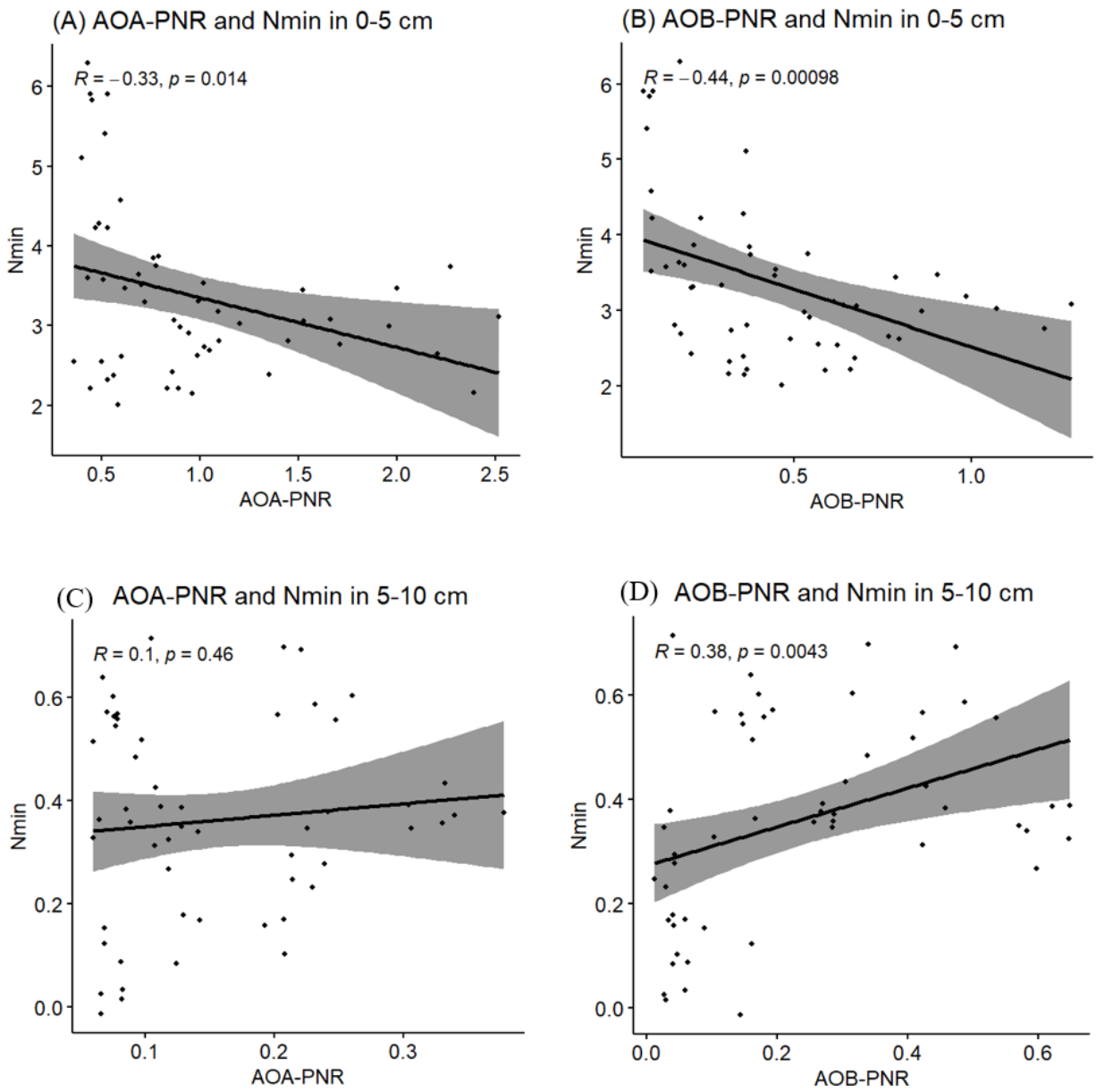

Fig. 4. A 2. Relationship between ammonia-oxidizers' potential nitrification rates and nitrogen mineralization rates

Table 5.A 1. Summary description of measured soil properties in terraced and unterraced lands across sites and hillslope positions

Soil parameters	Management practice	N	Mean	SEM	SD	Minimum	Maximum
Physico-chemical properties							
SOM (g kg^{-1})	Terraced	45	80.780	1.626	10.909	55.278	113.893
	Unterraced	45	89.335	1.968	13.203	61.455	114.460
SOC (g kg^{-1})	Terraced	45	26.088	1.755	11.770	8.801	50.667
	Unterraced	45	22.457	1.183	7.933	5.281	39.900
Total N (g kg^{-1})	Terraced	45	2.192	0.133	0.889	0.973	3.828
	Unterraced	45	1.638	0.111	0.742	0.941	3.828
pH_{KCL}	Terraced	45	3.912	0.036	0.240	3.570	4.390
	Unterraced	45	3.863	0.018	0.123	3.530	4.000
pH_{H2O}	Terraced	45	4.562	0.077	0.515	3.620	5.460
	Unterraced	45	4.358	0.060	0.399	3.620	5.380
C/N	Terraced	45	14.438	1.406	9.435	3.219	36.973
	Unterraced	45	16.250	1.097	7.362	1.686	29.820
AvP (mg kg^{-1})	Terraced	45	2.752	0.196	1.318	0.639	5.234
	Unterraced	45	1.521	0.072	0.482	0.810	2.605
TEA (%)	Terraced	45	5.785	0.191	1.282	3.680	8.800
	Unterraced	45	4.327	0.159	1.070	1.520	6.640
Sand (%)	Terraced	45	76.440	0.466	3.128	70.000	82.000
	Unterraced	45	74.498	0.858	5.758	58.000	83.000
Clay (%)	Terraced	45	15.742	0.683	4.580	8.000	26.000
	Unterraced	45	19.098	1.053	7.062	10.000	36.000
Silt (%)	Terraced	45	7.818	0.410	2.751	4.000	15.600
	Unterraced	45	6.404	0.371	2.488	2.000	12.000
WAS (%)	Terraced	45	56.516	1.121	7.519	49.091	85.714
	Unterraced	45	55.264	1.048	7.030	46.667	76.923
WSC (mg kg^{-1})	Terraced	45	53.933	5.344	35.852	9.521	154.959
	Unterraced	45	28.617	2.560	17.174	5.680	71.858
HWC (mg kg^{-1})	Terraced	45	626.871	54.434	365.155	327.259	1642.819
	Unterraced	45	385.892	19.732	132.366	220.350	751.374
WSNH4 (mg kg–1)	Terraced	45	7.672	1.847	12.390	1.032	45.065
	Unterraced	45	3.259	0.363	2.437	0.766	9.826
$WSNO_3$ (mg kg^{-1})	Terraced	45	17.679	1.830	12.276	1.085	46.399
	Unterraced	45	10.391	1.547	10.376	0.000	35.841
HWNtot (mg kg^{-1})	Terraced	45	28.227	1.758	11.791	9.678	54.874
	Unterraced	45	21.079	1.851	12.418	6.730	46.539
Microbial properties and processes							
RP (μg CO_2–C g^{-1} h^{-1})	Terraced	45	10.065	0.930	6.236	3.428	24.585
	Unterraced	45	6.431	0.370	2.481	2.157	11.947
MBC (mg C kg^{-1})	Terraced	45	4.959	0.742	4.976	0.407	19.355
	Unterraced	45	2.929	0.660	4.431	0.000	15.784
MBN (mg N kg^{-1})	Terraced	45	51.332	2.757	18.494	25.871	85.369
	Unterraced	45	37.540	1.544	10.360	22.527	61.168
MBP (mg P kg^{-1})	Terraced	45	0.552	0.057	0.379	0.198	1.861
	Unterraced	45	0.370	0.054	0.361	0.070	1.504
qCO2 (μgCO_2–C mg^{-1}MBC h^{-1})	Terraced	45	305.159	33.514	224.819	25.056	777.145
	Unterraced	45	141.863	16.854	113.058	7.818	390.888
qmic (mg MBC g^{-1} C_{tot})	Terraced	45	24.764	3.436	23.052	0.270	81.681
	Unterraced	45	14.907	1.879	12.603	0.444	60.849
Nmin (mg N $kg^{-1}d^{-1}$)	Terraced	45	5.043	0.602	4.039	0.376	15.819
	Unterraced	45	3.783	0.511	3.425	0.009	12.780
Nitnet (mg NO_3–N	Terraced	45	2.462	0.223	1.496	0.400	7.880

$kg^{-1}d^{-1}$)	Unterraced	45	6.609	1.556	10.436	0.520	63.870
Nitnet (mg NO_3–N $kg^{-1}d^{-1}$)	Terraced	45	67.620	7.789	52.251	4.810	184.680
	Unterraced	45	28.432	3.633	24.372	1.340	79.080
Nitrel (%)	Terraced	45	1.335	0.151	1.014	0.155	4.415
	Unterraced	45	0.869	0.146	0.976	0.117	3.479

Samples from three sites, three hillslope positions (upper, middle, and lower) in each management practice, and five samples per hillslope position. SEM: standard error of the mean; SD: standard deviation

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