Microbial response to restoration of tantalite mine soils in western Rwanda

Dissertation for the acquisition of the academic degree
Doktor der Agrarwissenschaften (Dr. agr.)

Submitted to the Faculty of Organic Agricultural Sciences (FB11)
University of Kassel

Dora Neina
Witzenhausen, April 2016

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Organic Plant Production and Agroecosystems
Research in the Tropics and Subtropics
&
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Dora Neina

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This work has been approved by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doktor der Agrarwissenschaften (Dr. agr.).

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Witzenhausen, --

(Dora Neina)

Preface

This Ph.D. research was conducted under the Coltan Environmental Management Project located in Central Africa and partly funded by the Volkswagen Foundation (Volkswagen Stiftung, Hannover, Germany) within the Africa Initiative on “Resources, their Dynamics, and Sustainability – Capacity Development in Comparative and Integrated Approaches”. The major part of the funding was provided, however, through a German Academic Exchange Service (DAAD) scholarship. The research focuses on tin-tungsten-tantalum mine wastelands of western Rwanda. The first chapter introduces the thesis and gives a background of the entire Ph.D. research as well as the research questions and objectives addressed in the study. Chapters 2, 3, and 4 contain manuscripts either submitted to or prepared for publication in peer-reviewed journals.

Chapter 2:

Neina, D., Buerkert, A., Joergensen, R.G. Effects of land use on soil microbial indices in tantalite mine soils, western Rwanda. Land Degradation & Development (In Press).

Chapter 3:

Neina, D., Buerkert, A., Joergensen, R.G. Microbial response to restoration of a Technosol amended with local organic materials. Soil Tillage and Research (Under review).

Chapter 4:

Neina, D., Buerkert, A., Joergensen, R.G. Estimating the potential of N and P supply by local organic materials in tantalite mine soils. Applied Soil Ecology (to be submitted).

Chapter 5 contains a general discussion, where I examined issues that were not sufficiently addressed in the main papers such as problems identified at the study sites, further comments on mine land restoration, the usefulness of the organic amendments used in the study, experimental limitations, and alternative solutions. The thesis is concluded with recommendations that may be useful for future use by the stakeholders of the mining business in the Great Lakes region of Central Africa.

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List of Acronyms

ADF	Acid detergent fiber
AERs	Anion exchange resins
Al	Aluminium
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
B	Boron
$BaCl_2$	Barium chloride
Be	Beryllium
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide (quick lime)
Cd	Cadmium
CEC	Cation exchange capacity
CEM	Coltan Environmental Management Project
$CHCl_3$	Chloroform
CIA	Central Intelligence Agency of the USA
CO_2	Carbon dioxide
Coltan	Columbite-tantalite
Cr	Chromium
Cs	Cesium
Cu	Copper
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DR Congo	Democratic Republic of Congo
DVB	Divinylbenzene
F	Fluorine
FAO	Food and Agriculture Organization of the United Nations
$FeNb_2O_6$	Ferrocolumbite
FYM	Farmyard manure
Ga	Gallium
Girinka	One cow per poor family program, Rwanda
GLR	Great Lakes Region of Africa
GMC	Gatumba Mining Concession
GMD	Gatumba Mining District
H_2O	Water
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
HSD	Honestly significant difference
IBM	International Business Machines
ICP-AES	Inductively coupled plasma atomic emission
IERs	Ion exchange resins

IMF	International Monetary Fund
INEAC	*Institut National de l'Etude Agronomique au Congo*
INERA	*Institut National des Etudes et Recherches Agronomique*
IPCC	Intergovernmental Panel on Climate Change
IUSS	International Union of Soil Sciences
K	Potassium
K_2SO_4	Potassium sulfate
KCl	Potassium chloride
k_{EC}, k_{EN}	Extractable portion of total carbon and nitrogen from microbial biomass
Li	Lithium
LCT family	Lithium-Cesium-Tantalum family
MBC	Microbial biomass carbon
$MgNb_2O_6$	Magnocolumbite
MgO	Magnesium oxide
MINAGRI	Rwanda Ministry of Agriculture and Animal Resources
MINEDUC	Rwanda Ministry of Education, Rwanda
MINETAIN	Société des Mines d'Etain du Rwanda-Urundi, Rwanda
MINIRENA	Rwanda Ministry of Natural Resources, Rwanda
$MnNb_2O_6$	Manganocolumbite
$MnTa_2O_6$	Manganotantalite
N	Nitrogen (total)
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
Nb	Niobium
NDF	Neutral detergent fiber
NH_4F	Ammonium Fluoride
Ni	Nickel
NISR	National Institute of Statistics of Rwanda
NYF family	Niobium-Yttrium-F-Signature family
P	Phosphorus
Pb	Lead
Rb	Rubidium
REMA	Rwanda Environmental Management Authority
Sc	Scandium
SMCRA	Surface Mining Control and Reclamation Act
Sn	Tin
SOC	Soil organic carbon
SOM	Soil organic matter
Ta	Tantalum
Th	Thorium
Ti	Titanium
U	Uranium
UN-DESA	United Nations Department of Economic and Social Affairs
USDA	United States Department of Agriculture
UV	Ultra violet

WHC	Water holding capacity
WHO	World Health Organization
WMC	Workable moisture content
WRB	World Reference Base (for Soil Resources)
Y	Yttrium
Zn	Zinc
Zr	Zirconium

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First of all, I wish to thank the Almighty God for carrying me on his wings across this domain.

I wish to express my sincere gratitude to Prof. Dr. Andreas Buerkert for granting me the opportunity to work in his group and for being patient with me even when things turned sour after the first year of fieldwork in Rwanda. I am also indebted to Prof. Dr. Rainer Georg Joergensen who allowed me to work in his laboratory even with no proof of my capability to handle the work. I really appreciate the fruitful discussions we often had. I particularly remember that on one occasion we spent over two hours discussing my data. Words cannot express my appreciation for the freedom you gave me throughout the study, the trust reposed in me and, more importantly, your commitment to my work. I am truly grateful.

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Summary

The Great Lakes Region (GLR) of Africa hosts about 12% of the African population with an annual growth rate of 2.9% against a continental average of 2.6% and a projected doubling of the population by 2050. The region has huge mineral wealth with a larger fraction in the Democratic Republic of Congo (DR Congo). Coltan, cassiterite (tin ore), and wolframite (tungsten ore) are abundant in the region and occur mostly as alluvial deposits, which makes them exploitable by artisanal methods. Artisanal mining and the coltan price hike and explosion in the last decade brought new opportunities but also many challenges to over 80% of the population whose livelihoods depend largely on agriculture and pastoralism. As a former hub of plantation agriculture, the GLR still has high agricultural potential due to productive soils and diverse climatic conditions ranging from tropical rainy to warm temperate climates. Besides providing new income opportunities to farmers, extensive artisanal mining has also had negative effects on the rural agricultural economy through new labor deficits in farming communities, loss of farmland, land degradation, food insecurity, a high cost of living, and reduced traditional export crop production. This occurs alongside secondary impacts that distantly affect the quality of air, water, soil, plants, animals, and human wellbeing. The situation is certainly multifaceted and calls for a holistic approach for short and long-term mitigation of such negative impacts of mining on livelihoods. With the complex effects of mining on rural social-ecological systems of the GLR, this study focuses on the effects of mine land restoration on soil microbiological quality.

The Gatumba Mining District (GMD) is a historic tin-tungsten-tantalum mining zone located at the eastern end of the GLR in the Western Province of the Republic of Rwanda. Over eight decades of coltan mining in the district created many areas of degraded mine wastelands. While some of the wastelands were afforested with pine and eucalyptus tree species over three decades ago, farmers directly cultivated others due to land scarcity. Farmyard manure (FYM) is often the sole fertilizer applied on the degraded fields although it is mostly inadequate to achieve the desired crop yields. Despite this, several multi-purpose plants such as *Tithonia diversifolia, Markhamia lutea*, and *Canavalia brasiliensis* thrive in the GMD and could serve as soil amendments. Previous studies and surveys in the area showed large yield increases of commonly grown crops on the tantalite mine soils fertilized with FYM, compost, and *Tithonia* biomass mixed with phosphate rock compared to un-mined soils. Regrettably, the potential for these local plant species to improve soil microbial properties, particularly in the tantalite mine soils, has been least investigated. The microbial response of these soils to the amendments is unknown given the effects of edaphic factors, environmental conditions, and substrate quality. Therefore this study aimed at investigating whether (a) the post-mining land uses had effects on the microbial properties of the tantalite mine soils, and whether there are soil properties that pose limitations to the microbial properties; (b) the potential of the organic soil amendments to initiate the microbial restoration of the Technosols; and (c) the estimation of nitrogen (N) and phosphorus (P) supply potential of the amendments in the short-term. The specific objectives of the study were to:

1. Evaluate the effects of land use on soil microbial indices of the tantalite mine soils (Study I);
2. Investigate the restorative effects of organic amendments on a Technosol (Study II); and
3. Estimate the short-term N and P supply potential of these soil amendments (Study III).

To answer these queries, six sites in the GMD were sampled (fresh soils, 0-20 cm) in five field replicates. These include an unmined native forest, two mine sites restored to pine and eucalyptus forests designated as pine and eucalyptus Technosols, an arable land, and two cultivated Technosols (Kavumu and Kirengo Technosols). The native forest and arable land were chosen as references. For the first study, the physicochemical properties of the soils were analyzed before a 28-day incubation (22°C) experiment. The incubated soils were analyzed for mineral N, soil microbial biomass C, N, P, and fungal ergosterol contents using standard methods. Study I revealed that the cultivated but degraded Technosols had adequate microbial biomass required to kick-start soil restoration. This led to Study II, which determined whether the Technosols could mimic graded mine soils (mine soils restored with topsoil replacement) for a rapid snap back towards pre-mine conditions. It consisted of a 12-week incubation (22°C) study of the arable soil and the Kavumu Technosol amended with FYM, *Canavalia* biomass, *Markhamia* leaf, and *Tithonia* biomass applied at 2.5 mg C g^{-1} soil. Carbon (C) and nitrogen (N) mineralization, microbial biomass, and fungal ergosterol contents were measured at four, eight, and twelve weeks of incubation using the methods applied in Study I. The remarkable effects of *Canavalia* biomass in Study II and the reported role of *Tithonia* biomass in P release merited further investigations of their role in N and P supply in the tantalite mine soils. Thus, in Study III, two 4-week laboratory incubation experiments each were conducted to determine potential mineralizable N and P mineralization involving soils from the six sites. The mineralizable N was estimated using a soil-sand mixture (1:1) amended with *Canavalia* and goat manure at 500 µg N g^{-1} soil. The P mineralization experiment comprised mixtures of soil and anion exchange resins (1:1) converted to bicarbonate form and amended with *Tithonia* biomass and goat manure at 330 µg P g^{-1} soil. The resin beads were separated from the soil by sieving the incubated soil-resin mixture through 0.4 mm nylon net bags followed by sedimentation and decantation. The separated resins were extracted with 0.5 M HCl in six successions and measured for phosphate content.

Study I addressed the question to what degree the afforested and cultivated Technosols differ in microbial biomass and activity. The results revealed that afforestation increased soil organic carbon (SOC) and total N contents in the pine and eucalyptus Technosols by 34-40% and 28-30%, respectively of that in the native forest soil. The basal respiration of the pine Technosol and native forest soil were similar compared with the eucalyptus Technosol, which was 11-14% lower than its forest counterparts. Conversely, the net N mineralization in pine and eucalyptus Technosols were 40 and 65% of that in the native forest soil, respectively. Soil microbial biomass C in the pine and eucalyptus Technosols were 33 and 48% of that in the native forest soil. The tree species differed significantly in microbial biomass and activity, exhibiting a trajectory towards recovery as depicted in a lower metabolic quotient. The cultivated soils were, however, similar in terms of the SOC and total N contents as well as microbial biomass and activity. The microbial indices were constrained by soil acidity, dithionite-extractable Al and low P availability.

Study II confirmed that the organic soil amendments can initiate biological restoration of the Technosols. The amendments substantially increased CO_2 efflux, N mineralization, and microbial properties compared with the non-amended soils. The application of *Canavalia* biomass increased CO_2 efflux by 340%, net N mineralization by 30-140%, and microbial biomass C and N by 240-600% and 240-380% ($P < 0.01$), respectively after four weeks of incubation compared with the non-amended soils. The addition of *Tithonia* biomass led to a major increase in ergosterol content (roughly 240%) as well as an increase of the ergosterol to microbial biomass C ratio. Due to its major responses to the amendments in terms of the measured biological parameters, the Kavumu Technosol showed a high potential for quick restoration of its soil quality. This observation is supported by high crop yields obtained in other studies involving amended Technosols.

In Study III, the N and P supply potential of the amendments in the tantalite mine soils was estimated after four weeks of incubation. The results showed that *Canavalia* biomass addition led to the highest (130 $\mu g\ g^{-1}$ soil, $P < 0.01$) mineralizable N in the Kavumu Technosol and the lowest in the native forest soil (-20 $\mu g\ g^{-1}$ soil). Conversely, the mineralizable N of goat manure was negative in all soils ranging from -2.5 μg N g^{-1} to -7.7 μg N g^{-1} soil except for the native forest soil. Interestingly, the immobilization of goat manure N in the "cultivated soils" was 30-70% lower than in the "forest soils" signifying an imminent recovery of the amended soils from N immobilization. This also suggests that careful management of the organic amendments is needed to tailor applications such that N immobilization and mobilization patterns are synchronized with plant N requirements. In the P mineralization experiment, the bicarbonate resins and the amendments increased the pH of the soil-resin mixture and the original soil pH of each site by approximately 1-2 units. The mineralization of goat manure P was three times that of *Tithonia*, constituting 61-71% of total P applied with little difference among sites. Phosphorus mineralization slightly decreased after four weeks of incubation and was attributed to sulfate competition as reflected in a negative correlation, which was steeper in the *Tithonia* treatment.

Discussions and recommendations tackled practical consequences of the research results. Firstly, liming will reduce soil acidity, improve P availability, and enhance soil biological quality. Apart from regular liming materials, wood ash may be an alternative for smallholder farmers due to its enrichment with alkali ions and P. Secondly, afforestation with mixed-species of fast-growing eucalyptus and legume or indigenous tree species are suggested to restore tantalite mine wastelands. Thirdly, each amendment used in this study played a unique role in C, N, and P mineralization and contributed substantially to microbial properties in the tantalite mine soils. Interestingly, the "N immobilizers" exhibited potentials for P release and SOC storage. Consequently, the combined use of the amendments in specific ratios, or co-composting prior to application is recommended to optimize nutrient release, microbial

biomass dynamics and soil organic matter accrual. Transport of organic inputs seems more feasible for smallholder farmers who typically manage small field sizes.

It is emphasized that most of this research was conducted under controlled laboratory conditions, which excludes interaction with environmental variables. Also fine fractions of the amendments were used compared with the usual practice of applying a mixture of predominantly coarser fractions. Therefore the biological dynamics reported in the studies here may not entirely reflect those of farmers' field conditions.

Zusammenfassung

Die Region der Afrikanischen Großen Seen (RAGS) ist heute Heimat für ungefähr 12% der afrikanischen Bevölkerung. Das jährliche Bevölkerungswachstum liegt dabei mit 2.9% über dem kontinentalen Durchschnitt von 2.6%, und Hochrechnungen gehen von einer Verdopplung der Bevölkerung bis zum Jahre 2050 aus. Als ehemaliges Zentrum der afrikanischen Plantagenwirtschaft besitzt die RAGS aufgrund fruchtbarer Böden und günstiger klimatischer Bedingungen noch immer ein großes landwirtschaftliches Potential. Gleichzeitig verfügt die Region aber auch über reichhaltige Vorkommen an mineralischen Rohstoffen, insbesondere Coltan, Kassiterit (Zinnerz) und Wolframit (Wolframerz), wobei ein Großteil hiervon in der Demokratischen Republik Kongo (DR Kongo) liegt. Viele dieser Rohstoffe treten als alluviale Ablagerungen auf, was es ermöglicht, sie mit einfachen, nicht industriellen Methoden abzubauen. Den über 80% der lokalen Bevölkerung, deren Existenz weitgehend von Ackerbau und Weidewirtschaft abhängt, konnte der Kleinbergbau sowie der extreme Anstieg der Coltanpreise auf dem Weltmarkt in den zurückliegenden zehn Jahren neue Einkommensmöglichkeiten eröffnen. Allerdings hat der umfangreiche Abbau von Bodenschätzen auch zahlreiche negative Auswirkungen auf die ländliche Agrarwirtschaft. Diese äußern sich heutzutage in Arbeitskräftemangel innerhalb bäuerlicher Gemeinschaften, dem Verlust von Ackerland, Bodendegradation, Ernährungsunsicherheit, höheren Lebenserhaltungskosten und verminderten Exporterlösen aus ackerbaulicher Produktion. Darüber hinaus ergeben sich weitere negative Folgen für die Umwelt, also für die Luft-, Wasser- und Bodenqualität, für die Natur sowie das menschliche Wohlergehen. Diese insgesamt sehr vielschichtige Situation verlangt nach einem ganzheitlichen Ansatz zur Abschwächung negativer Auswirkungen des Bergbaus auf die Existenzgrundlage der lokalen Bevölkerung. Vor dem Hintergrund der komplexen Folgen des Bergbaus für das ländliche sozial-ökologische System in der RAGS befasst sich diese Studie dabei in erster Linie mit den Auswirkungen der Rekultivierung von Bergbaulandschaften auf die mikrobiologische Bodenqualität.

Der in der Westprovinz Randas gelegene Gatumba Mining District (GMD) ist eine traditionelle Zinn-Wolframerz-Tantalit-Bergbauzone am östlichen Rand der RAGS. Der hier bereits seit mehr als 80 Jahren anhaltende Coltanbergbau hat dabei hat große Flächen devastierter Bergbaufolgelandschaften hinterlassen. Während einige dieser Brachflächen bereits vor mehr als drei Jahrzehnten mit Kiefern und Eukalyptusarten aufgeforstet wurden, erfolgte auf anderen aufgrund von Landknappheit eine direkte ackerbauliche Nutzung. Stallmist ist dabei oft der einzige Dünger, der auf den degradierten Feldern Anwendung findet, wobei dies meistens nicht ausreicht, um die erwünschten Ernteerträge zu erzielen. Trotzdem gedeihen im GMD zahlreiche zu unterschiedlichen Zwecken verwendete Pflanzen wie *Tithonia diversifolia, Markhamia lutea*, and *Canavalia brasiliensis* und dienen dabei gleichzeitig der Bodenverbesserung. Für die am häufigsten angebauten Kulturpflanzen wiesen frühere Untersuchungen und Erhebungen in dem Gebiet zum Beispiel deutlich höhere Erträge auf mit Stallmist, Kompost sowie mit *Tithonia*-Biomasse angereichertem Phosphatgestein gedüngten Tantalit-Abbauböden im Vergleich zu zuvor nicht bergbaulich genutzten Böden nach. Allerdings wurde das Potential dieser lokalen Pflanzenarten zur

Verbesserung mikrobieller Bodeneigenschaften, insbesondere Tantalit-Abbauböden, bislang kaum untersucht. Die mikrobiellen Reaktionen auf solche Bodenverbesserungsmaßnahmen vor dem Hintergrund der Auswirkungen von bodenbedingenden Faktoren, Umweltbedingungen und Substratqualität sind nicht bekannt. Die vorliegende Studie zielt daher darauf ab zu untersuchen, ob (a) die Bergbaufolgenutzung vor Ort Auswirkungen auf die mikrobiellen Eigenschaften der Tantalit-Abbauböden hat und welche Bodeneigenschaften einschränkend auf die mikrobielle Aktivität wirken können, (b) welches Potential die organische Bodenverbesserung für die mikrobielle Wiederherstellung solcher Technosole besitzt und (c) über welches Potential zur Verbesserung der Stickstoff-(N)- und Phosphor-(P)-versorgung diese Bodenverbesserungsmaßnahmen kurzfristig verfügen. Die spezifischen Zielsetzungen dieser Arbeit sind die folgenden:

1. Bewertung der Auswirkungen von Landnutzung auf bodenmikrobielle Indizes von Tantalit-Abbauböden (Studie I);
2. Untersuchung der wiederherstellenden Wirkung organischer Bodenverbesserungsmaßnahmen auf einen Technosol (Studie II); und
3. Einschätzung des Kurzzeitpotentials solcher Bodenverbesserungsmaßnahmen zur N- und P-Versorgung (Studie III).

Zur Beantwortung dieser Fragen wurden an sechs Standorte innerhalb des GMD Bodenproben (0-20 cm) in fünffacher Wiederholung genommen – auf einer vom Bergbau unberührten Waldfläche, auf zwei mit Kiefern- und Eukalyptuswald aufgeforsteten Flächen (im Weiteren Kiefern- und Eukalyptus-Technosol genannt), auf einer Ackerfläche und auf zwei Anbauflächen auf Technosol (im Weiteren Kavumu- und Kirengo-Technosolg genannt). Die Proben von den beiden vom Bergbau unberührten Wald- und Ackerflächen dienten dabei als Referenz. Für die erste Studie wurden die physikochemischen Bodeneigenschaften im Rahmen eines 28-tägigen Inkubationsexperiments (bei 22°C) analysiert. Unter Verwendung von Standardverfahren wurden die inkubierten Böden auf den Gehalt von mineralischem N, mikrobieller Biomasse, Kohlenstoff (C), N, P sowie Ergosterol untersucht. Die Ergebnisse von Studie I ergaben, dass die bebauten aber degradierten Technosole über ausreichend mikrobielle Biomasse verfügen, um eine Bodensanierung einzuleiten. Diese Erkenntnis führte zu Studie II, welche ermitteln sollte, ob die Technosole sortierten Abbauböden (durch Austauschen des Mutterbodens sanierte Abbauböden) in ihren Eigenschaften vor der bergbaulichen Nutzung gleichen würden. Im Rahmen eines 12-wöchigen Inkubationsexperiments (bei 22°C) wurden Proben von Ackerboden und mit Stallmist, *Canavali*-Biomasse, *Markhamia*-Blättern und *Tithonia*-Biomasse angereichertem Kavumu-Technosol (Aufbringung auf 2.5 mg C g^{-1} Boden) untersucht. Unter Anwendung der bereits in Studie I genutzten Methoden wurde die C- und N-Mineralisierung, die mikrobielle Biomasse sowie der Ergosterolgehalt der inkubierten Proben nach vier, acht und zwölf Wochen gemessen. Die in Studie II nachgewiesenen bemerkenswerten Auswirkungen der *Canavali*- und *Tithonia*-Biomasse auf die P-Freisetzung warfen die Fragestellung nach der Bedeutung der N- und P-Versorgung in Tantalit-Abbauböden auf. Hieraus ergab sich Studie III, in welcher im Rahmen von zwei vierwöchigen Inkubationsexperimenten die potentielle N- und P-Mineralisierung der sechs Standortböden ermittelt wurde. Die N-Mineralisierung wurde anhand eines mit *Canavali*-Biomasse und Ziegenmist angereicherten Boden-Sand-Gemisches (Verhältnis 1:1; Aufbringung auf 500 µg N g^{-1} Boden) abgeschätzt. Die Ermittlung der P-Mineralisierung erfolgte anhand eines mit *Tithonia*-Biomasse und Ziegenmist angereicherten Gemisches aus Boden und Anionenaustauscherharzen (Verhältnis 1:1; Aufbringung auf 330 µg P g^{-1} Boden). Durch das Sieben des inkubierten Boden-Austauscherharz-Gemisches durch einen Nylonnetzbeutel mit einer Maschenbreite von 0.4 mm wurden die Harzkügelchen separiert und anschließend durch Ablagerung und Dekantation separiert. Die Harzkügelchen wurden anschließend in sechs aufeinanderfolgenden Behandlungen mit 0.5 M HCL extrahiert und auf ihren Phosphatgehalt analysiert.

Studie I griff die Fragestellung auf, wie stark sich Technosole auf aufgeforsteten und ackerbaulich genutzten Standorten hinsichtlich ihrer mikrobiellen Biomasse und Aktivität unterscheiden. Die Ergebnisse zeigten, dass Wiederaufforstung zu einer Erhöhung des organische Kohlenstoffgehalts (SOC) sowie des N-Gehalts in Kiefern- und Eukalyptus-Technosolen um 34-40% bzw. 28-30% im Vergleich zu natürlichem Waldboden führt. Bezüglich der Basalatmung wiesen Kiefern-Technosol und natürlicher Waldboden vergleichbare Werte auf; dagegen fiel sie beim Eukalyptus-Technosol um 11-14% geringer aus als bei natürlich belassenem Waldboden. Die N-Mineralisierung der Kiefern- und Eukalyptus-Technosole betrug wiederum 40 bzw. 65% der in natürlichem Waldboden gemessenen Werte. Der C-Gehalt der mikrobiellen Biomasse in Kiefern- und Eukalyptus-Technosol lag bei 33 bzw. 48% der Werte in natürlichem Waldboden. Hinsichtlich der mikrobiellen Biomasse und Aktivität ergaben sich signifikante Unterschiede zwischen den beiden Baumarten. Dies lässt wiederum Rückschlüsse auf die Wiederherstellung zu, was sich in einem geringeren metabolischen Quotienten äußert. Demgegenüber waren die in den Technosolen gemessenen SOC- und N-Gehalte vergleichbar mit denen in ackerbaulich genutzten Böden. Die mikrobiellen Indizes wurden dabei durch den Säuregehalt des Bodens sowie durch mit Diothinit extrahierbares Aluminium (Al) und geringe P-Verfügbarkeit eingeschränkt.

Studie II bestätigte, dass organische Bodenanreicherung geeignet ist, die biologische Sanierung von Technosolen einzuleiten. Die Anreicherung führte zu einer substanziellen Erhöhung des CO_2-Abflusses, der N-Mineralisierung sowie der mikrobiellen Eigenschaften im Vergleich zu nicht angereicherten Böden. Bei vierwöchiger Inkubationszeit erhöhte die Zugabe von *Canavali*-Biomasse den CO_2-Abflusses um 340%, die Netto-N-Mineralisierung um 30-140% sowie den C- und N-Gehalt der mikrobiellen Biomasse um 240-600% bzw. 240-380% ($P < 0.01$) im Vergleich zu nicht angereicherten Böden. Die Zugabe von *Tithonia*-Biomasse führte zu einem starken Anstieg des Ergosterolgehalts (ungefähr 240%) sowie zu einer Erhöhung des Verhältnisses von Ergosterol zum C-Gehalt der mikrobiellen Biomasse. Aufgrund der gemessenen Auswirkungen der Anreicherung auf die biologischen Bodenparameter konnte dem Kavumu-Technosol ein großes Potential für eine schnelle Sanierung seiner Bodenqualität nachgewiesen werden. Diese Erkenntnis wird durch die in anderen Studien ermittelten hohen Ernteerträge auf angereicherten Technosolen bestätigt.

In Studie III wurde das N- und P-Versorgungspotential von angereicherten Tantalit-Abbauböden nach vierwöchiger Inkubationszeit ermittelt. Der höchste Anstieg der N-Mineralisierung nach Anreicherung mit *Canavali*-Biomasse war bei Kavumu-Technosol (130 µg g^{-1} Boden, $P < 0.01$) und der geringste bei natürlichem Waldboden (-20 µg g^{-1} Boden) zu verzeichnen. Demgegenüber verlief die N-Mineralisierung nach Anreicherung mit Ziegenmist negativ; mit Ausnahme von natürlichem Waldboden bewegte sie sich im Bereich zwischen 2.5 µg N g^{-1} und -7.7 µg N g^{-1} Boden. Dabei war die Immobilisierung von N aus Ziegenmist bei "ackerbaulich genutzten Böden" um 30-70% geringer als bei "Waldböden", was auf eine zeitnahe Erholung der angereicherten Böden von der N-Immobilisierung hindeutet. Hieraus ergibt sich die Empfehlung zu einer sorgsamen und gezielten Anwendung organischer Zusätze, um so die N-Immobilisierungs- und –Mobilisierungsmuster genau auf den N-Bedarf der Pflanzen zuzuschneiden. Im P-Mineralisierungsversuch erhöhten die Bikarbonat-Harze und Zusätze den pH des Gemisches aus Boden und Austauscherharzen sowie den pH der Originalproben von den sechs Standorten um etwa 1-2 Einheiten. Die P-Mineralisierung von Ziegenmist war dabei dreimal höher als die von *Tithonia*-Biomasse und betrug 61-71% des verwendeten Gesamt-P mit nur geringen Unterschieden zwischen den einzelnen Standorten. Nach vierwöchiger Inkubationszeit verringerte sich die P-Mineralisierung und korrelierte negativ mit dem Sulfatgehalt, insbesondere nach einer Anreicherung mit *Tithonia*-Biomasse.

Der abschließende Teil dieser Arbeit befasst sich mit Diskussionen und praktischen Empfehlungen, welche aus den gewonnenen Forschungsergebnissen abgeleitet werden können. Erstens wird eine Kalkdüngung angeregt, um den Säuregehalt der Böden zu senken und die P-Verfügbarkeit sowie die bodenbiologische Qualität zu erhöhen. Neben herkömmlicherweise für die Kalkdüngung verwendeten Materialien könnte Holzasche aufgrund ihrer Anreicherung mit Alkaliionen und P eine Alternative für die lokalen Kleinbauern darstellen. Zweitens wird die Aufforstung mit verschiedenen, schnell wachsenden Leguminosen und Eukalyptus- sowie einheimischen Baumarten zur Restaurierung der Tantalit-Bergbaufolgelandschaften empfohlen. Die dritte Empfehlung beruht auf der Erkenntnis, dass jeder der in dieser Studie angewandten Formen der Bodenanreicherung eine besondere Funktion bei der C-, N- und P-Mineralisierung zukam und somit wesentlich zu den mikrobiellen Eigenschaften in den Tantalit-Abbauböden beitrug. Interessanterweise zeigten dabei die "N-Immobilisierer" gleichzeitig auch Potential zur P-Freisetzung und SOC-Einlagerung. Folgerichtig wird auch die kombinierte Anwendung dieser Bodenanreicherungsmaßnahmen in spezifischen Verhältnissen oder die Co-Kompostierung vor der Anwendung empfohlen, um die Freisetzung von Nährstoffen, die Dynamik der mikrobiellen Biomasse sowie die Entstehung organischer Bodensubstanz zu optimieren. Gleichzeitig erscheint der Transport von Materialien zur organischen Bodenverbesserung eine für die lokalen Kleinbauern umsetzbare Maßnahme zu sein, da sie selbst zumeist nur kleine Feldgrößen bewirtschaften.

An dieser Stelle soll abschließend noch darauf hingewiesen, dass die meisten Untersuchungen dieser Forschungsarbeit unter kontrollierten Laborbedingungen durchgeführt wurden und somit keine Wechselwirkungen mit Umweltvariablen vorlagen. Zudem kamen Materialien zur Bodenanreicherung nur als Feinanteile zur Anwendung, während in der Praxis vorwiegend eine Mischung aus gröberen Fraktionen Verwendung findet. Daher können die in dieser Studie ermittelten biologischen Dynamiken von den bäuerlichen Feldbedingungen vor Ort abweichen.

1 General introduction

1.1 Background of the Great Lakes region of Africa

According to the U.S. Department of State (2015), the Great Lakes region (GLR) of Africa consists of the Democratic Republic of Congo (DR Congo), Burundi, Rwanda, and Uganda. The region is endowed with diverse natural resources including considerable deposits of cassiterite (tin ore), columbite-tantalite, and wolframite (tungsten ore) (Hayes and Burge, 2003; Jeníček and Grofová, 2015; MINIRENA, 2015). The largest fraction of the region's mineral wealth occurs in the DR Congo (Moyroud and Katunga, 2002; Global Witness, 2005). For instance, almost two-thirds of the global columbite-tantalite (coltan) reserves, representing about 80% of the reserves in Africa (Hayes and Burge, 2003; Global Witness, 2005), are found in this country. In 2014, the GLR accounted for 68% of the global coltan production mainly from the DR Congo and Rwanda, and a small fraction from Burundi (U.S. Geological Survey, 2015). The minerals are mostly concentrated in the eastern part (Figure 1) of the country (Moyroud and Katunga, 2002; Hayes and Burge, 2003) and occur as alluvial deposits in soft rocks, stream beds, national parks, agricultural land (Hayes and Burge, 2003; Global Witness, 2005), near rivers and riverbanks, savannah areas, and in forests and forest reserves (Moyroud and Katunga, 2002). Consequently, they can be readily mined by artisanal methods with simple tools such as pickaxes, shovels, and spades (Hayes and Burge, 2003; Global Witness, 2005). Mineral exploitation in the GLR dates back to pre-colonial times (Geenen, 2012), but most reliable records date from the 20th century (Hayes and Burge, 2003) when Belgium entered the region to exploit its rich mineral resources (Hayes and Burge, 2003). After the end of colonialism, the mineral exploitation, which was mostly industrial and publicly owned, collapsed due to financial crises and state administrative breakdown (Hayes and Burge, 2003; Geenen, 2012), leading to the spread of an informal (artisanal mining) sector which today accounts for over 90% of the mineral production in the region (Geenen, 2012). Artisanal mining is said to have emerged as a communal activity controlled by seasonal alluvial processes and market availability (Phimister, 1974) and it has often become an important economic activity for many stakeholders (Geenen, 2012).

1.2 Agricultural potential of the Great Lakes region of Africa

To assess the extent of effects of mining on the agricultural sector, it is essential to recognize the agricultural potential of the GLR and its role in the livelihoods of the inhabitants. This region holds 12% of the African population with an annual growth of 2.9% compared with the African average of 2.6%. The populations of these countries, except Rwanda, are expected to double by 2050 (UN-DESA, 2015; Central Intelligence Agency, 2015a). Before mining became widespread in the region, agriculture and pastoralism were the dominant livelihood strategies employing between 80 and 90% of the population (REMA, 2011; Maass et al., 2012; Jeníček and Grofová, 2015).

In addition to its rich biodiversity, minerals, and non-mineral resources, the GLR has a high agricultural potential because of the potentially productive soils and favorable climatic conditions. The soils vary widely partly reflecting the effects of the climatic diversity which includes (i) tropical rainy climates with an average monthly air temperature above 18ºC categorised into areas with constant moisture throughout the year, those with a short dry season and heavy rains during the rest of the year, and the remainder having a savannah climate with a dry winter season; and (ii) warm temperate climates at higher altitudes with average monthly temperatures below 18ºC (but above 0ºC), which are either humid with a dry winter season or humid with rainfall throughout the year (Batjes, 2008). The major Reference Soil Groups in the region are Ferralsols comprising over 50% of the soils while Acrisols make up 25%. The rest are 8% Cambisols, 6% Arenosols, 5.5% Gleysols, 4% Lixisols, and 2% Nitisols (Batjes, 2008) with minor fractions of Vertisols, Andosols, Luvisols, and Alisols (Batjes, 2008; van Ranst et al., 2010).

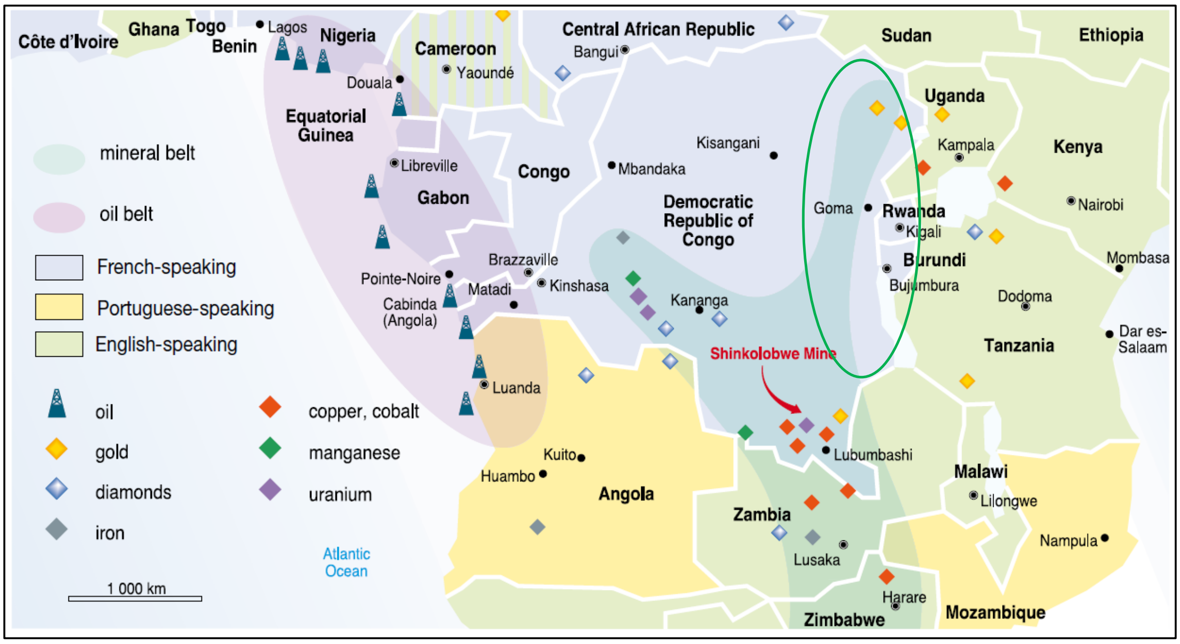

Figure 1. Circled (green) area showing mineral resources of the GLR concentrated in eastern DR Congo (Source: Nellemann et al., 2010).

The high agricultural potential of this region stimulated the installation of plantation agriculture by Belgium during colonial times. The first plantations of rubber (*Hevea brasiliensis* Müll Arg.), oil palm (*Elaeis guineensis* Jacq.), cocoa (*Theobroma cacao* L.) and coffee (*Coffea arabica* L.) trees were installed in the late 19th century in the Mayumbe region (Baert et al., 2012). Subsequently, sugar cane (*Saccharum officinarum* L.), compulsory cash crops such as cotton (*Gossypium spp*. L.) and groundnuts (*Arachis hypogea* L.), food crops such as cassava (*Manihot esculenta* Crantz), maize (*Zea mays* L.), and banana (*Musa spp*. L.) were also widely grown (Baert et al., 2012). The massive agricultural development led to the creation of the National Institute for Agronomy in the Belgian Congo (*Institut National de l'Etude Agronomique au Congo* (INEAC) in Yangambi in the Orientale Province of the DR Congo, now known as the National Institute for Agronomic Studies and Research (*Institut National des Etudes et Recherches Agronomique* (INERA) specialized to handle all aspects of agriculture. After independence in 1960, agricultural development suffered a near collapse due to political and economic instability (Baert et al., 2012). Nevertheless, the Orientale, North, and South Kivu Provinces of the DR Congo are still among the top productive areas in Africa (Moyroud and Katunga, 2002) where up to three harvests per year can be obtained (Moyroud and Katunga, 2002; Lecoutere et al., 2009).

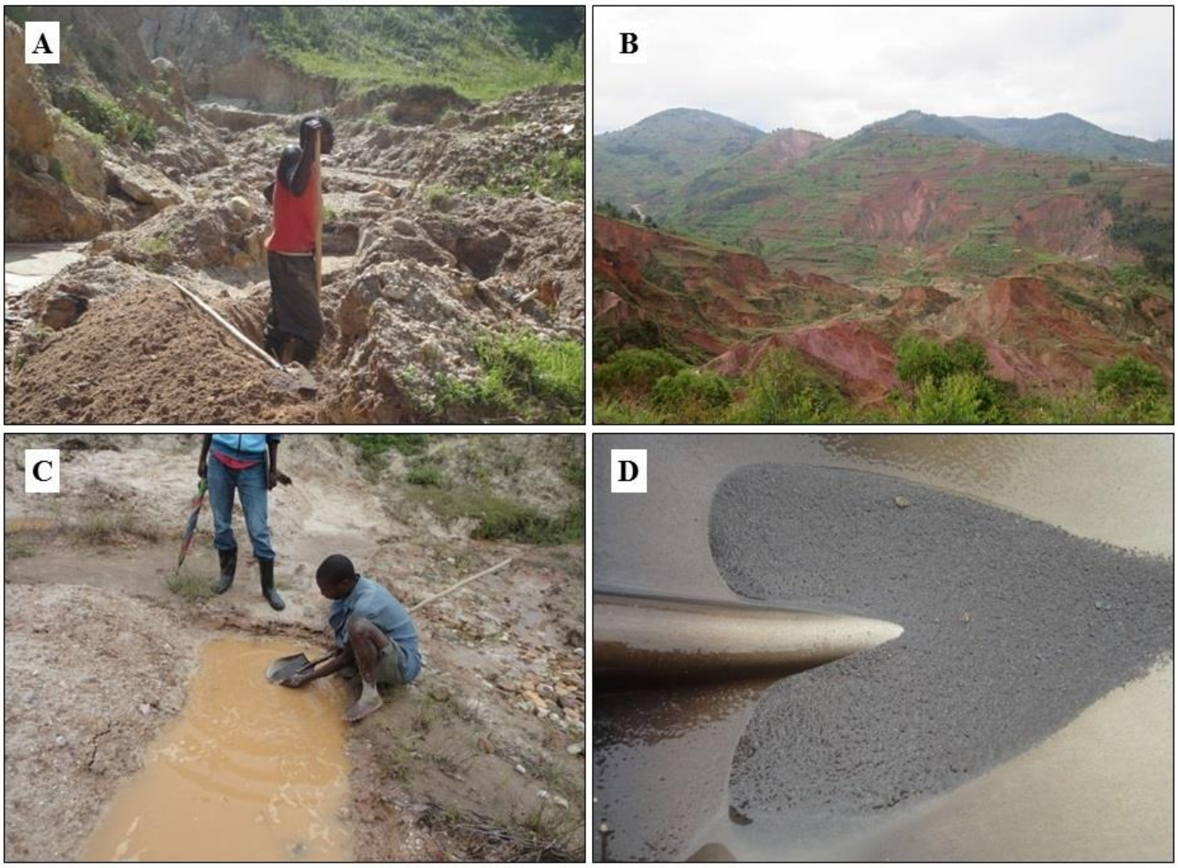

Figure 2. Features of artisanal mining in the GLR depicting (A) an active artisanal mine site, (B) a hummocky landscape from coltan mining, (C) coltan panning in a water channel, and (D) coltan ore retained on a shovel.

1.3 The effects of mining on agriculture in the Great Lakes region of Africa

The global tantalum shortage in the year 2000 led to sudden price hikes and a coltan boom (Hayes and Burge, 2003; Global Witness, 2005) in the GLR with increased interest in the "new path to instant wealth" (Geenen, 2012). The effects of artisanal coltan mining (Figure 2) on agriculture in the GLR can be identified as primary and secondary effects. The primary effects result from the sudden influx of the workforce to mining centers (Moyroud and Katunga, 2002; Hayes and Burge, 2003; International Alert, 2010) to experience a "new wealth status". This is partly driven by desperation to escape poverty since people involved in mining became better off (Hilson, 2009; Geenen, 2012). Artisanal mining attracted many farmers of the GLR who abandoned farming and pastoralism for mining, which they have tagged as a "lifeline" (Geenen, 2012). As a result, a deficit of productive farm labor occurred in the farming communities (International Alert, 2010). Moreover, some farmers turned their mineral-rich farms into mine sites while other farmers' relatives mined their farms (Pole Institute, 2001; International Alert, 2010) leading to the loss of existing farmlands and traditional livelihoods, degradation of land quality, and eventually a breakdown of the rural economy. In the Gatumba Mining District of western Rwanda, for instance, it is common to find desperate artisanal miners encroach on farmlands with crops still growing. This has severely reduced traditional export crop production (Lecoutere et al., 2009). The unregulated nature of artisanal mining (Figure 2) evokes environmental risks, particularly because of weak legal frameworks required for consistent post-mining management (rehabilitation). Consequently, many quarries, denuded land surfaces from land clearing, and rugged hills (Figure 2B) that are vulnerable to mudslides, landslides, erosion, and loss of fertile topsoil are scattered in the region (Biryabarema, 2008; Duskova et al., 2014). This can threaten an entire watershed ecosystem because such areas are barely usable for agriculture unless major infrastructural measures are employed for resurfacing and revegetation.

The secondary effects of mining on agriculture in the GLR result from combined detrimental effects on the quality of air, water, soil, plants, animals, and eventually human well-being. In many rural communities there is a strong link between access to land and food security (Lecoutere et al., 2009) because of heavy dependence on agriculture. A break in this link affects household incomes and food supplies. Reports indicate that artisanal mining has led to food shortages in former food producing areas (Lecoutere et al., 2009) through the abandonment of farming and a population surge linked with a sudden rise in food demands by the new mining communities (Global Witness, 2005). These have aggravated the existing food insecurity, malnutrition, and unemployment and even led to increases in food prices in urban centers (Moyroud and Katunga, 2002). An additional threat to the rural economy and food security is armed military men linked with mining camps who reportedly plunder crops and cattle (Moyroud and Katunga, 2002; Lecoutere et al., 2009; Maass et al., 2012). A study conducted by Maass et al. (2012) suggests that most farmers in the Kivu Provinces of the DR Congo have diversified into small livestock such as rabbits, cavies, chicken, sheep, and goats to mitigate such effects. Although artisanal mining has generated “easy money”, created other economic activities such as petty trading, provided basic needs, supported children's education, and enhanced agricultural activities for some individuals (Geenen, 2012b), its secondary impacts can be debilitating in many areas of the GLR. The absence of financial institutions, insecurity, and moral decadence are common problems associated with mining communities in the GLR (Geenen, 2012). These may plunge residents into alcoholism and other acts of indiscipline. Moreover, armed military men reportedly harass artisanal miners with assorted taxes both at mine sites and en route to trading centers (Global Witness, 2005; Nellemann et al., 2010) as well as road tolls and bribes (Nellemann et al., 2010). They are also reported to torture, rape and kill residents, and destroy personal property (Global Witness, 2005). Ultimately, these incidencies may lead to health, social, and mental problems, which have detrimental effects on agricultural activities and productivity. Beside the socio-economic factors, open mine pits may serve as temporary sinks for rainwater, breed mosquitoes, and worsen the existing poor health and sanitary conditions. Also, the ground sluicing and gravimetric processing methods used in artisanal mining have caused sedimentation in nearby rivers and streams (Flügge et al., 2008; Jeníček and Grofová, 2015). Thus, residents of some communities may have to trek for long distances in search of good drinking water. Moreover, mining-related water pollution may destroy aquatic life and interrupt the natural food chain. Additionally, explosives used in some mine sites are also reported to cause respiratory problems among miners and inhabitants of neighboring communities (Moyroud and Katunga, 2002).

Mining and attendant conflicts in the GLR remain a huge threat to agriculture and the ecosystem at large. This may, in some cases, leave people more impoverished than before (Pole Institute, 2001). As a result, stringent measures are required to mitigate these multidisciplinary and multi-sectoral effects in the short and long run. After the non-renewable mineral resources have been exhausted, a partial reconstruction of the landscape will be required to restore the agricultural potential of the region. In subsequent sections, this study will focus on the important historic tin-tungsten-tantalum mining zone in the Western Province of the Republic of Rwanda known as the Gatumba Mining District (GMD), and specifically address restorative measures in soils affected by former tantalum mining.

1.4 Geochemical composition and mining of tantalum ore in the GMD

Knowledge about the geochemical composition of the ore is an important prerequisite for effective post-mining management since the mine wastes determine, to a large extent, the direction and success of mine ecosystem restoration. Tantalum ore, occurring as columbite-tantalite (niobite-tantalite), is mostly associated with granitic pegmatite bodies either as muscovite class pegmatites or as rare-earth class pegmatites (Černý, 1992). The pegmatite bodies are subdivided into three families: (i) the LCT (Lithium-Cesium-Tantalum) family comprising Li, Rb, Cs, Be, Sn, Ga, Ta > Nb, (B, P, F), (ii) the NYF (Niobium-Yttrium-F signature) family marked by Nb > Ta, Ti, Y, Sc, REE, Zr, U, Th, F signature, and (iii) the mixed LCT + NYF family consisting of granite and pegmatite groups with mixed characteristics (Černý, 1992). Columbite-tantalite has the general formula AB_2O_6, where position A is typically occupied by Fe^{2+} and Mn^{2+}, and to a lesser extent, by Mg^+ and trivalent cations. The B position is mainly occupied by Nb^{5+} and Ta^{5+}, and subordinately, by Ti^{4+} and Sn^{4+}. These orthorhombic minerals include the end-members ferrocolumbite ($FeNb_2O_6$), manganocolumbite ($MnNb_2O_6$), manganotantalite ($MnTa_2O_6$), and magnocolumbite ($MgNb_2O_6$). Ferrotantalite $[(Fe>Mn)(Ta>Nb)_2O_6]$ is a member of the columbite-tantalite group in the classification of niobium-tantalum oxides (Mulja et al., 1996).

The GMD (Figure 3) is located between the Gitarama and Kabaya granite bodies of the Kiberan belt with a network of pegmatites stretching over 5 km between Kirengo, Ruhanga, Gakumba and Kavumu (Dewaele et al., 2011). These pegmatites belong to the LCT family (Flügge et al., 2008) with a tantalum-rich columbite $[(Fe, Mn)(Ta>Nb)_2O_6]$ containing about 52 to 86% by weight of Ta_2O_5 (Flügge et al., 2008, 2009). Therefore, tantalite is the term used to describe the mine soils in this study although the global name Technosols (IUSS Working Group WRB, 2014) is applied in most cases. The pegmatites bodies are white and have variable grain sizes ranging from aplite to giant size crystals and consist of alkali feldspars (albite and microcline), muscovite, and quartz (Lehmann et al., 2014; Nieder et al., 2014). Among the alkali feldspars, potassium feldspars are geochemical indicators of pegmatite zoning (Shearer et al., 1985). The columbite-tantalite together with cassiterite occur in states of primary mineralization in quartz veins, greisens (muscovite-associated cassiterite mineralization) and pegmatites, and (2) secondary mineralization in alluvial deposits

(Dewaele et al., 2011). Such ore are mined with a combination of drilling and blasting with technical equipment and manual methods with simple tools such as hammers, chisels, and pickaxes (Schütte et al., 2011). Secondary alluvial deposits, also called placer deposits (Els and Eriksson, 2006), allow exploitation of the minerals at shallow depths (Els and Eriksson, 2006). This, coupled with the workability of deeply weathered pegmatites enhance their exploitation by artisanal methods (Melcher et al., 2008; Dewaele et al., 2011; Melcher et al., 2015).

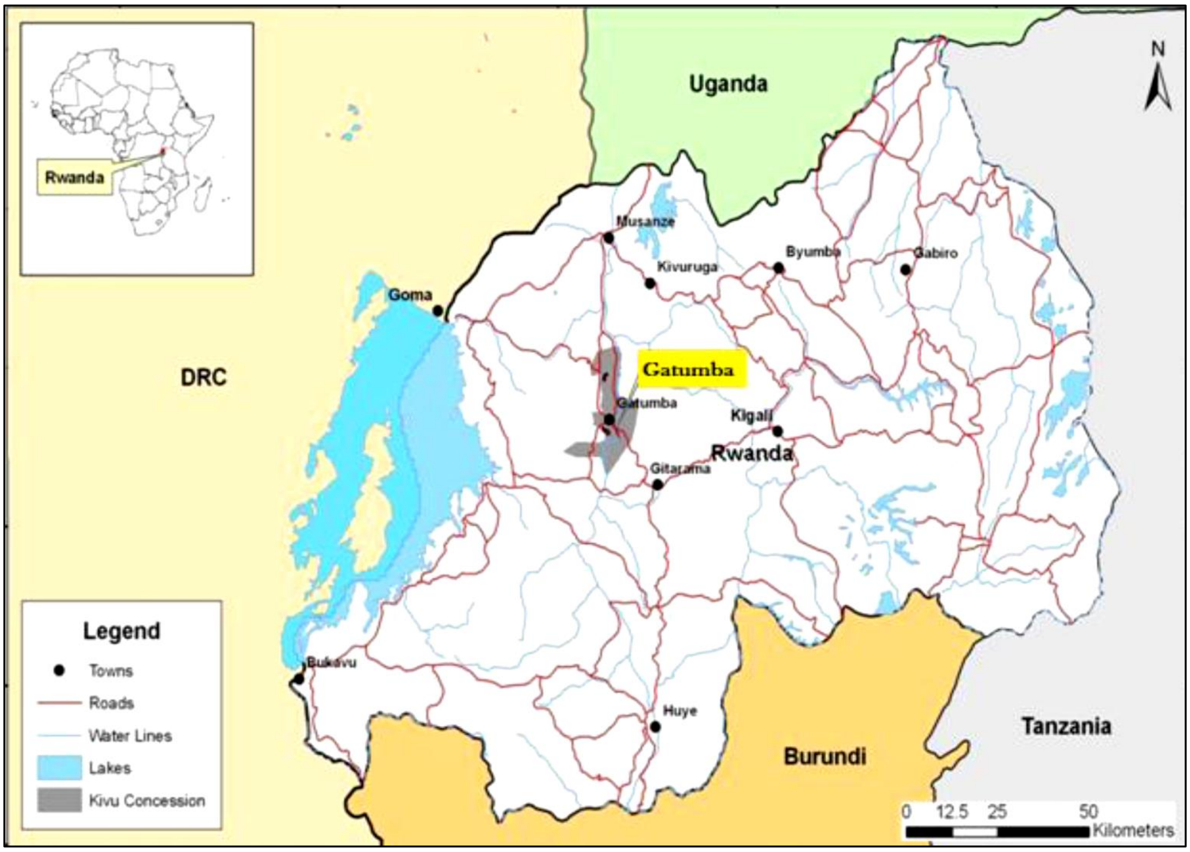

Figure 3. Position of the former Gatumba Mining Concession in Rwanda, Central Africa (Source: MINIRENA, 2015)

The Rwandan mining sector constitutes 26% of the industrial sector and is the second foreign export earner of the country's economy after tourism contributing over 40% of the export revenues (REMA, 2009). Records show that since 1999, the sector has grown by of at least 10% annually (MINIRENA, 2010). In the GMD, the major minerals mined are cassiterite (SnO_2), coltan, and wolframite (MINIRENA, 2015). The coltan deposits are of alluvial and eluvial origin mined by semi-industrial and artisanal mining (Schütte et al., 2011; Nieder et al., 2014). Mining and processing involve gravity separation of the ore from non-ore mineral components through ground sluicing and panning to isolate the ore (Nieder et al., 2014) because of the high density of niobium-tantalum (4.5 to 8.3 g cm^{-3}) minerals (Nikishina et al., 2014) compared with 2.65 g cm^{-3} for most soil minerals. This is aided by water flowing through channels downhill without chemical or metallurgical treatments (Nieder et al., 2014).

Figure 4. Water channels used to pan coltan ore and subsequent erosion (A) contribute to sedimentation of the Nyabarongo River (B) and (C) in Rwanda (Source: REMA, 2011)

1.4.1 Environmental effects of tantalite mining

The environmental effects of mining depend on the type of ore mined, geochemical parameters and composition, the depth and extent of mining, ore processing methods employed, and the pre-mine environment. The obvious features of mining in Rwanda are denuded landscapes caused by the clearing of land causing damage to the entire ecosystem (Biryabarema, 2008). Generally, the natural bedding of the landscape has been disrupted by mining together with erosion and mudslide, leaving an irregular hummocky (Figure 2B) landscape (Flügge et al., 2008). Debris of rocks and mine wastes (MINIRENA, 2015), ditches, trenches, gullies (Duskova et al., 2014), and quarries are also widespread in the area. The ground sluicing and panning (Figure 4) of the ore contributes to siltation and high turbidity of adjacent rivers (Figure 4B and C). Since the mining processes do not employ chemical or metallurgical treatments (Nieder et al., 2014), there are no risks of cyanide and heavy metal pollution, which pose a danger to the environment in mining operations elsewhere (Flügge et al., 2009; Nieder et al., 2014). Previous analyses of water samples from the neighboring streams and rivers revealed no hazardous concentrations of Cr, Cu, Ni, Zn, Cd, and Pb according to WHO guidelines (Nieder et al., 2014). However, uranium (3.7-32 mg kg^{-1}) and arsenic (14-101 mg kg^{-1}) concentrations were high in stream sediments near former mine sites but low in stream waters (Flügge et al., 2008). The actual origin these elements is still a mystery as they can be traced to primary hydrothermal, secondary supergene dispersion, and recent mobilization from intensive agricultural activities (Flügge et al., 2008). Although the current heavy metal concentration may not pose a danger to the environment, the continuous release of these metals into water bodies may lead to their accumulation in some aquatic organisms and finally the food chain.

1.5 Climate, soils, and food security

Rwanda is popularly known as “the land of a thousand hills” because of its mountainous relief broadly classified into four categories as: (a) the Congo-Nile Ridge dominated by eight volcanoes ranging from 3474-4507 m, (b) the Central Plateau consisting of steep hills (2000-1500 m) dissected by valleys, (c) the Eastern Lowlands dominated by depressed relief with undulating landscape and altitudes between 1100-1500 m, and (d) the Bugarama Plains of southwest Rwanda (part of the Great Rift Valley) with an average altitude of 900 m a.s.l. (REMA, 2011). The rainfall distribution is bimodal with alternating short dry seasons. Usually, the highlands have more rains (> 2000 mm p.a.) than the lowlands (< 1000 mm p.a.), giving a range of humid to semi-arid rainfall regimes. This climatic diversity allows cultivation of crops suited for tropical and temperate conditions. Therefore, three agricultural seasons per year are possible. The main crops cultivated in Rwanda are banana, cassava, sweet potato (*Ipomoea batatas* (L.) Lam.), potato (*Solanum tuberosum* L.), soybean (*Glycine max* (L.) Merr.), common bean (*Phaseolus vulgaris* L.), and pea (*Pisum sativum* L.) (Verdoodt and van Ranst, 2003b).

The dominant Reference Soil Groups in Rwanda are Cambisols, Ferralsols, Acrisols, and Alisols (Verdoodt et al., 2010). However, the soils in the GMD are more diverse partly due to the additional influence of mining and hydrology. Reetsch et al. (2008) found Anthrosols, Cambisols, Gleysols, Fluvisols, Nitisols, Lixisols and Umbrisols in the area. Over half of Rwandan soils have been developed from shale in association with quartzite, and to some extent, granite. In some parts of western Rwanda, shale and granite transformed slightly into schist, mica schist, and micaceous granite. About 30% of Rwandan soils have deep soil profiles (>1 m) and argillic B-horizon as the key development stage with clay contents ranging from 35-60% (Verdoodt and van Ranst, 2003a). The soils are strongly weathered and dominated by low activity clay minerals such as kaolinite leading to low cation exchange capacity (Reetsch et al., 2008). About 75% of the soils (Yamoah et al., 1990) have pH-H_2O ranging from 4.7-6.0, but mostly below 5.5 (Verdoodt and van Ranst, 2003a; Reetsch et al., 2008) and are associated with Al saturation and low phosphorus (P) availability. Intense leaching led to low base saturation and low basic cation content in 50% of the soils (Verdoodt and van Ranst, 2003a) Additionally, soil organic carbon (SOC), total nitrogen (N), and available P contents are generally low, but vary from 0.9-32.8%, 0.028-12.9% (Reetsch et al., 2008; Verdoodt et al., 2010), and 0.00-372 mg kg^{-1} (Verdoodt et al., 2010), respectively.

1.1 Existing and potential soil fertility amendments

Rwanda is the most densely populated country in mainland Africa (REMA, 2011) with an annual population growth of 2.6% according to 2015 estimates. About 71% of the population lives in rural areas with high dependence on land for their livelihoods (Central Intelligence Agency, 2015b). The scarcity of agricultural land in the country, particularly in the GMD requires the use of mine wastelands for farming (Figure 5). Over 60% of the Rwandan population hold a farm size of 0.7 ha, while at least 25% have less than 0.2 ha per farming household (Bucagu et al., 2008; International Monetary Fund, 2008). The land scarcity is worsened by poor soil fertility which is the second cause of poverty in Rwanda (International Monetary Fund, 2008). Thus, 36-40% of the households in the area suffer from high food insecurity (REMA, 2009). According to the FAO (1996), "food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life". Food insecurity in Rwanda can be described as a consequence of the combined influence of climate, geomorphology and relief, soil fertility, population growth, and human activities. Therefore, measures that improve soil fertility under the given socio-ecological conditions enhance food security.

Figure 5. Cultivated Technosols in the Gatumba Mining District of Rwanda showing small amounts of manure (A) ready to be applied before planting.

In most parts of Rwanda, inorganic fertilizers are seldom used (Yamoah et al., 1990) because of limited access and financial constraints. Farmyard manure (FYM), animal dung mixed with straw, feed refusals and other organic leftovers, is the typical soil amendment at the farm level. It is estimated that 83% of the households in the GMD own livestock (NISR, 2011), but in few numbers. Through the Girinka program (Rwanda's one cow per poor family policy; MINAGRI, 2006), virtually every household has a cow, which is the source of the scanty FYM (Figure 5). A substantial quantity of compost from municipal solid waste is also available, but inaccessible to many smallholder farmers in GMD due to financial and transport constraints. The zero-grazing policy also allows goat manure collection to some extent, but in small amounts compared with FYM. The low manure availability hampers crop production in the area.

To supplement FYM as a fertilizer, several multi-purpose plant species thrive in the area which could serve as soil amendments. These include *Tithonia diversifolia* (Hemsley) A. Gray, *Markhamia lutea* (Benth.) K. Schum., and *Canavalia brasiliensis* (Mart. ex Benth.). *Tithonia* (Mexican sunflower) is an invasive plant adapted to the Gatumba mine sites (Gakwerere et al., 2013). It is well-known for nutrient scavenging capacity and its biomass has proved useful in improving soil nutrient levels and increasing crop yields (Drechsel and

Reck, 1998; Gachengo et al., 1999; Kwabiah et al., 2003; Olabode et al., 2007). *Canavalia* is a drought-tolerant legume cover plant adapted to low soil fertility and a wide range of soil pH levels (4.3-8.0; Schloen et al., 2015). It thrives in the GMD, covers the ground within two months after sowing and can suppress weeds. It is also a widespread component of the fallow vegetation (Schloen et al., 2015) and has been reported to promote C sequestration in surface soils of a no-tillage system in Brazil (Carvalho et al., 2014). In the highlands of Rwanda, *Canavalia* has been found to produce more biomass and fix more N than other leguminous covers (Yamoah and Mayfield, 1990). *Markhamia* is an indigenous tree species and ranks among the five top-priority agroforestry species in tropical Africa (Owino, 1992). It is virtually extinct within the farming communities (Mugunga, 2008), but contains substantial amounts of plant nutrients (Silas et al., 2012). Together with *Ficus thonningii* (Blume), *Markhamia* provides good bee forage, is a source of high-quality timber, fuelwood, and charcoal (World Agroforestry Centre, 2013) and its leaves have medicinal value (Kernan et al., 1998). Unfortunately, the potential of these native plant species to improve soil microbial properties, particularly in the tantalite mine soils of Central Africa are heavily under-investigated.

1.2 Research questions and objectives

A survey conducted within the Coltan Environmental Management Project funded by the Volkswagen Foundation (Volkswagen Stiftung) in the area indicated the potential of yield increases in bean, cassava, cocoyam and sweet potato due to the application of animal manure to mine land (R. Diogo, unpublished data). Similar findings were reported by Ndoli et al. (2013) from soybeans fertilized with *Tithonia* biomass mixed with phosphate rock and triple super phosphate. In the context of these attempts to improve crop yields, little attention is given to the role of microorganisms in mine soil quality improvement although much work has been done on the biological quality of arable soils (Adeboye et al., 2006; Adeboye et al., 2011). As an essential soil component, microorganisms mediate C cycling and nutrient release in terrestrial ecosystems. However, it is unknown how the tantalite mine soils of the GMD will respond to the amendments available for soil reconstitution under the different edaphic factors, environmental conditions, and variations in substrate quality.

After decades of mining, the afforested and cultivated mine wastelands in the GMD provide an ideal case of post-mining land reclamation useful to study the effects of applied soil amendments on biological soil quality. The tantalite mine soils are denoted here as Technosols (IUSS Working Group WRB, 2014). The afforested ones are referred specifically referred to as pine and eucalyptus Technosols while the cultivated ones are Kavumu and Kirengo Technosols. The key questions addressed in this study were: What are the effects of these post-mining land uses on the microbial properties of the tantalite mine soils, and which soil properties pose limitations to these microbial properties? How can the application of organic amendments contribute to restoring the biological quality of the Technosols? Can the N and P supply potential of the amendments applied to the tantalite mine soils be estimated in the short-term? To address the questions, the objectives of the study were to:

1. Evaluate the effects of land use on soil microbial indices of the tantalite mine soils (Chapter 2);
2. Investigate the restorative effects of the amendments in a Technosol (Chapter 3); and
3. Estimate the short-term N and P supply potential of the amendments (Chapter 4).

The last chapter provides an integrated discussion on critical soil fertility constraints, concludes the study, recommends possible mitigations of the constraints, and presents an outlook for future research.

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2 Effects of land use on microbial indices in tantalite mine soils, western Rwanda

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2.1 Abstract

Rare metal mining in western Rwanda created degraded mine wastelands. Some of them were restored by afforestation with pine and eucalyptus forests while others have been cultivated alongside arable lands, owing to limited land availability. We assessed the effects of these land uses on microbial biomass and activity (basal C and net N mineralization) through a laboratory experiment with fresh soils (0-20 cm) of an unmined native forest, two Technosols afforested with pine and eucalyptus forests, an arable soil and two cultivated mine soils (Technosols). Afforestation increased soil organic C (SOC) and total N in pine and eucalyptus Technosols, reaching 34-40% and 28-30% respectively of that in the native forest. The tree species differed significantly in microbial biomass and activity, exhibiting a trajectory towards recovery depicted in a lower metabolic quotient. Basal respiration of pine Technosol and native forest soil were similar compared with eucalyptus Technosol, exhibiting 11 and 14% less of its forest counterparts. Conversely, net N mineralization in the pine and eucalyptus Technosols were 40 and 65% of that in native forest soil, respectively. Soil microbial biomass C in the pine and eucalyptus Technosols was 33 and 48% of that in the native forest soil. A decreased SOC availability to soil microorganisms promoted fungal ergosterol as an indicator for saprotrophic fungi at low soil pH. The arable soil and cultivated Technosols did not differ significantly in SOC, N, and microbial biomass and activity. The soil biological quality was constrained by soil acidity, dithionite extractable Al, and low P availability.

Keywords: Bacal C respiration, ergosterol, metabolic quotient, microbial biomass, Technosols

2.2 Introduction

It is well documented that mining exerts potential environmental impacts (Zipper et al., 2011; Baer et al., 2012), which may cause great ecosystem impairments (Bradshaw, 1997; Baer et al., 2012) by reducing soil, water and air quality, and biodiversity. The degree of impairment depends predominantly on the type and extent of mining, mineral ore exploitation, processing methods, and geochemical composition of the overburden (Bradshaw, 1997; Kim et al., 2007). Surface mining is generally more destructive, causing loss of soil organic matter (SOM), the core integrity of soil health and quality. According to IPCC (1998), degraded lands lose 50% of SOM in non-degraded or native vegetation. A higher fraction is presumably lost from mine sites, depending on the extent and depth of mining, and whether top- and subsoils are replaced during post-mining closure. SOM deficit reduces the operational biological community and energy required for continuous nutrient cycling in mine ecosystems. As the storehouse of most major nutrients, SOM provides the energy of life for microorganisms (Craswell and Lefroy, 2001) and maintains the functional continuity of the soil plant atmosphere-continuum. Moreover, mine soils are often characterized by unfavorable pH, high bulk density, and toxicity from pyrite-associated heavy metals (Bradshaw, 1997; 2000; Ussiri and Lal, 2005), which present environmental challenges for plant growth and establishment.

To offset these challenges in mine soils, the Forest Reclamation Approach has been widely employed (Zipper et al., 2011) to restore the functional integrity of mine soils. Previous research shows that afforestation accelerates ecological succession in degraded lands (Bradshaw, 1997, 2000; Zipper et al., 2011) because of the trees' capacity to forage plant nutrients beyond the root zone (Bradshaw, 2000), deposit biomass for subsequent decomposition, and increase biological weathering of parent material (Boyle and Voigt, 1973). For instance, in reclaimed coal mine sites of western Pennsylvania, SOM was the key element in natural succession and correlated with the microbial community structure (Brenner et al., 1984) since it enhanced microbial proliferation and activity, and facilitated plant nutrient supply. However, the effects of specific tree species on mine soil properties differ (Jandl et al., 2007; Nsabimana et al., 2008; Chodak and Niklińska, 2010b) mainly because of differences in biomass quality.

Mining of tin-tungsten-tantalum ore begun in Rwanda during the early twentieth century (MINIRENA, 2010) in a predominantly artisanal manner, generating large degraded mine wastelands, particularly in Rwanda's Western Province. Around 1910, Belgians instituted afforestation programs to rehabilitate degraded lands (Biggelaar and Gold, 1996), control erosion, create buffer zones, natural reserves, and meet escalating demands for wood products (Roose and Ndayizigiye, 1997; Nsabimana et al., 2008). This led to widespread planting of exotic tree species like *Eucalyptus spp.*, *Pinus patula* (Scheide. ex Schltdl. & Cham.), *Cupressus lusitanica* (Mill.) (Biggelaar and Gold, 1996; Mugunga, 2008; Nsabimana et al., 2008), *Grevillea robusta* A. Cunn. ex R. Br. and *Persea gratissima* C.F. Gaertn. (Biggelaar and Gold, 1996) on mined sites throughout the country. Previous studies in southern Rwanda (Nsabimana et al., 2008) showed that after 34-80 years of afforestation, the planted trees led to major improvements in SOC and total N. Beside the afforested mine sites, other tantalite mine sites in western Rwanda have been cultivated alongside arable and marginal lands because of ever increasing pressure on land.

Despite major research devoted to mine land reclamation, few have focused on biological mine soil quality (Dutta and Agrawal, 2002; Šourková et al., 2005; Anderson et al., 2008; Chodak and Niklińska, 2010a). Such studies were mostly conducted in temperate regions, but are very scarce in the tropics, particularly in sub-Saharan Africa. A recent review by Joergensen (2010) suggests very limited research on biological soil quality in Africa, especially in mine soils. While most mine soils are often associated with acid mine drainage and high heavy metal content, the tantalite mines soils of the Gatumba Mining District (GMD), in western Rwanda have low heavy metal contents and the latter pose no danger to the environment (Lehmann et al., 2008). This is advantageous and offers a competitive advantage for effective restoration of these sites.

In this study, we investigated whether differences exist between basal C respiration at the sites, and whether this was related to microbial biomass of restored (afforested) tantalite mine soils and cultivated ones (also Technosols). Additionally, we examined edaphic factors that may have posed limitations to the microbial biomass and activity. We hypothesized that, the afforested and cultivated Technosols differ in microbial biomass and activity due to the different management approaches and a reduced SOM availability. To resolve these queries, we conducted a laboratory study using fresh soil samples from the sites as a basis for future restoration experiments of Technosols with locally available organic amendments.

2.3 Materials and methods

2.3.1 Study sites and soil sampling

We selected six sites at the Gatumba Mining District in the Western Province of Rwanda, located between the Congo-Nile Ridge and the Central Plateau with altitudes between 2500-3000 and 2000-1500 m a.s.l., respectively to suite conditions described above. The Gatumba Mining District is located near the Nyabarongo River between longitudes 29°37” and 29°40” E, and latitudes 1°53” and 1°56”S. Hills, mountains, and grassy uplands dominate the area with average temperatures between 18° and 21°C (REMA, 2011). The rainfall distribution is bimodal averaging 1320-2400 mm per annum. The dominant soil types are Anthrosols, Cambisols, Fluvisols, Gleysols, Lixisols, Luvisols, Nitisols, and Umbrisols (Reetsch et al., 2008; IUSS Working Group WRB, 2014).

The sites (Figure 6) comprised an unmined native forest, pine and eucalyptus Technosols, an arable land, and Kavumu and Kirengo Technosols. The native forest and arable land were chosen as references for the afforested and cultivated Technosols.

a) The native forest refers to an unmined natural forest reserve in Muhanga District, Southern Province, Rwanda. *Chrysophyllum gorungosanum* Engl., *Polyscias fulva* (Hiern) Harms, *Macaranga neomildbraediana* Lebrun, *Maesa lanceolate* Forssk., *Dombeya torrida* J.F. Gmel., and *Albizia gummifera* (J.F. Gmel.) C. A. Sm. dominate this forest.

b) The pine Technosol denotes an afforested mine wasteland established around 1982 (Gatumba Mining Concession, personal communication, 6 May 2013), comprising *Pinus patula* (Scheide. ex Schltdl. & Cham.) forest on a hill with 45-50° slope at Nganzo in the Sitwe cell. Tree stumps and branches were found on this site, indicating recurrent tree felling for wood products. A turf of fine roots and litter cemented by mosses covered the forest floor.

c) The eucalyptus Technosol is an afforested mine wasteland of about 30 years old (C. Rwabahizi, personal communication, 6 May 2013) located on 30-40° slope adjacent the Ruhanga coltan quarry near Rusumo village. Older trees had been cut down leading to new growth.

d) The Kavumu Technosol is located in the Kavumu coltan quarry. It was an experimental site for the Coltan Environmental Management Project and farmed by the residents. At the time of sampling, it was used for a cassava experiment with compost, wood ash and human urine as soil amendments.

e) The Kirengo Technosol is a mined site cultivated for several years and used by the Coltan Environmental Management Project.

f) The arable land refers to a farm in Kavumu village cultivated with beans, green peas, sweet potatoes and cassava in a mixed pattern.

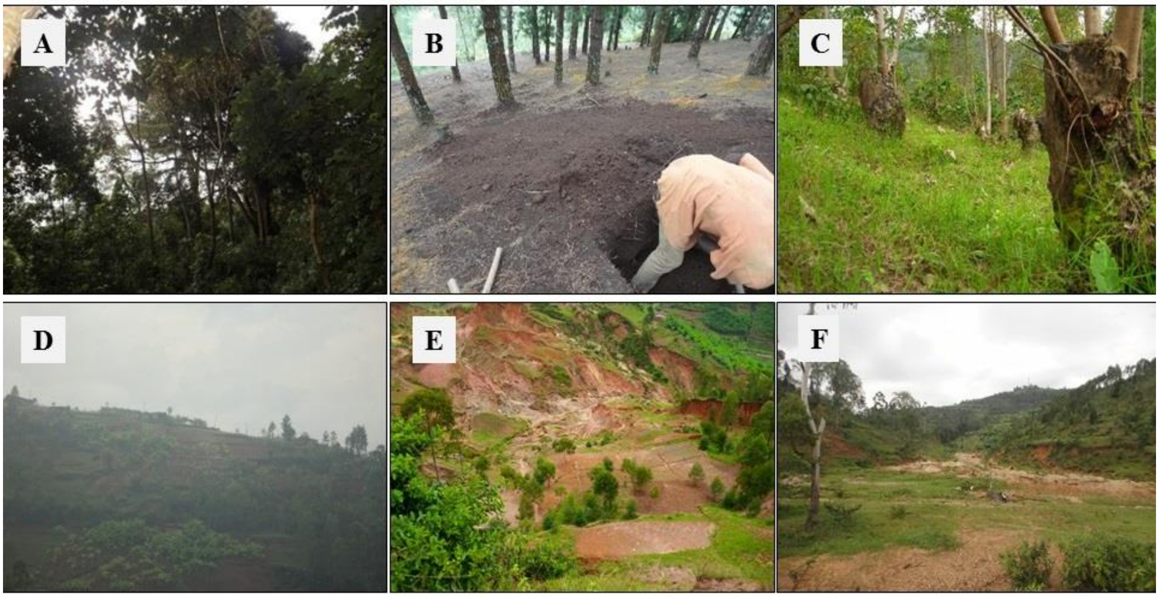

Figure 6. Images of the study sites showing the native forest (A), pine Technosol (B), eucalyptus Technosol (C), arable land (D), the Kavumu Technosol (E), and the Kirengo Technosol (F) at the Gatumba Mining District in Western Rwanda.

A stratified random sampling method was used to capture the soil variability along the slopes. First we marked out five strata of 20 m width on each site and randomly collected five bulk samples to 20 cm depth. Sampling rings were cored to 20 cm depth for bulk density determination. The samples were sieved (< 2 mm), transported to Germany and stored at 4°C until experimentation.

2.3.2 Physicochemical soil analysis

Soil pH, bulk density, and texture were measured with a probe in 1:2.5 soil-water ratio, gravimetrically at 105°C, and by the pipette method after oxidation of SOM with H_2O_2 respectively. The contents of C and N were determined by dry combustion using a Vario MAX CHN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Calcium carbonate ($CaCO_3$) was not present, thus C content equaled SOC. Oxalate- and dithionite-extractable Al (Al_{ox}, Al_d) and Fe (Fe_{ox}, Fe_d) were determined as described in Blume et al. (2011). Total P was determined by microwave (MLS GmbH, Leutkirch im Allgäu, Germany) digestion in aqua-regia (32% HCl, 65% HNO_3). The extracts were analyzed by ICP-AES (Varian VISTA, Varian Inc., Melbourne, Australia). We determined resin P by shaking 4 g dry weight soils in 100 ml bidistilled water with strong base anion exchange membranes (Sartobind Q, Sartorius Stedim Biotech, Göttingen, Germany) at 175 rev min^{-1} for 20 h and eluted the samples with 25 ml 0.5 M HCl for 1 h. The extracts were measured on a FLUOstar Omega Microplate Reader (BMG Labtech, Ortenberg, Germany) at 882 nm.

2.3.3 C and N mineralization

Soils (80 g DW) were weighed into 500 ml glass jars, adjusted to 50% water holding capacity and pre-incubated for one week. Glass vials containing 5 ml 0.25 M NaOH were placed at the bottom of the jars and closed tightly to trap CO_2. Glass jars were randomly placed in an incubator at 22°C for 28 days. Evolved CO_2 was measured at 7, 14, 21, and 28 days after precipitation of Na_2CO_3 with 5 ml of saturated $BaCl_2$ and back-titrated with 0.25 M HCl in the presence of phenolphthalein indicator. Inorganic N (NH_4^+ and NO_3^-) was measured at day 0 and at day 28 by extracting 10 g soil with 40 ml 0.5 M K_2SO_4 after shaking at 200 rev min^{-1} for 30 min and filtering with folded filter paper (hw3, Sartorius Stedim Biotech GmbH, Göttingen, Germany). The extracts were measured on a continuous flow analyzer (Evolution II, Alliance Instruments GmbH, Ainring, Germany). Net N mineralization was estimated from the difference between inorganic N at days 0 and 28.

2.3.4 Soil microbial biomass

Microbial biomass C and N were estimated by the fumigation-extraction method using 0.5 M K_2SO_4 as extractant (Brookes et al., 1985). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and $k_{EC} = 0.45$ (Wu et al., 1990). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and $k_{EN} = 0.54$ (Brookes et al., 1985).

Soil microbial biomass P was also measured by the fumigation-extraction method (Brookes et al., 1982), using Bray-I extractant, i.e. 0.025 M HCl + 0.03 M NH_4F (Khan and Joergensen, 2012). From each jar, 6 g (on an oven-dry basis) soil was divided into three subsamples. The first subsample (non-fumigated) was extracted with 40 ml Bray I solution by shaking at 150 rev min^{-1} for 30 min. The suspension was centrifuged for 10 min at 2000 *g* and filtered (hw3). The second subsample (non-fumigated P recovery) was spiked with KH_2PO_4 at 25 μg P g^{-1} soil while the last subsample was fumigated as described above. Both groups were extracted as the non-fumigated samples and measured on a FLUOstar Omega Microplate Reader at 882 nm. Microbial biomass P was calculated as E_P / k_{EP} / recovery, where E_P = (PO_4-P extracted from fumigated soil) - (PO_4-P extracted from non-fumigated soil) and k_{EP} = 0.40.

The fungal cell-membrane component ergosterol was extracted from 5 g (on an oven dry basis) moist soil with 100 ml ethanol by oscillated shaking at 250 rev min^{-1} for 30 min according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

2.3.5 Statistical analysis

The results presented in the tables are arithmetic means of each land-use type and refer to an oven-dry basis (105°C, 24 h). Normality of residuals was tested by the Shapiro-Wilk test and homogeneity of variance by the Levene's test. If necessary, non-normally distributed data were ln or square root transformed before one-way ANOVA with sites as the main factors. Paired t-tests were applied where necessary. Means are expressed on dry soil weight basis (n = 5). The significant differences between the land use types was tested by a one-way ANOVA, followed by the Holm-Sidak method as a pair-wise multiple comparison test. Multiple regression models were calculated between soil microbial properties and independent variables that were selected from physical, chemical and microbial soil properties by stepwise forward regression analysis. All regression models were tested for normality, constancy of variance, absence of correlation between the residuals (Durban-Watson statistics) and absence of multi-collinearity, calculating the variance inflation factor (VIF). Variables were removed from the model if the VIF value exceeded 4.0. All statistical analyses were performed using SigmaPlot 13.0 (Systat Inc., San José, CA, USA).

2.4 Results

2.4.1 Post-mining land use effects on soil properties

The soils were strongly acid and grouped into "cultivated soils" (arable soil and cultivated Technosols) with pH around 5, afforested Technosols with pH above 4, and the native forest soil with pH below 4 (Table 1). An outlier of pH 8 was observed in one replicate of Kavumu Technosol, because of wood ash applied during a cassava experiment leading to a higher average pH for this site. The "cultivated soils" formed a trio of high bulk density soils compared with the "forest soils". Clay contents ranged from 24-50% and soil texture comprised sandy clay loams for the pine, Kavumu, and Kirengo Technosols, and clayey loams for arable soil, native forest soil, and eucalyptus Technosol.

Resin-P ranged from 0.6 to 1.2 μg P g^{-1} soil with the highest value in the native forest soil (Table 2). Bray-I-P was high in the arable soil and Kavumu Technosol, but low in eucalyptus Technosol. Phosphorus recovery was relatively high in Kirengo Technosol and low in the afforested Technosols. The dithionite-extractable Fe content, Fe_d, of the soils was generally higher (17-45 mg g^{-1} soil) than dithionite-extractable, Al_d (2.2-5.3 mg g^{-1}

soil). Meanwhile, dithionite-extractable, Al_{ox} and Fe_{ox} were higher in the "forest soils" than in the "cultivated soils" (Table 2). The ratios of oxalate to dithionite Al and Fe ranged from 0.16-0.59 and 0.10-0.36 respectively, with consistently high ratios in the "forest soils".

Table 1. Soil pH, bulk density, and particle size distribution in the native forest soil, and afforested Technosols, arable soil, and the cultivated Technosols of the Gatumba Mining District, western Rwanda.

Site	pH (H_2O)	Bulk density (g cm^{-3})	Sand	Silt	Clay
				%	
Native forest	3.7 c	0.9 b	29	25	46
Pine Technosols	4.4 b	1.1 b	63	9	28
Eucalyptus Technosols	4.5 b	1.0 b	36	22	42
Arable land	4.9 a	1.3 a	39	11	50
Kavumu Technosol*	5.6 a	1.2 a	51	16	33
Kirengo Technosol	5.2 a	1.3 a	67	9	24
CV (± %)	*0.4*	*9*	*47*	*44*	*38*

CV = pooled coefficient of variation between replicate field samples (n = 5). Different letters indicate a significant difference between means within a column (Tukey HSD, $P < 0.05$). *Contained an outlier of pH 8 in sample from a plot applied with of wood ash during an on-going experiment. The mean pH without the outlier was 5.0.

The SOC content differed (t-test, $P < 0.001$) among the sites and grouped into "forest soils" and "cultivated soils" (Table 3), where the native forest soil had more than twice the SOC of the afforested Technosols and about six-fold that of "cultivated soils". The N content followed the pattern of SOC and barely differed between the arable soil and cultivated Technosols. Consequently, the C/N ratios ranged from 11-18. The pine Technosol had the highest C/N ratio while the arable soil had the lowest. Total P followed no peculiar trend, but the native forest soil had the largest value (Table 3), whereas the pine Technosol had the lowest. The C/P ratio was relatively high in the native forest soil and pine Technosol, moderate in the eucalyptus Technosol and low in the "cultivated soils".

2.4.2 Post-mining land use effects on soil microbial activity and biomass

Cumulative CO_2 grouped into "forest soils" with high CO_2 efflux compared to "cultivated soils" with a significantly (t-test, $P < 0.01$) lower CO_2 efflux (Table 3). Net N mineralization also formed two groups ranging from 11-40 µg g^{-1} soil. The SOC correlated with net N mineralization ($r = 0.90$, $P < 0.01$). Soil pH, dithionite-extractable Al and C/P ratio explained 74% ($P < 0.001$) whereas Bray-I-P, silt and dithionite-extractable Al contents, C/N and C/P ratios explained 83% ($P < 0.001$) of the variability in CO_2 efflux and net N mineralization respectively (Table 4). The ratio of CO_2 efflux to net N mineralization varied from 8 to 36 with the highest and lowest values in arable soil and native forest soil, respectively (Table 3).

Table 2. Contents of total P, resin P, Bray-I-extractable P, dithionite- and ammonium oxalate-extractable Fe and Al in native forest soil, pine and eucalyptus Technosols, arable soil, and cultivated Technosols of the Gatumba Mining District, western Rwanda

Site	Total P	Resin-P	Bray-I-P	Recovery	Fe_d	Al_d	Fe_{ox}	Al_{ox}
		($\mu g\ g^{-1}$ soil)		(% Bray-I-P)		($mg\ g^{-1}$ soil)		
Native forest	760 a	1.2	6.6 a	32	44 ac	5.3 a	9.2 a	1.8 a
Pine Technosol	320 c	0.6	6.7 a	19	17 bc	3.7 bc	6.1 ab	2.2 a
Eucalyptus Technosol	500 b	1.0	1.3 b	20	34 ac	3.5 bc	7.3 ab	1.5 ab
Arable land	550 ab	0.8	7.0 a	31	45 a	4.9 ac	4.5 b	0.8 b
Kavumu Technosol	420 b	0.5	7.1 a	32	44 ac	4.2 ab	5.3 ab	0.9 b
Kirengo Technosol	610 ab	0.7	4.8 a	43	23 ac	2.2 b	4.2 b	0.7 b
CV (± %)	*18*	*70*	*80*	*50*	*21*	*24*	*36*	*35*

CV = pooled coefficient of variation between replicate field samples (n = 5). Different letters indicate a significant difference between means within a column (Tukey HSD, $P < 0.05$)

Table 3. Contents of SOC, total N and their ratios, cumulative CO_2, net N mineralization (N_{min}) and their ratios, and metabolic quotient (qCO_2) in native forest soil, pine and eucalyptus Technosols, arable soil, and cultivated Technosols of the Gatumba Mining District, western Rwanda

Site	**SOC**	**Total N**	**SOC/ total N**	**SOC/ total P**	**ΣCO_2-C**	**Net N_{min}**	**ΣCO_2-C/ net N_{min}**	**qCO_2**
	(mg g^{-1} soil)				**(µg g^{-1} soil 28 d^{-1})**			**(mg CO_2-C g^{-1} MBC h^{-1})**
Native forest	62.2 a	5.0 a	12 c	82 a	294 a	40 a	8 b	0.67 d
Pine Technosol	24.7 b	1.4 b	18 a	78 a	301 a	26 ab	13 a	2.26 b
Eucalyptus Technosol	21.4 b	1.5 b	14 b	43 b	264 a	16 b	21 a	1.28 c
Arable land	10.9 c	1.0 c	11 c	18 c	143 c	12 b	36 a	2.50 b
Kavumu Technosol	10.2 c	0.9 c	12 c	19 c	178 bc	12 b	19 a	4.26 a
Kirengo Technosol	8.7 c	0.6 c	14 b	18 c	191 ab	11 b	19 a	4.15 a
CV (± %)	*44*	*44*	*7*	*36*	*26*	*61*	*50*	*52*

CV = pooled coefficient of variation between replicate field samples (n = 5). Different letters indicate a significant difference between means within a column (Tukey HSD, $P < 0.05$).

Table 4. Multiple linear regression models (stepwise forward method) between the microbial properties and other soil properties of the soils from the Gatumba Mining District, western Rwanda

	ΣCO_2-C	**Net N_{min}¥**	**Microbial biomass C¥**	**Ergosterol¥¥**	**qCO_2¥**
Intercept	-361.9**	0.771	2.427***	-0.316*	0.881*
Predictor 1	50.4 pH***	0.982 SOC/P_{total}¥***	0.986 SOC¥***	0.282 SOC/P_{total}¥***	0.284 pH***
Predictor 2	-17.1 Al_d*	0.495 Bray-I-P¥***			-0.876 MBC/SOC***
Predictor 3	121.2 SOC/P_{total}¥***	0.040 Silt***			2.463 Al_d**
Predictor 4		-0.379 Al_d***			1.532 N_{total}¥***
Predictor 5		-0.101 SOC/N_{total}*			
R^2 (%)	73.7***	83.3***	81.3***	62.3***	92.4***

¥ln-transformed, ¥¥square root-transformed

N_{min} = Net N mineralization

P_{total} = total P

N_{total} = total N

***$P < 0.001$

**$P < 0.01$

* $P < 0.05$

Table 5. Contents of microbial biomass C, N, P and their ratios, microbial biomass as %SOC, ergosterol as well as ergosterol to microbial biomass C in the native forest soil, afforested Technosols, arable soil, and the cultivated Technosols of the Gatumba Mining District, western Rwanda

Site	**Microbial biomass**				**C/N**	**C/P**	**Ergosterol ($\mu g\ g^{-1}$ soil)**	**Ergosterol/ MBC[*] (%)**
	C	**C**	**N**	**P**				
	($\mu g\ g^{-1}$ soil)	**(% SOC)**	**($\mu g\ g^{-1}$ soil)**					
Native forest	654 a	1.1	108 a	71 a	6.4	10	0.79 a	0.13
Pine Technosol	213 b	0.9	40 b	35 b	5.3	10	0.97 a	0.50
Eucalyptus Technosol	312 b	1.5	52 b	36 b	6.6	9	0.75 a	0.25
Arable land	134 c	1.1	29 b	9 c	4.0	19	0.21 b	0.19
Kavumu Technosol	126 c	1.2	47 b	9 c	4.0	27	0.25 b	0.38
Kirengo Technosol	103 c	1.2	30 b	11 c	5.0	24	0.14 b	0.19
CV (± %)	*53*	*29*	*57*	*55*	*38*	*61*	*47*	*63*

CV = pooled coefficient of variation between replicate field samples (n = 5). Different letters indicate a significant difference between means within a column (Tukey HSD, $P < 0.05$). Paired t-test showed significant ($P < 0.001$) differences between microbial biomass C, N and P ratios.

[*] MBC: microbial biomass C

Microbial biomass C (MBC) grouped into relatively high abundance in the native forest soil, moderate in the afforested Technosols, and low in the "cultivated soils" (Table 5). The last two groups contained 33 and 48%, and 16-20% of native forest soil MBC. The eucalyptus Technosol had the highest MBC content among the Technosols while Kirengo Technosol had the lowest. Despite the high MBC content in the "forest soils", its contribution to SOC was barely different from that of the "cultivated soils". In the eucalyptus Technosol, the MBC contributed more to SOC than in the pine Technosol. Soil pH negatively correlated ($r = 0.79$, $P < 0.01$) with MBC compared with a positive correlation with SOC ($r = 0.92$, $P < 0.01$). SOC content was the best predictor ($R^2 = 0.81$, $P < 0.001$) of MBC (Table 4). Unlike MBC, microbial biomass N exhibited a slightly different trend where native forest had over twice ($P < 0.05$) the amount in the other soils. Microbial biomass N varied between 29 and 108 µg g^{-1} soil, with the highest and lowest values in native forest and arable soils, respectively. Thus, the microbial biomass C/N ratio ranged from 4.0-6.6, but showed no substantial disparity among sites. The microbial biomass P content was highest ($P < 0.05$) in the native forest soil, constituting 1.6-10.9% of total P with an average of 5.5% having microbial biomass C/P ratios from 9-27.

The metabolic quotient, qCO_2, varied significantly ($P < 0.001$) among sites and formed a profile of low qCO_2 in the native forest soil, moderate in the afforested Technosols as well as the arable soil, and high in the cultivated Technosols (Table 3). It was negatively correlated ($r = 0.45$-0.9, $P < 0.01$) with all the soil properties and microbial indices except for soil pH. Soil pH, the ratio of MBC to SOC, Al_d and N were the predictors of qCO_2 ($R^2 = 0.92$, $P < 0.001$; Table 4). The ergosterol content formed a trio with more ergosterol ($P < 0.05$) in the "forest soils" than in the "cultivated soils" (Table 5). The ratio of SOC to total P accounted for the variability ($R^2 = 0.62$, $P < 0.001$) in ergosterol content in the soils (Table 4). The contribution of ergosterol to MBC (%) was negatively correlated with the clay ($r = 0.46$, $P < 0.05$) content (Figure 7a) and the ratio of MBC to SOC (%) ($r = 0.37$, $P < 0.05$; Figure 7b).

2.5 Discussion

2.5.1 Post-mining land use effects on soil properties

The influence of specific tree species was reflected in the SOC and N contents of pine and eucalyptus Technosols. This corroborates findings of Nsabimana et al. (2008) and Wasige (2014) in afforested sites of southern Rwanda, although Nsabimana et al. (2008) found higher contents in some eucalyptus forests. Age differences, and recurrent tree felling (Jandl et al., 2007) may have affected C accrual as it is a common phenomenon in the afforested Technosols. Meanwhile, a 6-year old mine soil restored to *Acacia mangium* Willd. forest contained only 20% SOC, but as much as 84% of N in a native forest soil (Neina, 2006). Conversely, the resemblance of SOC and N contents in the "cultivated soil" is characteristic of some arable fields in Rwanda (Reetsch et al., 2008) and may be attributed to low organic nutrient inputs coupled with excessive seasonal nutrient export. After 3-years of restoration to an oil palm (*Elaeis guineensis* Jacq.) plantation, a mulched and fertilized mine soil of Ghana contained 16 mg SOC g^{-1} and 1.3 mg N g^{-1} soil compared with 18 mg SOC g^{-1} and 1.2 mg N g^{-1} in an adjoining native forest (Neina, 2006). Top- and subsoil replacement during restoration amplified SOC and N contents, suggesting the major role of soil composition before restoration. This, alongside the distinct effects of pine and eucalyptus plantations further substantiates the effects of specific tree species and soil management in mine soil restoration.

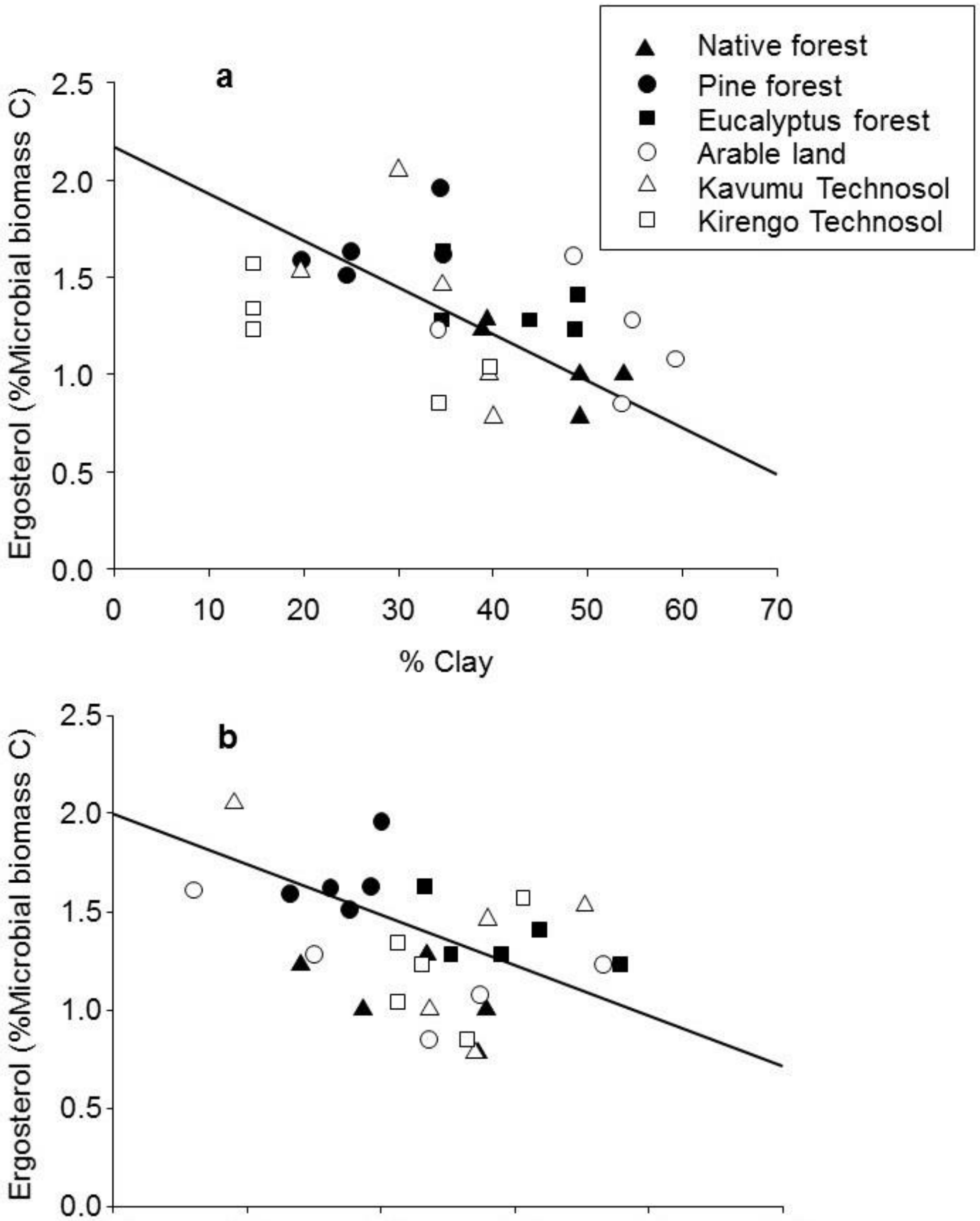

Figure 7. Linear Relationships between ergosterol as fraction (%) of microbial biomass C and (a) % clay content ($y = -0.01x + 1.75$; $r = -0.46$, $P < 0.05$) as well as (b) microbial biomass C as %SOC ($y = -0.44x + 1.74$; $r = -0.37$, $P < 0.05$) of soils from the Gatumba Mining District, western Rwanda

The observed soil acidity is controlled by mica-schist and granite parent material, which is widespread in Rwandan soils (Verdoodt and van Ranst, 2003). This is further aggravated by organic acids produced during SOM decomposition, especially in forest soils (Chodak and Niklińska, 2010b). The acidity supposedly limited P availability as seen in resin and Bray-I-P values, and corroborates Reetsch et al. (2008). Further weathering of the parent material may have led to substantial amounts of total and active Al and Fe contents. Their ratios suggests that the oxides are mostly amorphous (Kaempf and Schwertmann, 1983) forms, which regulate P availability and organometallic complexes and have implications for microbial biomass and activity.

2.5.2 Post-mining land use effects on soil microbial activity

The effect of post-mining land uses was reflected in microbial activity by default grouping into "forest" and "cultivated" soils, subsequently making a distinction between the tree species. One major edaphic constraint in the study sites was soil pH as manifested in its negative correlation with CO_2 efflux and net N mineralization, which corroborates results of Anderson and Domsch (1993), and Chodak and Niklińska (2010b). Differences in net N mineralization of pine and eucalyptus Technosols could have been spurred by extremes of metabolizable C. In the "cultivated soils", the net N mineralization lagged behind decomposition as seen in the ratio of cumulative CO_2-C to net N mineralization, which may be caused by insufficient N for microbial release. Generally, stress from low P availability and soil acidity seemed to compel microbes to divert SOC to respiration in order to offset high energy demands for cell replication and maintenance (Odum, 1969; Witter et al., 1993). Furthermore, SOM may have been protected by existing organo-Al complexes and restricted access by microbes.

2.5.3 Post-mining land use effects on microbial biomass

Microbial biomass is a very sensitive fraction of SOM readily affected by even slight changes in soil conditions (Powlson and Brookes, 1987; Joergensen, 2010). We found less MBC in the afforested Technosols than reported by Nsabimana et al. (2004) for 20-year old South African *Eucalyptus grandis* W. Hill ex Maiden forest probably because of age differences, litter quality, sampling depth, soil fertility and management practices. Low MBC in a pine forest soil was previously reported by Nsabimana et al. (2004) and Chodak and Niklińska (2010b), and attributed to antimicrobial effects of polyphenols in pine needles. Although eucalyptus also contains polyphenols, a higher MBC may have resulted from mycorrhiza colonization as eucalyptus is typically associated with both ecto- and arbuscular mycorrhiza (Wang and Qiu, 2006; Pagano and Scotti, 2008).

The results also show that MBC values in the "cultivated soils" are inferior to those of the sub-humid tropical and guinea savanna (Nsabimana et al., 2004; Adeboye et al., 2006; Adeboye et al., 2011), probably due to shallow sampling depth (10 cm) and higher nutrient inputs in those soils. Consequently, we found a low microbial contribution to SOC only near the lower limit of 0.9-2.8% (Anderson and Domsch, 1993; Chodak and Niklińska, 2010b; Joergensen, 2010). The ergosterol contents of the tropical soils were generally similar to those of temperate arable and forest soils (Djajakirana et al. (1996), but considerably above those of secondary tropical forest stands on slightly acid soils in the Philippines (Salamanca et al., 2002). The negative effects of MBC to the SOC ratio on the contribution of ergosterol to MBC suggests that a declining SOC availability to soil microorganisms promotes saprotrophic fungi at low soil pH (Joergensen and Wichern, 2008). This negative relationship between MBC to SOC ratio and the contribution of ergosterol to MBC has not been observed in slightly acid soils (Salamanca et al., 2002).

As argued earlier, the microbial stress associated with soil acidity, low available P, and organo-Al complexes may have affected the metabolic quotient $q\mathrm{CO_2}$ (Anderson and Domsch, 1993; Anderson, 2003). In a healthy soil ecosystem, the microorganisms are energy-efficient and reserve more C for biomass production (Odum, 1969; Anderson, 2003). The native forest may represent such an ecosystem while the pine and eucalyptus forests suggest a trajectory towards a stabilized ecosystem. The profound effects of soil pH on microbial biomass and activity are reflected in the fact that, under similar environmental conditions, fungi have optimal growth at 1-2 pH units below bacteria due to their distinct cell structure and vacuoles (Pina and Cervantes, 1996). Additionally, soluble Al toxicity occurs at low pH where Al binds to DNA, ATP, cell membranes or walls (Pina and Cervantes, 1996) and affect normal biological functions. This may have been the case at higher ratios of $\mathrm{Al_{ox}}$ to $\mathrm{Al_d}$ in our forest soils.

SOC and N reserves, plant species, rhizosphere and soil pH, soil texture and structure modulate microbial population, diversity and activity (Wardle, 1992), which to some extent determine ecological restoration. According to Odum (1969) ecological restoration is an orderly, reasonably directional, predictable, and community controlled process, where the physical environment determines the pattern and rate of change, and often sets limits to how far development can go. This was also observed at our study sites.

2.6 Conclusions

This study revealed differences between the afforested and the cultivated Technosols with clear superiority of SOC, and N contents as well as microbial indices in the affrested Technosols, suggesting improvement in their biological quality. However, for real ecosystem restoration, the choice of tree species should be related to their capacity to sequester SOC and increase soil nutrients. Exclusive establishment of fast-growing tree species may not offer multiple ecosystem services and thus mixed stands may be beneficial. The cultivated Technosols and arable soil did not differ significantly in SOC, N, and microbial biomass and activity. These are stressed ecosystems that demand intensive use of organic amendments to provide substrates for growth and maintenance of the microbial population. Soil acidity is a major challenge in the study sites, which require liming or wood ash application since wood ash has about half $\mathrm{CaCO_3}$ equivalent and is common in most households and schools in the area because of heavy dependence on firewood and charcoal as the main energy sources for cooking. However, this approach would obviously be limited to very small field sizes.

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3 Microbial response to restoration of a Technosol amended with local organic materials

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3.1 Abstract

Due to land scarcity many farmers of western Rwanda cultivate tantalite mine soils with sole reliance on farmyard manure (FYM) as fertilizer. However, there is limited knowledge of the effects of FYM and organic amendments on soil microbial properties. We therefore investigated the effects of FYM, *Canavalia brasiliensis* and *Tithonia diversifolia* biomass, and *Markhamia lutea* leaf litter on C and N mineralization and microbial properties in a Technosol (unrestored mine soil) and an arable soil in a laboratory study. Fresh soils (0-20 cm) were amended and incubated for twelve weeks. C mineralization was measured weekly by alkali trap while mineralized N and microbial properties were determined from the incubated soils after four, eight, and twelve weeks of incubation. The amendments substantially increased ($P < 0.01$) CO_2 efflux, N mineralization, and microbial properties compared to non-amended soils. *Canavalia* and FYM mineralized N while *Markhamia* and *Tithonia* immobilized it. After four weeks of incubation, *Canavalia* increased CO_2 efflux by 340%, net N mineralization by 30-140%, and microbial biomass C and N by, 240-600%, and 240-380%, respectively, compared with non-amended soils. *Tithonia* showed the largest increase in ergosterol content by roughly 240% and the greatest contribution of ergosterol to microbial biomass C with marginal effects on microbial biomass C and N. The Technosol had the greatest responses to the amendments and thus showed a high potential for a quick restoration of soil quality. The amendment effects also indicate high potential for soil C storage and nutrient release.

Key words: *Canavalia brasiliensis, Markhamia lutea,* microbial biomass, N mineralization, tantalite mine soils, *Tithonia diversifolia*

3.2 Introduction

The core principle of the Surface Mining Control and Reclamation Act of 1977 (Torbert and Burger, 2000) is to restore the integrity of mine lands to pre-mine or better conditions in order to offer ecosystem services. Consequently, the concept of mine land reclamation spread over the globe becoming an alternative to the tradition of mine land abandonment in the mining industry. This triggered an upswing in research on mine ecosystem restoration involving afforestation-reforestation with and without soil amendments, depending on the end land use. In this context, amendments such as fly ash (Ram and Masto, 2010), biosolids, animal manure (Tam, 1987; Gardner et al., 2010; Larney and Angers, 2012), crop residues, compost (Larney et al., 2005; Tejada et al., 2006) as well as pulp and paper mill wastes (Larney et al., 2005; Larney and Angers, 2012) have been advocated to improve the chemical, physical, and biological quality of mine soils. Regrettably, most of the approaches and research on mine ecosystem restoration referred to case studies in North America, Europe, and Australia (Chodak and Niklińska, 2010), with only a few from emerging economies, particularly in Asia. There is paucity of such studies in Africa where mining has occurred for centuries and has recently experienced exponential growth rates (United Nations Economic Commission for Africa, 2011). Furthermore, most of the amendments employed are largely industrial, agro-based, and municipal solid wastes, which have been mobilized through systematic mechanisms in quantities large enough for field application. Such mechanisms are unrealistic in many parts of Africa.

In the Gatumba Mining District of western Rwanda, most tantalum mine wastelands are not restored before agricultural use but are directly cultivated alongside arable and marginal lands due to high population density coupled with land scarcity. The problem is often aggravated by poor soils with high acidity (pH < 5.5) and nutrient mining (Yamoah et al., 1990) caused by minimal nutrient inputs as well as continuous nutrient exports through crop harvests. Due to limited access to commercial fertilizers, farmers rely solely on scanty poor-quality farmyard manure (FYM) to improve crop yields. Yet, there is an abundance of multi-purpose plant species, which could be utilized for soil quality improvement. One of such species is *Tithonia diversifolia* (Hemsley) A. Gray, commonly called Mexican sunflower. *T. diversifolia* is abundant in East Africa and is known for its ability to scavenge and concentrate nutrients in its biomass which has proved useful in compost quality enhancement (Drechsel and Reck, 1998), crop yield increase (Gachengo et al., 1999; Jama et al., 2000; Olabode et al., 2007), and P mineralization (Kwabiah et al., 2003). Further, the legume cover crop *Canavalia brasiliensis* (Mart. ex Benth.) is also prevalent in Rwanda. It is reported to be a high N_2-fixing, virgorously growing legume cover crop recommended for the tropical highlands of Rwanda (Yamoah and Mayfield, 1990). *C. brasiliensis* has been widely studied in Latin America (Cobo et al., 2002; Carvalho et al., 2014) for its contribution to C and N sequestration and crop improvement in Brazilian agro-ecosystems.

While a substantial portion of Rwanda is covered by Forest Reserves and National Parks, which aim at conserving biodiversity, many of the indigenous tree species are extinct in the rural setting where farming is prevalent. One such tree species is *Markhamia lutea* (Benth.)

K. Schum. ranked among the five top-priority agroforestry species in tropical Africa (Owino, 1992). *M. lutea* is almost extinct in GMD (C. Rwabahizi, personal communication, May 2013) although it contains substantial amounts of plant nutrients (Silas et al., 2012). So far, the potential of these native species to improve soil microbial properties, particularly in mine soils has been least explored.

Recent studies (Ndoli et al., 2013) and surveys (Diogo, 2012; unpublished) conducted on the (columbite-tantalite) tantalite mine soils of the GMD revealed that the application of FYM, compost, and *T. diversifolia* biomass greatly increased yields of climbing bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* (L.) Merr), cassava (*Manihot esculenta* Crantz), cocoyam (*Xanthosoma sp*. Schott.), and sweet potato (*Ipomea batatas* (L.) Lam.) on mine soils compared with un-mined soils. However, the extent of microbial proliferation and mineralization of these organic amendments in the mine soils is unknown. Interestingly, the tantalite mine soils are dominantly pegmatite substrates and do not contain appreciable quantities of heavy metals harmful to the environment. They are, however, enriched with potassium (K) and have near-neutral soil pH conditions (Lehmann et al., 2014). This bestows the spoils with great prospects for restoration towards sustainable crop production. In his benchmark study, Bradshaw (1996) proposed comprehensive laboratory experiments as a prerequisite to real-world restoration efforts. Until now, data from such experiments with respect to the use of native organic materials under native environmental conditions, are scanty although they could serve as a guide to planning restorative measures for mine soils.

A previous study (Chapter 2) on microbial properties revealed that during the period of cultivation, the tantalite mine soils attained initial microbes required to kick-start nutrient cycling. Globally, reclamation laws require the salvage of top and subsoils before mining for subsequent replacement on mine spoils prior to revegetation (Larney et al., 2005) to restore biological activity and promote plant succession. Therefore, mine soils restored with top- and subsoil replacement are called *graded* mine soils (Akala and Lal, 2001) whereas their counterparts without such replacements are *ungraded* mine soils. With our study, we wanted to ascertain whether the cultivated tantalite mine soils could mimic *graded* mine soils to enhance rapid snap back towards pre-mine or similar conditions. We specifically aimed to investigate (1) the increase in microbial biomass, and (2) C and N mineralization in a tantalite mine soil, termed as Technosol (IUSS Working Group WRB, 2014), amended with FYM, *C. brasiliensis*, *M. lutea*, and *T. diversifolia*. We further postulated that local organic materials are a feasible option to enhance the biological quality of the Technosols in GMD by stimulating microbial properties and activity to release essentials plant nutrients. However, because of their unique biochemical characteristics, the amendments may exert distinctive effects on microbial C and N turnover.

3.3 Materials and Methods

3.3.1 Soils

To unravel the query presented earlier, we selected an arable soil and a Technosol both (Figure 6) from Kavumu village in the GMD, western Rwanda. The arable soil was a clayey loam from a farm that had been cultivated with climbing beans, green peas (*Pisum sativum* L.), sweet potatoes, and cassava in a mixed pattern and amended with FYM usually before planting. A previous study (Chapter 2) showed that the arable soil had a pH-H_2O of 4.9, a bulk density of 1.3 g cm^{-3}, 10.9 mg g^{-1} soil organic carbon (SOC), 1.0 mg total N g^{-1}, 134 µg g^{-1} microbial biomass C (MBC), and 0.2 µg ergosterol g^{-1} soil. The Technosol was a sandy clay loam located in the Kavumu coltan quarry and had a pH-H_2O of 4.9, a bulk density of 1.2 g cm^{-3}, 10.2 mg g^{-1} SOC, 0.9 mg total N g^{-1}, 126 µg MBC g^{-1}, and 0.3 µg ergosterol g^{-1} soil. The residents of the GMD cultivated the Kavumu coltan quarry aside experiments installed by the Coltan Environmental Management Project. From March to July 2013, climbing beans and sweet potatoes had been cultivated on the site with FYM and biomass of *Tephrosia vogelii* (FYM: 5-10 t dry matter ha^{-1}; FYM + Tephrosia (1:1): 5-10 t dry matter ha^{-1}) as amendments. At the time of soil sampling, a cassava trial was installed on the site. The amendments used were compost, wood ash, and stored human urine. The wood ash slightly raised the pH-H_2O (Neina and Dowuona, 2013), yielding an average pH of 5.6. We sampled fresh soils (five field replicates each) from the top 20 cm using a stratified sampling method because of the sites' steep topography. The samples were sieved (< 2 mm) and stored at 4°C until use. Further, we undertook a 12-week laboratory study after amending the soils with the aforementioned organic amendments. We chose an incubation temperature of 22°C to reflect the annual average temperature of the GMD, Rwanda.

3.3.2 Local organic amendments

In addition to FYM, the most widely used soil amendment in the GMD, we chose the biomass of *C. brasiliensis* and *T. diversifolia*, and *M. lutea* leaf litter (Figure 8). These represent the available soil amendments for local green manuring (*C. brasiliensis* and *T. diversifolia*) or agroforestry systems (*M. lutea*). In the context of this study, the amendments are termed FYM, *Canavalia*, *Tithonia*, and *Markhamia*. The *Canavalia* biomass was collected from field trials of the Coltan Environmental Management Project. For *Markhamia*, recent litter fall from several trees was composite (trees are almost extinct and scarce) while *Tithonia* biomass was collected from farmlands, wayside, and marginal lands. The FYM, which comprised mainly cattle slurry and straw, was obtained from organic farms in Schwäbisch-Hall, southern Germany where clover (*Trifolium spp.*) was the main source of fodder (Wentzel and Joergensen, 2015).

3.3.3 Sample preparation and analysis

The leaf litter and biomass were air-dried while FYM was oven-dried at 60°C, milled, and sieved to 1 mm. Total contents of C and N were measured by dry combustion, using a Vario MAX CHN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The amendment pH was measured in a 1:10 substrate-water ratio with an electrode (SI Analytics GmbH, Mainz, Germany). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin fractions were measured using a semi-automated ANKOM Fiber Analyzer (ANKOM Technology, Macedon, New York, NY, USA) based on the principles of van Soest (1991). Total Phosphorus (P) was measured photometrically on a Hitachi U-2000 spectrophotometer (Hitachi Ltd, Tokyo, Japan) according to the ammonium molybdate method (Gericke and

Kurmies, 1952) after di-acid digestion, whereas total potassium (K) content was measured on a flame photometer (IL 543, Instrumentation Laboratory Inc, Bedford, MA, USA) from the same extracts. Extractable NO_3-N and NH_4-N were measured on a Metrohm 850 ion chromatograph (Metrohm AG, Herisau, Switzerland) after extracting 0.5 g substrate with 50 ml 0.1 M $CaCl_2$ solution.

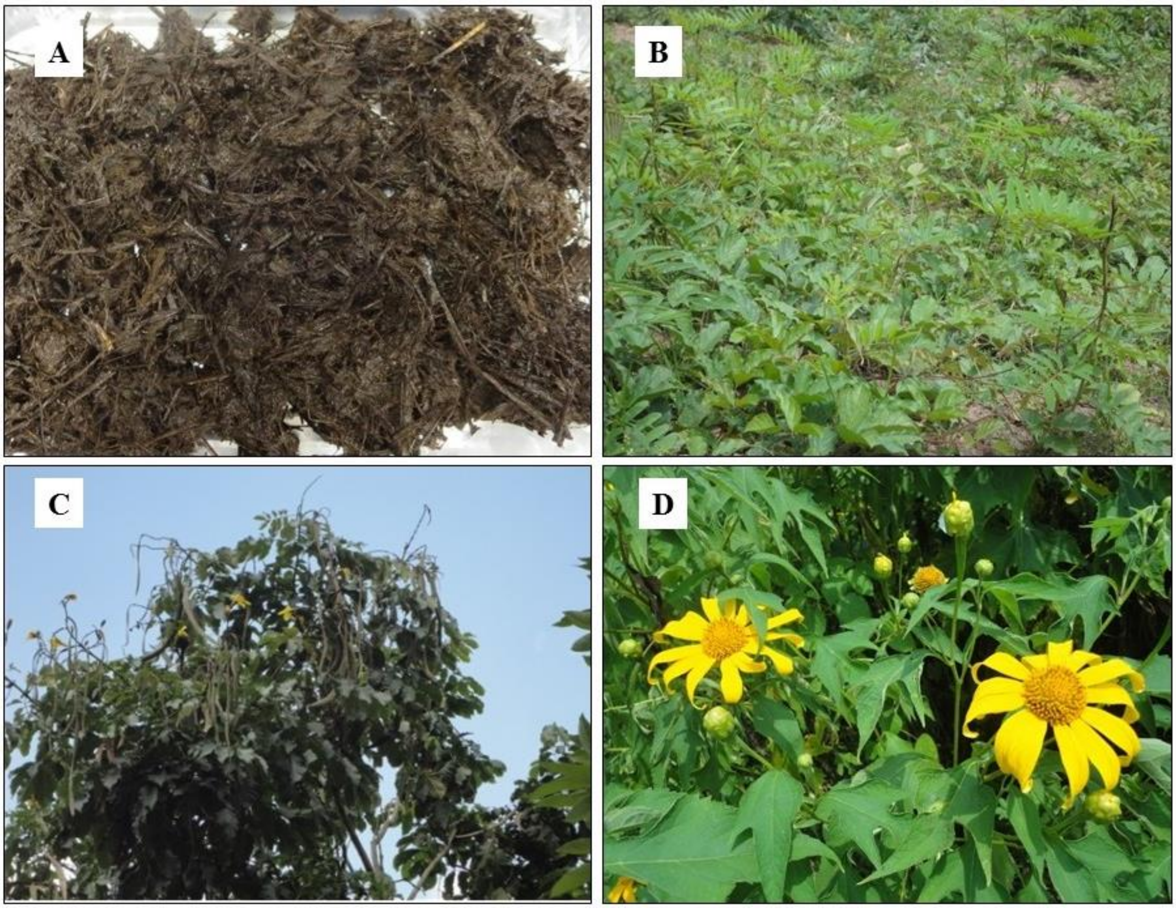

Figure 8. Soil amendments used for the experiment showing (A) FYM, (B) *Canavalia brasiliensis*, (C) *Markhamia lutea*, and (D) *Tithonia diversifolia* at the Gatumba Mining District, western Rwanda.

3.3.4 C and N mineralization experiment

The experiment comprised the arable soil and the Technosol with five field replicates and treatments each including: a) non-amended soil (control), b) FYM, c) *Canavalia*, d) *Markhamia*, and e) *Tithonia*. The amendments were applied at 2.5 mg C g^{-1} soil to 150 g soil (dry weight basis) in 600 ml beakers and thoroughly mixed after adjusting to 50% water holding capacity. Afterwards, the amended soils were split in three portions into 50 ml polyethylene cups (Sarstedt AG & Co, Nümbrecht, Germany) for subsequent destructive sampling. The cups were placed in 1.5 L glass jars. Additionally, glass vials, containing 20 ml of 0.5 M NaOH and 10 ml of bi-distilled water, were placed in each jar to trap CO_2 and to maintain humidity. The jars were randomly arranged and incubated at 22°C for twelve weeks. Evolved CO_2 was determined weekly by titration after precipitation of Na_2CO_3 with 5 ml of saturated $BaCl_2$ and back-titrating with 0.5 M HCl in the presence of phenolphthalein indicator. After four, eight, and twelve weeks of incubation, one cup of soil each was removed from the incubated jars to determine mineralized N and microbial properties. After each sampling period, the evolved CO_2 was calculated based on the quantity of soil remaining in the jars. Mineralized N (NO_3^- and NH_4^+) was determined by extracting 10 g of fresh soil with 40 ml 0.5 M K_2SO_4 for 30 min by shaking at 200 rev min^{-1} and filtering with folded filter paper (hw3, Sartorius Stedim Biotech GmbH, Göttingen, Germany). The extracts were measured for NO_3^- and NH_4^+ on a Segmented-flow Analyzer (Evolution II, Alliance Instruments GmbH, Ainring, Germany). Net N mineralization was estimated by subtracting the pre-incubation mineral N content from post-incubation values.

3.3.5 Soil microbial response to organic amendments

Microbial biomass C and N were estimated by the fumigation-extraction method with 0.5 M K_2SO_4 extractant (Brookes et al., 1985) as described by Khan et al. (2009). For each sample, 20 g fresh soil were divided into two portions of 10 g. One portion was fumigated with ethanol-free chloroform in the dark at 25°C for 24 h. The non-fumigated samples were immediately extracted with 40 ml 0.5 M K_2SO_4 for 30 min by shaking at 200 rev min^{-1} and filtered through folded filter paper (hw3). The fumigated samples were extracted in a similar manner as the non-fumigated ones. Organic C and total N in the extracts were measured on a multi N/C analyzer (Analytik Jena AG, Jena, Germany). Microbial biomass C and N (µg g^{-1} dry soil) were calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985).

The fungal cell membrane component ergosterol was extracted from 5 g fresh soil with 100 ml distilled ethanol after shaking for 30 min at 250 rev min^{-1} (Djajakirana et al., 1996). The soil-ethanol suspension was filtered through a Whatman GF/A glass fiber and filtered into round bottom flasks. The filtrate was evaporated to near dryness at 40°C on a rotary evaporator. Subsequently, the extracted ergosterol was concentrated in 10 ml methanol and quantitatively measured using reverse phase HPLC followed by UV detection at 282 nm.

3.3.6 Statistics

Means presented are expressed on an oven-dry weight basis (105°C, 24 h). The assumptions of ANOVA (analysis of variance) were checked and data with non-normally distributed residuals were either log or square root transformed before analysis. The means were compared using General Linear Models. Significant differences were determined at 5%, using Tukey's HSD (Honestly Significant Difference) test. The stepwise forward method of multiple linear regression was used to determine the effects of biochemical properties of the amendments on CO_2 efflux and net N mineralization. The regression model was tested for normality, constancy of variance, absence of correlation between the residuals (Durban-Watson statistics) and absence of multi-collinearity, and the variance inflation factor (VIF). Variables were removed from the model if the VIF value exceeded 4.0. All data were analyzed with SPSS version 20 (IBM Corporation, New York, NY, USA) (Field 2009).

3.4 Results

3.4.1 Amendment quality

Initial contents of total and extractable inorganic N were in the order: FYM > *Canavalia* > *Markhamia* > *Tithonia* and *Canavalia* > FYM > *Markhamia* > *Tithonia*. Consequently, the C/N ratio followed the trend *Tithonia* > *Markhamia* > *Canavalia* > FYM while the lignin/N ratio was ordered as *Tithonia* > *Markhamia* > FYM > *Canavalia*. *Canavalia* had the highest hemicellulose, but lowest NDF, ADF, and lignin contents (Table 6).

3.4.2 C and N mineralization

The CO_2 efflux from both soils grouped into high *Canavalia*, moderate *Markhamia*, FYM and *Tithonia*, and low non-amended soil CO_2 effluxes. Cumulative CO_2 efflux during the twelve weeks showed additive effects of soil and amendment whereby the Technosol produced 7-34% more CO_2 ($P < 0.05$) than the arable soil (Figure 9). The amendments significantly ($P < 0.001$) increased cumulative CO_2 efflux in both soils. Compared with non-amended soils, *Canavalia* had the largest increase by roughly 340% whereas FYM had the smallest by 170% in comparison with non-amended soils. The fraction of applied C mineralized as CO_2-C in the Technosol differed in the order *Canavalia* (27%) > *Markhamia* (19%) > *Tithonia* (17%) > FYM (15%), whereas in the arable soil, it arrayed as *Canavalia* (26%) > *Tithonia* (16%) > *Markhamia* (13%) > FYM (12%). The hemicellulose content explained 57% of the variability in CO_2 efflux.

Table 6. Biochemical characteristics of FYM, *Canavalia* biomass, *Markhamia* leaf litter, and *Tithonia* biomass used as amendments during the twelve-week incubation experiment with soils from Gatumba Mining District, western Rwanda. Data are means of three replicates with pooled coefficient of variation (CV_{pooled}) except acid detergent fiber, neutral detergent fiber, hemicellulose lignin, and extractable NO_3-N and NH_4-N, which had four replicates.

Variable[*]	**FYM**	***Canavalia***	***Markhamia***	***Tithonia***	**CV_{pooled} (%)**
pH-H_2O (1:10)	9.0	6.2	6.8	6.4	0.1
Total C	375	438	469	452	0.1
Total N	30	29	24	16	0.9
C/N	13	15	20	29	1.0
Total P	7.4	2.2	1.9	1.8	4.7
Total K	55.3	12.1	3.2	3.3	1.7
Extractable NH_4-N	23.6	81.8	12.8	12.6	1.1
Extractable NO_3-N	4.1	5.5	0.0	0.0	5.2
NDF	565	537	670	547	2.9
ADF	364	333	481	384	2.2
Hemicellulose	173	232	190	162	5.8
Lignin	228	108	263	215	4.9
Lignin/N	7.7	3.9	11.1	14.1	3.2

[*] All units are expressed in mg g^{-1} except extractable NH_4-N

In contrast to the CO2 efflux, net N mineralization occurred in non-amended soils, FYM, and Canavalia compared with net N immobilization in Markhamia and Tithonia treatments (Figure 10). Amendment and repeated measures (time of incubation) interacted ($P < 0.01$) whereby FYM released the largest amount of N in week four whereas Canavalia dominated N release in subsequent weeks ($P < 0.01$). Comparing both soils, net N mineralization in the Technosol marginally exceeded that of the arable soil. In the Technosol, Canavalia increased net N mineralization by 90-140% ($P < 0.001$) whereas in the arable soil it increased by only 30-70% compared with non-amended soils throughout the incubation period. For FYM, net N mineralization in the Technosol increased by roughly 90% and by only 40% in the arable soil compared with non-amended soils (Figure 10). The fraction of N immobilized by Markhamia and Tithonia slightly fluctuated, showing no statistical differences between them. Markhamia immobilized around 70% and 50% of non-amended soil N, whereas Tithonia immobilized 60% and 30% in the Technosol and the arable soil, respectively. The lignin/N ratio and ADF were the best predictors of net N mineralization and immobilization (73%, $P < 0.001$).

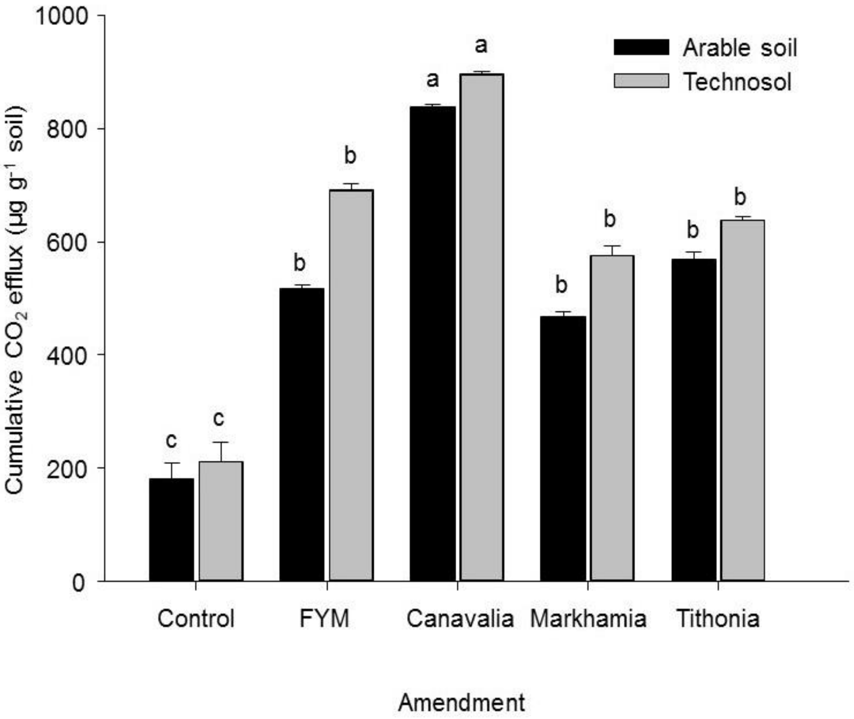

Figure 9. Cumulative CO_2 efflux during a twelve-week incubation of the arable soil and a Technosol from Gatumba Mining District, western Rwanda amended with FYM, *Canavalia*, *Markhamia,* and *Tithonia.* Soil and amendment did not interact ($P > 0.05$), but each influenced ($P < 0.001$) cumulative CO_2 efflux whereby *Canavalia* effects were largest. Bars ($n = 5 \pm$ one standard error of the mean) of similar fill with different letters indicate significant differences (Tukey HSD, $P < 0.05$) between treatments in each soil type.

3.4.3 Soil microbial biomass response to organic amendments

The application of local organic amendments significantly increased microbial biomass C, microbial biomass N, and ergosterol contents in the arable soil and the Technosol within four weeks of incubation. This was followed by either a steep or steady decline depending on the soil and the amendment. The average main effects of the amendments indicated that the respective microbial biomass C and N contents of the arable soil were roughly 20% ($P < 0.001$) larger than those of the Technosol (Table 7), mostly due to the initially higher microbial biomass in the arable soil. At the end of the incubation period, *Canavalia* had the greatest ($P < 0.001$) mean increase in microbial biomass C and N (Table 7), averaging roughly 300% in comparison with the non-amended soil while *Tithonia* had the least increase of 200%. The Technosol responded more strongly to the amendments than the arable soil. For instance, in week four, *Canavalia* treatment remarkably increased ($P < 0.001$) the microbial biomass C by 600% and 240% compared to non-amended soils in the Technosol and arable soil, respectively, but decreased it to 120% in both soils at the end of the incubation period (Figure 11). FYM and *Tithonia* treatments had the least but similar effects on soil microbial biomass C, which increased by 230% and 140% in the Technosol and in the arable soil, respectively.

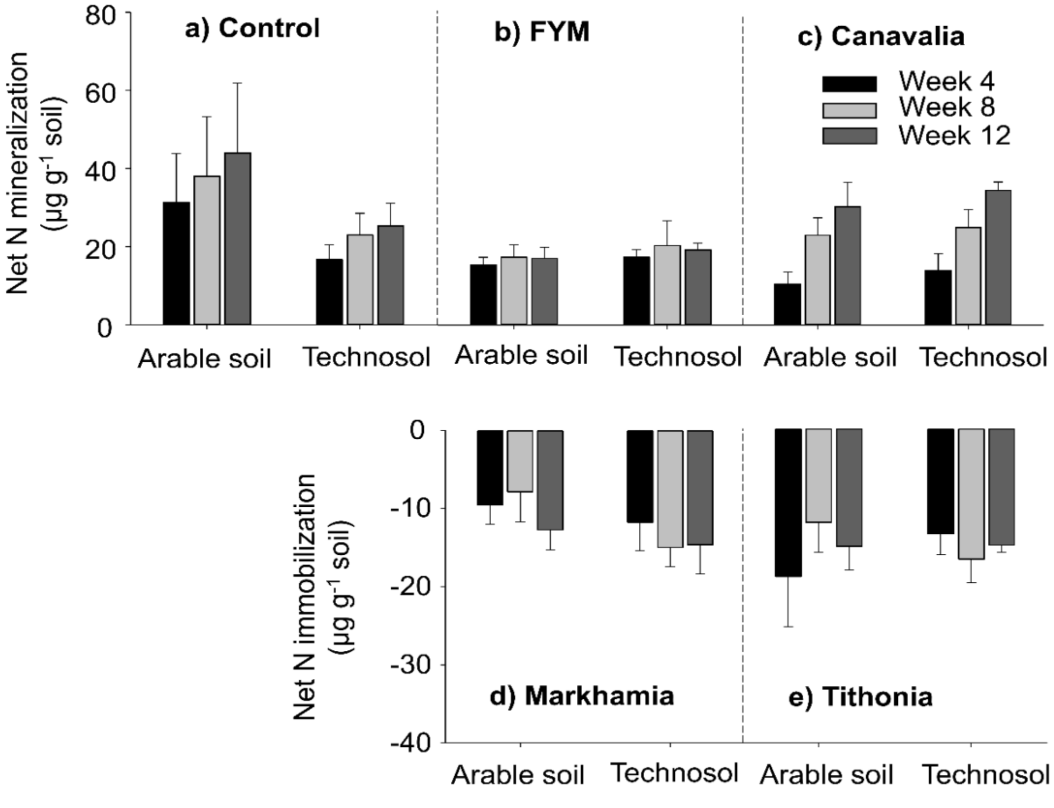

Figure 10. Net N mineralization and immobilization of three successive periods during a twelve-week incubation of non-amended control soil, FYM, *Canavalia*, *Markhamia,* and *Tithonia* in the arable soil and a Technosol from Gatumba Mining District, western Rwanda. Error bars denote one standard error of the mean (n = 5). Amendment and repeated measures interacted ($P < 0.01$), and repeated measure influenced ($P < 0.001$) net N mineralization while soil, amendment, and repeated measures interacted ($P < 0.01$) in net N immobilization.

This similarity subsequently disappeared with a steeper decrease in FYM, particularly in the arable soil. The General Linear Model showed interaction effects of soil × amendment (P < 0.01), and amendment × repeated measures (time of incubation) (P < 0.001) on microbial biomass C indicated by a larger increase in FYM treatment of the Technosol during the last eight weeks. Among the amendments, only Canavalia and FYM treatments of the Technosol maintained a steady decrease in microbial biomass C till the end of incubation, suggesting a sustained maintenance of the microbial population. Similarly, the microbial biomass N of Canavalia increased by 380% in the Technosol and by 240% in the arable soil. Tithonia biomass had the smallest effects on microbial biomass N with a roughly 130% increase in both soils. An interesting observation was a generally steady decrease in soil microbial biomass of the arable soil compared to the Technosol. The microbial biomass C/N ratio varied around an average of 5.8, showing only the effects of soil and repeated measures ($P < 0.01$). The non-amended soils and Markhamia had the highest and lowest ratios, respectively (Table 7).

Table 7. Main effects of microbial biomass C and N, their ratios, ergosterol, and ergosterol to microbial biomass C ratio of an arable soil and a Technosol from Gatumba Mining District, western Rwanda during three successive periods following amendment with FYM, *Canavalia*, *Markhamia*, and *Tithonia*. Means (n = 5) in columns with the same letters indicate significant differences (Tukey HSD, $P < 0.05$).

Factor	**Microbial biomass** **C**	**N**	**C/N**	**Ergosterol**	**Ergosterol/ MBC (%)**
	(µg g^{-1} soil)			**(µg g^{-1} soil)**	
Soil					
Arable soil	189 a	33 a	6.0 a	0.48	0.26 b
Technosol	152 b	28 b	5.5 b	0.53	0.45 a
Treatment					
Control	85 c	15 c	6.0	0.25 d	0.45 a
FYM	174 b	32 b	5.6	0.44 c	0.27 b
Canavalia	245 a	43 a	5.8	0.59 b	0.26 b
Markhamia	176 b	32 b	5.5	0.48 c	0.30 b
Tithonia	171 b	30 b	5.8	0.77 a	0.51 a
Repeated measures					
Week 4	172	32	5.4 b	0.61 a	0.59 a
Week 8	171	31	5.7 bc	0.46 b	0.29 b
Week 12	169	28	6.1 ac	0.44 b	0.28 b
Probability values					
Soil	*<0.001*	*0.001*	*0.006*	*NS*	*<0.001*
Amendment	*<0.001*	*<0.001*	*NS*	*<0.001*	*<0.001*
Repeated measures	*NS*	*NS*	*0.008*	*<0.001*	*<0.001*
Soil × Repeated measures	*0.001*	*<0.001*	*NS*	*NS*	*<0.008*
CV (%)	*39*	*39*	*22*	*47*	*68*

MBC = microbial biomass C

The average main effects showed that *Tithonia* had the greatest increase in ergosterol content, averaging 68% while FYM had the smallest increase of only 43% in comparison with non-amended soils (Table 7). During the first four weeks, *Tithonia* biomass increased the ergosterol content by roughly 240% in both soils compared with non-amended soils. FYM had the least increase of 90% in both soils. These declined steadily in the arable soil and sharply in the Technosol. The contribution of ergosterol to microbial biomass C was significantly higher in the Technosol than in the arable soil. Like the ergosterol content, *Tithonia* most strongly increased the contribution of ergosterol to microbial biomass C, particularly in the Technosol.

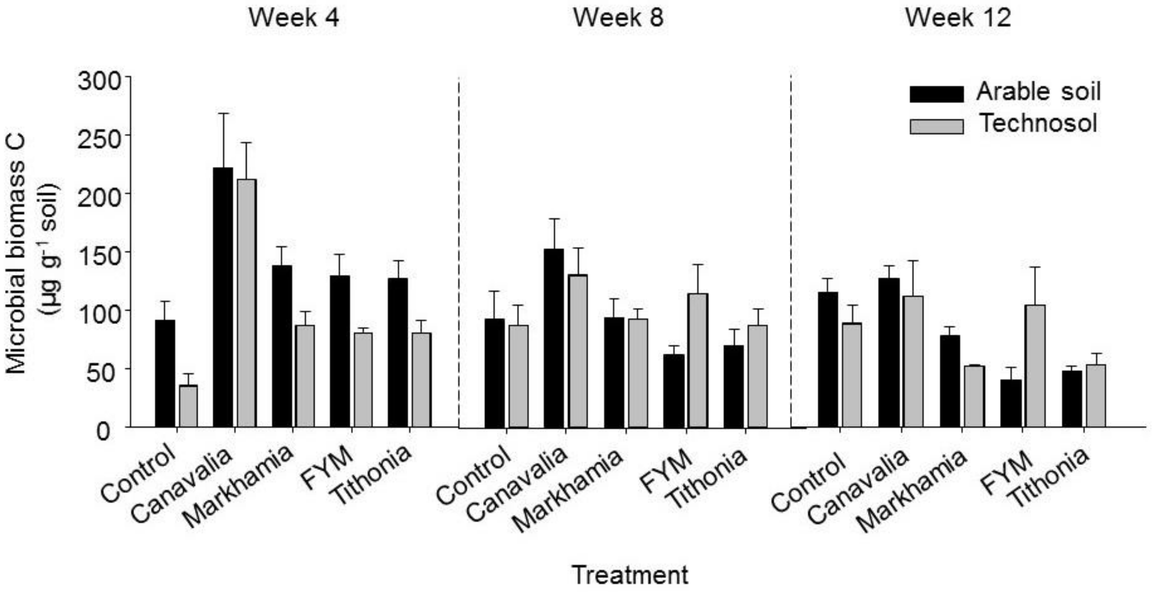

Figure 11. Microbial biomass C content of three successive periods during a twelve-week incubation of the arable soil and a Technosol from GMD, western Rwanda amended with FYM, *Canavalia*, *Markhamia,* and *Tithonia*. Soil and amendment ($P < 0.01$) and repeated measures and amendment ($P < 0.001$) interacted. Means (n = 5 ± one SEM) are exclusive of non-amended soil.

3.5 Discussion

The study answered our question to what degree the Technosol acquired microbial properties similar to those of graded mine soils during the period of cultivation. It further affirmed that the organic materials available in the Gatumba Mining District are a feasible option to improve the biological quality of the Technosol and release essential plant nutrients. An earlier study (Chapter 2) established that the Technosol and the arable soil did not differ significantly in microbial biomass C and N as well as ergosterol contents though the Technosol had slightly less microbial biomass C, but higher microbial biomass N and ergosterol contents than the arable soil. In this study, we observed a greater response of the Technosol to the organic amendments through microbial properties and C and N mineralization in comparison with the non-amended soils, particularly during the first few weeks of incubation. Thus, the Technosol exhibited a great potential for restoration through the organic amendments. This can be attributed to various reactions between the intrinsic soil properties and C inputs expressed in two main propositions: (i) the effects of ergosterol and low resin-P contents in the Technosol, and (ii) the effects of prevailing soil pH (Khalil et al., 2005) together with interim soil pH increase caused by alkalinity of amendments (Xu et al. 2006; Butterly et al. 2013).

The first proposition may be attributed to pre-incubation ergosterol content (Chapter 2) of the Technosol, which may have increased the CO_2 efflux. Previously, the ergosterol content explained 48% ($P < 0.01$) of the total variability in the CO_2 efflux of native SOM, whereas the total microbial biomass C explained only 34% ($P < 0.05$; Neina, unpublished data). As an indicator of saprotrophic fungi, the ergosterol content shows the overarching role of fungi, particularly during the initial phase of decomposition (Djajakirana et al., 1996; Blagodatskaya and Anderson, 1998). This is attributed to the C utilization efficiency of fungi (Al-Busaidi et al., 2014). Moreover, P deficiency in the Technosol might have triggered a higher CO_2 efflux as the Technosol had 24% less total P and 37% less resin-P content than the arable soil (Chapter 2). In the second proposition, the CO_2 efflux seems to have coupled with net N mineralization whose pattern suggests not only the effect of the ergosterol content, but of soil pH. Our previous study on the microbial properties of the soils suggested a negative correlation between soil pH and microbial biomass and activity (Chapter 2), which corroborates many studies (Khalil et al., 2005), but contrasts a weak positive correlation found between soil pH and net N mineralization (Khalil et al., 2005). This discrepancy may be caused by a wider pH range (4.3 to 7.8) of soils used (Khalil et al., 2005) compared with a narrow (3.7 to 5.6) range for our soils. Subsidiary to the main propositions, cation enrichment in the Technosol, originating from unweathered soil minerals, particularly K (Lehmann et al., 2014) and wood ash amendment (Neina and Dowuona, 2013) could have had positive effects on soil microorganisms. Overall, the response of the Technosol to the local organic amendments seems to reflect the relatively large crop yields obtained from amended Technosols in Gatumba compared with amended arable soils (Ndoli et al., 2013; Bizimana et al. and Diogo, unpublished data). This is further supports the view of Larney and Angers (2012) that degraded soils will show the greatest benefits from applied organic amendments.

The study affirmed our assumption that the amendments will exert distinct effects on microbial C and N turnover because of their unique biochemical characteristics. More specifically, we found that hemicellulose, lignin/N ratio, and ADF influenced amendment-derived C and N mineralization, alongside soil properties and experimental conditions. The initial N content, lignin, polyphenols (Palm and Sanchez, 1991; Constantinides and Fownes, 1994), C/N and lignin/N ratios, NDF, and ADF (Cobo et al., 2002; Vahdat et al., 2011) are frequently encountered among the C quality indices known to produce large variations in C and N mineralization.

Large CO_2 efflux and N release from *Canavalia* treatment with 27% lost in applied C agree with high C mineralization in legumes and fresh leaves as reported by Mafongoya et al. (2000) and Xu et al. (2006). Drechsel and Reck (1998) also reported higher CO_2-C in legume green manure (*Calliandra*) than in FYM during a mineralization experiment on composted shrub prunings in Rwanda. This could be explained by the high amount of labile C, an interim soil pH increase caused by the alkalinity of *Canavalia*, and the initial ammonification of organic N (Xu et al., 2006). Surprisingly, the cumulative CO_2 efflux of *Tithonia* rather lagged behind that of FYM and contradicts the results of Drechsel and Reck (1998) and Cobo et al. (2002) who found a higher decomposition in *Tithonia* than in green manure (*Canavalia* and *Calliandra*). The results of Cobo et al. (2002), however, revealed larger C/N and lignin/N ratios, NDF, and ADF contents in *Canavalia* than in *Tithonia*, which probably accounted for the reverse trend in decomposition. Further, Jama et al. (2000) observed that *Tithonia* decomposes more rapidly when applied as a green manure, suggesting that the form of biomass application to soil is of prime importance for nutrient release. Thus, the lag in the CO_2 efflux of *Tithonia* treatment may be attributed to the initial drying of the biomass.

Previous research on organic soil amendments in the Tropics set 2.0-2.5% N as the critical levels below which N immobilization should occur (Jama et al., 2000; Palm et al., 2001). This explains the patterns of net N mineralization by *Canavalia* and FYM compared to net N immobilization by *Tithonia* and *Markhamia* observed in our study. The initial N content of *Markhamia* was 2% while that of *Tithonia* was 1.6%, thus clearly below the critical level. The case of our *Tithonia*, however, contradicts previous studies which showed high nutrient release and enhanced crop yield (Gachengo et al., 1999; Jama et al., 2000; Ndoli et al., 2013). This contradiction could be caused by differences in the initial N content and other biochemical properties. Further, the *Tithonia* biomass used in other studies, e.g. Gachengo et al. (1999), Jama et al. (2000), and Olabode et al. (2007) contained higher nutrient contents and may have been grown on relatively fertile soils. The quality of *Tithonia* biomass used in our study seems to be affected by poor soil fertility, season of harvesting, and possibly drying since dehydration reportedly increases the polyphenol content of *Tithonia* biomass and affects nutrient release (Mafongoya and Nair, 1997; Otuma et al., 1998; Jama et al., 2000). Moreover, the net N immobilization observed in *Markhamia* and *Tithonia* treatments could be caused by overriding effects of other parameters such as lignin/N ratio and ADF, which predicted 73% variability in net N mineralization and immobilization. Although we did not determine the polyphenol content in the amendments, previous work shows that *Markhamia* and *Tithonia* are widely used as medicinal plants (Kernan et al., 1998; Jama et al., 2000; Owoyele et al., 2004) and are enriched with different types of polyphenols (Cowan, 1999). This may have caused net N immobilization through the formation of nitrosamine compounds predominantly in acid soil conditions. This process may also depend on the type and amount of soluble polyphenolic present (Palm and Sanchez, 1991).

Many researchers (Saviozzi et al., 2002; Salamanca et al., 2006; Al-Busaidi et al., 2014) have consistently reported increases in soil microbial biomass C, N, and ergosterol following the application of organic amendments to soils. These findings agree with our results and suggests the rapid reaction of a consortium of active r-strategists, which utilize energy in labile C of fresh organic material. When the labile C is exhausted, the initially dormant K-strategists, which are adapted to consuming more polymerized C compounds, dominate the decomposition process (Fontaine et al., 2003). This may explain the mechanism behind the remarkable effect of *Canavalia* treatment on soil microbial biomass and ergosterol. Our observation also corroborates the results of Salamanca et al. (2006) who found a strong increase in microbial biomass C and ergosterol in tropical forest soils amended with *Calliandra* litter. The immense response of microbial biomass to soybean, cowpea (*Vigna unguiculalata* Walp.), and *Centrosema pascuorum* rotations has also been reported by Adoboye et al. (2006). Among the amendments, only *Canavalia* and FYM treatments in Technosol maintained a steady increase in microbial biomass C during the incubation period, suggesting a potential of these amendments to sustain the soil microbial population.

Frequently, amendment effects are high at the early stages and decline afterward either in a steep or steady manner depending on the biochemical and nutrient composition (Tejada et al., 2006). With increasing application rates, the microbial biomass increases proportionally to a peak (Gardner et al., 2010) and subsequently decreases at a diminishing rate (Bastida et al., 2008). Studies by Martens et al. (1992) and Saviozzi et al. (2002) showed no additional effects of amendment after large initial effects under regular applications at short intervals. These findings were obtained from long-term field experiments compared to our laboratory study of only twelve weeks. It is, therefore, uncertain how long the effect of the amendments on soil microbial properties observed in our study will last in the field. However, it was obvious from the general steep decline that repeated applications at short intervals may be required to maintain the described amendment effects. Additionally, the amount of applied C lost as CO_2 efflux (12-27%) from the soils within twelve weeks of incubation may be a substantial loss and may seem unsustainable for soil organic matter accrual, particularly in the Technosol. However, the situation may be different under field situations because of variable ambient settings and the quality or condition of amendments used. Meanwhile, it is assumed that after an initial boost of organic amendments, a self-sustaining effect can be attained once a SOC building soil management system is adopted (Larney et al., 2005; Larney and Angers, 2012). Unlike the N-rich FYM used in this study, the FYM in Gatumba is of poor quality because the fodder is dominantly elephant grass (*Pennisetum purpureum* Schumach) and may show a different effect. Nonetheless, the distinct performance of each amendment coupled with the general steep decline in soil microbial biomass of Technosol, suggests prospects for optimum benefits from integrated use in repeated applications. Consequently, an integrated soil fertility management approach is required for sustainable crop production and a sound C and N turnover in the Gatumba Technosols.

3.6 Conclusions

The Technosol of Gatumba Mining District holds sufficient microbial biomass required for the cycling of organic amendments and to release plant nutrients as a starting point of restoration. *Canavalia* was identified as a good soil amendment that can produce multiple benefits of sustaining soil microbes and nutrient release. The application of *Markhamia* and *Tithonia* led to net N immobilization. This raises concern for their use in the field but also indicates optimum benefits through integrated use of the amendments. It should be emphasized that this research was conducted under controlled conditions, which may differ from those in the field though extrapolation into field conditions is possible. Nevertheless, uncertainties remain about the maximum application rate of the amendments, either singly or combined, required to sustain microbial biomass in the Technosol in order to reach a reference state, and the time span before such a state is reached. As most soil environmental concerns are geared towards C sequestration, a debate is stirred up for considerations for a balance between "SOC builders" and "nutrient mineralizers" in the long-term if the intensive use of organic materials is expected. Opportunities also exist to compare the specific microbial responses to *Tithonia* grown on different soil types (mined and un-mined soils) and fertility gradients.

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4 Estimating the potential of N and P supply by local organic materials in tantalite mine soils of western Rwanda

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4.1 Abstract

Aims N and P remain the most limiting plant nutrients in tropical soils. In western Rwanda, land scarcity compels farmers to cultivate on degraded tantalite mine soils. The situation is worsened by low soil fertility and limited access to commercial fertilizers. We estimated short-term mineralizable nitrogen (N) and phosphorus (P) mineralization of local organic materials in the soils compared with a native forest and an arable land through a four-week incubation experiment at 22°C.

Methods Mineralizable N was estimated from soil-sand mixtures amended with *Canavalia brasiliensis* biomass and goat manure. The samples were leached fortnightly with 200 ml of 0.01 M $CaCl_2$. P mineralization was estimated from soil-resin mixtures amended with *Tithonia diversifolia* biomass and goat manure. The resins were separated from the soils through sieving, sedimentation, and decantation and eluted with 0.5 M HCl to estimate phosphate content.

Results Among the treatments, *Canavalia* had the highest mineralizable N with the highest (130 $\mu g\ g^{-1}$ soil, $P < 0.01$) and lowest (-20 $\mu g\ g^{-1}$ soil) in the Kavumu Technosol and the native forest soil, respectively. The mineralizable N of goat manure was negative. P mineralization in goat manure was triple the amount in *Tithonia*, constituting 61-71% of applied total P.

Key words: Anion exchange resins; *Canavalia brasiliensis*; mineralizable N; P mineralization; soil-resin mixture; *Tithonia diversifolia*

4.2 Introduction

Amidst prevalent land scarcity, farmers in subtropical Rwanda largely rely on poor-quality farmyard manure (FYM) for nitrogen (N) and phosphorus (P) supply of their crops. The quantity of FYM is insufficient because of limited fodder availability and corresponding livestock number per household. The situation is worsened by low soil fertility which is ranked the second cause of poverty (International Monetary Fund, 2008) among the 90% of the Rwandan population who are still engaged in subsistence agriculture (Central Intelligence Agency, 2015). Consequently, farmers in the Gatumba Mining District (GMD) of western Rwanda are also compelled to cultivate degraded tantalite mine soils. However, some neophyte plants thrive in the district and may supplement the poor-quality FYM to improve N and P supply in the tantalite mine soils. *Tithonia diversifolia* (Hemsley) A. Gray, a green manure known to scavenge plant nutrients, has been found to improve soil nitrogen (N), phosphorus (P), and potassium (K) contents, enhance crop yields (Kwabiah et al., 2003; Olabode et al., 2007; Ndoli et al., 2013), and increase soil fungi ergosterol content (Chapter 3). Meanwhile, high yielding legume cover *Canavalia brasiliensis* (Mart. ex Benth.) also had substantial effects on soil microbial biomass, and C and N mineralization in an arable soil and a Technosol (Chapter 3).

Many studies on plant nutrient dynamics have used ion exchange resins (IERs) to mimic the soil mineral exchange surface. In recent times, advancements in IERs, such as self-integration systems for cumulative nutrient leaching (Bischoff et al., 1999; Predotova et al., 2011; Siegfried et al., 2011), have proved useful in estimating field nutrient balances. Often, the IERs systems, customized in the form of net bags or cartridges, create barriers between the soil and the resins and reduce direct contact between them. Mixed soil-resin systems are rarely used. To the best of our knowledge, few studies (Saeed et al., 1994; Friedel et al., 2000) have employed soil-resin mixtures in nutrient mineralization studies. However, the use of such mixtures necessitates separation (Friedel et al., 2000) of the tiny beads from the soil to estimate the amount of nutrients adsorbed on the exchangers, which until today remains a challenge. Thien and Myers (1991) first separated IERs beads from soil, using density separation in sucrose solution through centrifugation. Thereafter, studies that either utilized this approach or advanced it are scarce. The method of Thien and Myers (1991), however, may not be applicable in many cases because (i) highly aggregated and amended soil-resin mixtures may require additional effort to gradually destroy the aggregates during homogenization before centrifugation, (ii) limited access to vortex and reciprocating shakers, and centrifuges may be limiting, (iii) the method is energy-demanding, (iv) the use of acetone in the procedure poses health risks, and (v) removal of excess water from resins after separation is an extra task. These limitations call for a simple environmentally friendly adaptation of the separation method suited for a wide range of situations.

Literature review shows a huge amount of research on C and N mineralization, but few exist on P mineralization. This is probably due to challenges associated with considerable P-fixation by positively charged soil minerals. It is most severe in high P-fixing tropical soils, where a substantial fraction of mineralized P is reportedly fixed or immobilized by soils minerals and microbes (Mafongoya et al., 2000; Azeez and van Averbeke, 2010a). To resolve this dilemma in P mineralization studies, soil-resin mixtures could be an alternative. Yet, these mixtures are also tagged with a bottleneck, i.e., the preferential selection of ions with diverse diffusion coefficients and high affinity for the exchanger, which is likely to affect the functioning of ion exchangers as a temporary sink for the target ion. For instance, chloride has a high affinity for strong base anion exchangers than phosphate (Skogley et al., 1996; The Dow Chemical Company, 2015) and is likely to be held more tightly, restricting phosphate adsorption on the exchanger. To offset this phenomenon, anion exchange resins (AERs) are often converted to bicarbonate form to enhance P adsorption (Sibbesen, 1978; Lajtha, 1988).

Meanwhile, plant roots release bicarbonate to offset ion imbalances (Riley and Barber, 1969; Gahoonia and Nielsen, 1992; Hinsinger, 2001) in the rhizosphere. Interestingly, the affinity of bicarbonate to mineral constituents is closer to that of phosphate compared with organic acids such as citrate, oxalate, and malate (Hinsinger, 2001). This *rhizosphere bicarbonate principle* has been applied in resin P analysis by Sibbesen (1978) where AERs in bicarbonate form extracted more P (Sibbesen, 1978; Lajtha, 1988) compared with their counterparts, and correlated well with plant P uptake (Sibbesen, 1978). A model involving direct contact between soil and AERs in bicarbonate form to mimic root-soil interaction in the rhizosphere could allow to study P mineralization in high P-fixing soils. Basically, the mineralized P is readily fixed on the AERs instead of the soil minerals, serving as a temporary sink for the phosphate, which can subsequently be eluted and analyzed.

The major tasks in this study were to assess the potential of *Canavalia* and *Tithonia* biomass and goat manure to supply adequate N and P in the tantalite mine soils of western Rwanda in a short-term laboratory study. We particularly wanted to answer the following questions: (a) Are there differences in short-term potential mineralizable N of *Canavalia* and goat manure amended to the soils? (b) Can the rhizosphere bicarbonate principle be used to estimate short-term P mineralization in the soils through soil-resin mixtures amended with *Tithonia* and goat manure? (c) How large are soil-specific effects of organic amendments on N and P mineralization? (d) What is the effect of soil pH change on P mineralization? In this study, we estimated the potential mineralizable N of *Canavalia* and goat manure, using a modified (Maqsood et al., 2013) method of Stanford and Smith (1972). We further tested a simple method of separating AERs from incubated soil-resin mixtures to investigate P mineralization from *Tithonia* and goat manure amended to the soils. We hypothesized that (i) the potential mineralizable N is influenced by amendment quality and site (abiotic and biotic soil properties), (ii) bicarbonate on the AERs can easily be exchanged by phosphate mineralized from the amendments, and (iii) the bicarbonate resins and the amendments will increase soil pH and reduce the affinity of soil minerals for phosphate in the acidic soils. Therefore, when these resins are mixed with high P-fixing soils and incubated, they will readily serve as a temporary sink for recently mineralized P. To test this we set up two experiments for potential mineralizable N and P mineralization.

4.3 Materials and Methods

4.3.1 Sites, soils, and amendments

Soils were collected from six sites in the GMD of the Western Province of Rwanda, a historic columbite-tantalite mining concession since the 20th century. The mining concession is located near the Nyabarongo River between longitudes 29°37” and 29°40” E, and latitudes 1°53” and 1°56”S at an altitude between 1500 and 3000 m a.s.l. The landscape is hilly with predominantly grassy uplands and average temperatures between 18° and 21°C (REMA, 2011). The rainfall distribution is bimodal averaging 1320-2400 mm per annum. The dominant soils types in the area are Anthrosols, Cambisols, Fluvisols, Gleysols, Lixisols, Luvisols, Nitisols, and Umbrisols (Reetsch et al., 2008; IUSS Working Group WRB, 2014).

The sites comprised an unmined native forest, two Technosols afforested with pine and eucalyptus, an arable land, and two cultivated Technosols (Kavumu and Kirengo Technosols). The native forest and arable land were chosen as references for forests and cultivated mine soils. From each site, five field replicates of fresh mineral soils were sampled from 0-20 cm depth. The litter layer at the forest sites were removed before sampling. The soils were strongly acid ranging from 3.7 in the native forest soil and from 5.6 in the Kavumu Technosol. The clay content ranged from 24-50% and soil texture were sandy clay loams for the pine and cultivated Technosols, and clayey loams for the native forest soil, eucalyptus Technosol, and arable soil (Chapter 2). The native forest soil, pine and eucalyptus Technosols had SOC contents of 62, 25, and 21 mg g^{-1} soil while their total N contents were 5.0, 1.4, and 1.5 mg g^{-1} soil, respectively. Conversely, the SOC contents of the arable soil, Kavumu Technosol, and Kirengo Technosol were 10.9, 10.2, and 8.7 mg g^{-1} soil with total N contents of 1.0, 0.9, and 0.6 mg g^{-1} soil (Chapter 2). Resin-P ranged from 0.6-1.2 µg P g^{-1} soil with the highest in native forest soil. The dithionite-extractable Fe, Fe_d, content of the soils was generally higher (17-45 mg g^{-1} soil) than dithionite-extractable Al, Al_d (2.2-5.3 mg g^{-1} soil). The oxalate-extractable Fe and Al contents ranged from 4.2-9.2 mg g^{-1} soil and 0.7-2.2 mg g^{-1} soil, respectively and were higher in the “forest soils” (native forest soil and afforested Technosols) than the “cultivated soils” (arable soil and cultivated Technosols). This corresponded to ratios of oxalate-extractable to dithionite-extractable Al and Fe in the range of 0.10-0.59, which were also high in the “forest soils” (Chapter 2).

The amendments used were *Canavalia* and *Tithonia* biomass and goat manure. The goat manure was obtained from an experiment on Batina bucks reared in Sohar, northern Oman. The bucks were fed with 60% hay from Rhodes grass (*Chloris gayana* Kunth.) and 40% barley meal. *Canavalia* biomass was obtained from field trials of the Coltan Environmental Management Project while *Tithonia* biomass was collected from farmlands, wayside, and marginal areas in the GMD. The amendments were air-dried, milled and sieved to 1 mm before use.

4.3.2 Amendment analysis

Total C and N contents in the amendments were measured by dry combustion, using a Vario MAX CHN analyzer (Elementar GmbH, Hanau, Germany). The pH was determined in a 1:10

substrate-water ratio with an electrode (SI Analytics GmbH, Mainz, Germany). Total P was determined by dry combustion at 550°C for 5.5 h followed by di-acid digestion and colorimetric measurement on a Hitachi U-2000 spectrophotometer (Hitachi Ltd., Tokyo, Japan) according to the vanadomolybdate method (Gericke and Kurmies, 1952). Water-extractable P was measured in extracts of 0.5 g substrates shaken in 50 ml bi-distilled water for 1 h. Nitrate, ammonium, and sulfate contents were measured on a Metrohm 850 Ion Chromatograph (Metrohm AG, Herisau, Switzerland) after extracting 1 g each with 50 ml 0.1 M $CaCl_2$ at 200 rev min^{-1} for 1 h.

4.3.3 Quartz sand

The quartz sand (0.4-2.0 mm) was pre-washed twice in 0.1 M HCl, soaked in fresh solution overnight, thoroughly washed again, rinsed thrice with bi-distilled water and oven-dried (40°C) dried before use.

Table 8. Physical and chemical properties of strong base Amberjet™ 4200 Cl^- AERs (The Dow Chemical Company, 2015) used for the P mineralization experiment.

Functional group	Trimethyl ammonium
Physical form	Yellow translucent spherical beads
Matrix	Styrene divinylbenzene copolymer
Ionic form	Cl^-
Exchange capacity of wetted bed ($mmol_c$ ml^{-1})	≥1.3
Particle size (mm)	0.60-0.80
Type	I
Operational pH range	0-14
Strength of exchange	Strongly basic
Moisture holding capacity (%)	49-55
Shipping weight (g L^{-1})	670
Specific gravity	1.06-1.08
Cross linking (%)	1
Activity (mmol g^{-1})	3.5-4.5
Selectivity of anions	
Bicarbonate	6.0
Phosphate	5.0
Nitrate	65
Sulfate	85

4.3.4 Pre-conditioning of anion exchange resins

The AERs, Amberjet™ 4200 in chloride form, were obtained from the DOW™ Chemical Company (Midland, MI, USA). Table 8 shows the physical and chemical properties of the resins. Before the experiment, the resins were preconditioned by washing and drying at 40°C till constant weight, sieved through 0.4 mm Sefar Nytal® (Sefar AG, Heiden, Switzerland) nylon net to eliminate bead sizes less than 0.4 mm, and converted from chloride form to bicarbonate form (Sibbesen, 1978; Lajtha, 1988). First, the resins were shaken in 0.5 M

$NaHCO_3$ on a rotating shaker for 1.5 h at 100 rpm, strained, replaced with a fresh solution and left to stand overnight. A second shaking was done the next day with two more flushes of 0.5 M $NaHCO_3$ followed by three rinses with bi-distilled water. The converted resins were dried at room temperature to 43% moisture content defined as workable moisture content (WMC). At WMC, the uneven distribution of water in wetted resins is avoided. It also enhances quantitative transfer of resins from one receptacle to another without loss of beads adhered to surfaces during sample preparation. The resins were then packed in Ziploc® plastic bags and stored under cool conditions until use.

4.3.5 Potential mineralizable N

The short-term incubation study to determine potential mineralizable N comprised the soils from the six sites and three treatments, including (1) Control: non-amended soil, (2) *Canavalia*, and (3) goat manure. For each treatment 30 g soil (105°C oven-dry weight basis) were adjusted to 60% WHC and pre-incubated for one week. Subsequently, the soils were mixed with 30 g acid-washed quartz sand (1:1 ratio) and amended with 500 µg N g^{-1} soil. The mixed samples were packed in 100 ml polyethylene syringes lined with glass wool (Merck KGaA, Darmstadt, Germany) and sand at the bottom and covered with glass wool. The syringes were placed on a leaching unit (Figure 12) connected to a vacuum pump and pre-leached according to the method of Stanford and Smith (1972) modified by Maqsood et al. (2013) to remove existing inorganic N before incubation. Subsequently, the samples were randomly arranged on wooden racks (Figure 12A) and incubated at 22°C for four weeks. Mineralized N was leached from soil pores with 200 ml 0.01 M $CaCl_2$ fortnightly and filtered through Whatman No. 42 filter paper. An N-free nutrient solution was used to replace leached nutrients during each sampling period. The leachates were analyzed for NO_3^- and NH_4^+ on a continuous flow analyzer (Evolution II, Alliance Instruments GmbH, Salzburg, Austria) while extractable C was measured on a multi N/C analyzer (Analytik Jena AG, Jena, Germany). Once the mineral N was already pre-leached before incubation, net N mineralization of the amendments was estimated using the difference between mineralized N in non-amended and amended soils. The short-term potential mineralizable N was estimated from cumulative net N mineralization using the equation: $N_o = N_t / (1 \times 10^{-kt/2.303})$, where, N_o = potential mineralizable N (µg g^{-1}), N_t = cumulative net N mineralization (µg g^{-1}), and k = mineralization rate constant ($week^{-1}$). Stanford et al. (1974) estimated decay rate constants of $k = 0.03$ and 0.13 for non-amended soils incubated at 25 and 35°C, but we chose $k = 0.13$ because the decay rate was expected to be relatively high for amended soils, particularly during the first four weeks.

4.3.6 P mineralization

Before the main experiment, a pretest was conducted with the native forest soil to set parameters. The main experiment consisted of soils from the sites mentioned earlier with three treatments, including (i) Control: non-amended soil-sand mixture, (ii) *Tithonia*, and (iii) goat manure. Thirty grams dry weight of the fresh soils were adjusted to 80% WHC, pre-incubated for one week, and mixed with 30 g WMC resins (1:1 ratio). The soil-resin mixtures were amended with 330 µg P g^{-1} soil. Since the resin and ion exchange reactions require sufficient moisture, 12 ml bi-distilled water was added to offset additional water requirements for *Tithonia* and 5 ml for goat manure and control treatments respectively. The mixture was transferred into 120 ml polyethylene bottles (Sarstedt AG, Nümbrecht, Germany), loosely fitted with lids, randomly placed, and incubated at 22ºC for four weeks. Plastic bowls containing distilled water were placed in the incubator to maintain humidity. The samples were destructively sampled fortnightly to separate the resin beads from the soil.

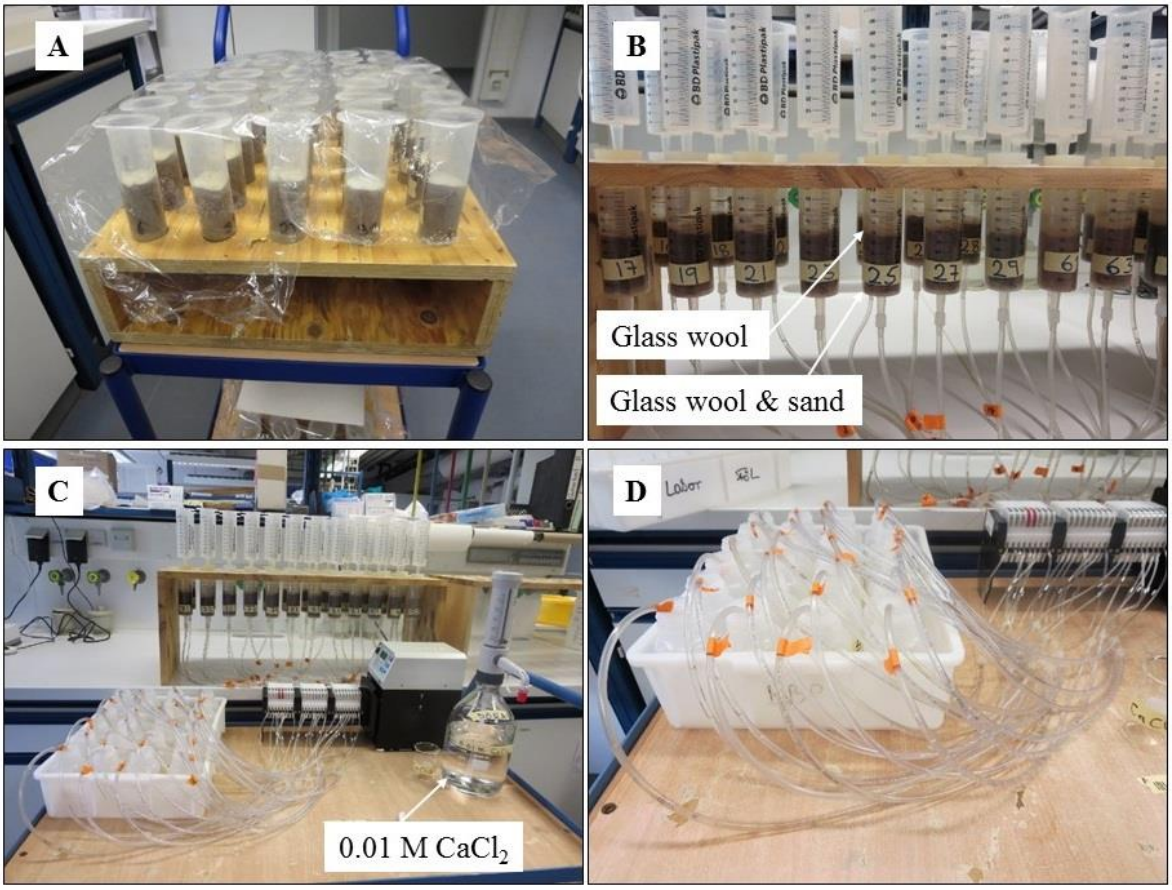

Figure 12. Experimental setup for potential mineralizable N of soils from the Gatumba Mining District, western Rwanda.

4.3.7 Separation of resin beads from soil

The separation of the resin beads from the soil involved sieving the soil-resin mixture through 0.4 mm nylon net bags of 9 × 13 cm size followed by decantation. Figure 13 shows a summarized version of the separation process. We chose 0.4 mm mesh size to eliminate particles below 0.4 mm and retain resin beads and soil particles above 0.4 mm. During each sampling period, the incubated soil-resin mixture was weighed and homogenized with an equivalent volume of bi-distilled water (1:1 ratio). The homogenized sample was carefully emptied into a net bag held over a beaker fitted with a funnel and sieved to collect the suspension for pH measurement. The bag content was then held upright under running distilled water to purge colloids and particles below 0.4 mm while retaining resins in the bag. Soil aggregates were also crumbled to enhance sieving after which the bag content was again returned to the beaker. With additional water and stirring, the beaker was tilted and held for at least 10 seconds to allow resins, heavy soil particles, and aggregates to settle at the bottom before decanting suspended organic materials. This procedure was repeated thrice. The resin beads were finally transferred into the net bag aided by additional water, repeated swirling, and decantation. Soil aggregates that settled at the bottom were further broken to reveal remnant resins since light aggregates can easily float over resin beads. The bag content was rinsed till clear water was obtained and gently pressed between the palms to drain off excess water. The open end of the bag was sewn to avoid bead loss during successive elution events. Generally, the 99% recovery of resin beads from non-amended soils and over 100% for amended soils seemed to be an overestimation because some amendment particles having similar weight and size with the resin beads were retained in the net bag.

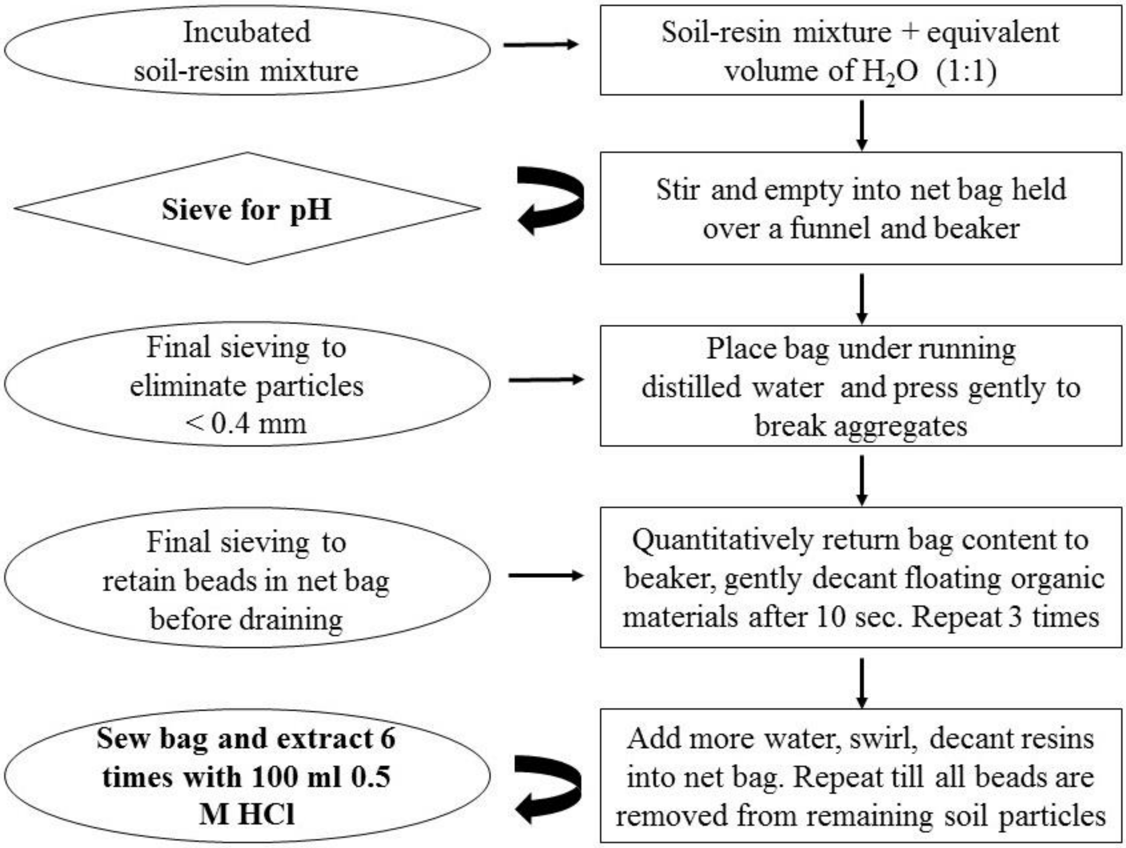

Figure 13. Flow chart showing steps involved in the separation of anion exchange resin beads from the incubated resin-soil mixture during the four-week P mineralization experiment with soils from the Gatumba Mining District, western Rwanda.

4.3.8 Extraction of phosphate from incubated resins

The sewn resin bags were placed in 250 ml polyethylene bottles and extracted with 100 ml 0.5 M HCl for 60 min in six successions, pooled together and homogenized. Fifty milliliters (50 ml) of the pooled extract was centrifuged at 3000 *g* for 5 min and filtered (hw3, Sartorius Stedim Biotech GmbH, Göttingen, Germany). The phosphate concentration was determined photometrically at 882 nm after coloring with ammonium molybdate and ascorbic acid, using a FLUOstar Omega microplate reader (BMG Labtech, Ortenberg, Germany). To estimate the recovery of phosphate extracted from the resins, two separate groups of five replicates of 30 g WMC resins were spiked with at 250 µg KH_2PO_4-P g^{-1} resins. In addition to blank resins, the spiked resins were extracted in a similar manner as the separated resins. The amount of P mineralized was calculation as: P (µg g^{-1} soil) = (S-B)/(E×D_f)×(V/S_d), where *S* and *B* are the sample and blank absorbances, respectively, *E* = extinction coefficient (slope of linear equation), D_f = dilution factor, *V* = volume of extract (ml) and S_d = oven-dry weight of soil (g).

4.3.9 Statistical analysis

The data were tested for normality of residuals and transformed where necessary. First, two-way analysis of variance (ANOVA) was conducted for data with normally distributed residuals using Site and Repeated measures (incubation time) as factors. Then for each treatment, one-way ANOVA and Kruskal-Wallis (for non-normally distributed residuals) test were used to separate means between sites followed by Scheffe's test ($P < 0.05$) and Mann-Whitney ($P < 0.013$) for normal and non-normally distributed residuals, respectively. The Pearson correlation was used to determine the relationship between amendment properties soil pH and mineralized P in the amendment during the incubation period. All statistical analyses were conducted using SPSS version 20 (IBM, New York, NY, USA; Field, 2009).

4.4 Results

4.4.1 Potential mineralizable N

The application of high-quality *Canavalia* biomass and low-quality goat manure led to net N mineralization and immobilization (Figure 14), which differed significantly among sites ($P < 0.001$) throughout the incubation period. The potential mineralizable N (Figure 15) followed the pattern of cumulative net N mineralization ($r = 1$, $P < 0.001$). *Canavalia* had largest mineralizable N (130 µg N g^{-1} soil, $P < 0.01$), which occurred in the Kavumu Technosol whereas the native forest soil had the lowest and negative mineralizable N of -20 µg N g^{-1} soil. Conversely, the potential mineralizable N of goat manure was negative for all soils, except for the native forest soil, but did not differ significantly among the sites. It was generally higher in the "cultivated soils" ranging from -2.5 µg N g^{-1} soil in the Kavumu Technosol to -7.7 µg N g^{-1} soil in the pine Technosol.

Within the first two weeks of incubation, the *Canavalia* treatment mineralized N in the "cultivated soils", but led to N immobilization in the "forest soils" (Figure 14). Conversely, the application of goat manure led to net N immobilization in all soils except the native forest soil, but it was not significantly different from its counterparts. Cumulative net N mineralization of *Canavalia* treatment ranged from 310-670% ($P < 0.01$) in the "cultivated soils" and only 20-160% in the "forest soils" compared with the non-amended soils (Figure 14). The largest and smallest *Canavalia* N were mineralized in the Kavumu and pine Technosols, respectively, while cumulative net N immobilization occurred in the native forest soil. In the goat manure treatment, cumulative net N immobilization grouped into low net N immobilization in the "cultivated soils" and high net N immobilization in the afforested Technosols. In contrast, the goat manure mineralized 7% N in the native forest soil in comparison with non-amended soils, but did not differ from its counterparts. In the non-amended soils, cumulative net N mineralization decreased with time and differed ($P < 0.001$) among sites, showing the largest and smallest values in the native forest soil and the Kavumu Technosol, respectively. Meanwhile, extractable C (Figure 16) showed treatment × site interaction effects ($P < 0.001$). In the amended soils, extractable C decreased by a range of 3-50% in the native forest and arable soils as well as the cultivated Technosols, in contrast to 10% increase in the afforested Technosols. However, it decreased with time increased with time in the non-amended soils except in the Kirengo Technosol.

Table 9. Chemical characteristics of Canavalia and Tithonia biomass and goat manure used for the mineralizable N and P mineralization experiments with samples from the Gatumba Mining District, western Rwanda.

Variable	**Unit**	***Canavalia***[*]	***Tithonia***[*]	**Goat manure**	**CV (%)**
pH		6.2	6.4	7.8	0.1
Total C	(mg g^{-1})	438	452	448	0.2
Total N	(mg g^{-1})	29	24	17	1.4
Total P	(mg g^{-1})	2.2	1.8	9.3	3.7
C/N		15	29	27	1.5
C/P		199	251	48	3.5
Water soluble P	(mg g^{-1})	NM	0.5	2.4	1.3
Extractable NH_4-N	(µg g^{-1})	81.8	12.6	17.2	1.3
Extractable NO_3-N	(mg g^{-1})	5.5	BD	BD	3.0
Extractable SO_4-S	(mg g^{-1})	3.5	2.2	1.0	2.8
Lignin	(mg g^{-1})	108	215	124	5.3
Lignin/N		3.9	14.1	7.5	-

[*]Data from Chapter 3; CV = pooled coefficient of variation; NM = not measured; BD = below detection

4.4.2 Change in pH of the soil-resin mixture during incubation

The pH of the incubated soil-resin mixture increased with time and confirms our hypothesis that the bicarbonate resins and the amendments will increase pH. After two weeks of incubation, the bicarbonate resins increased the pH in comparison with the non-amended samples (Table 10). The pine Technosol had the largest pH increase of 1.9 units in comparison with the original soil pH while the Kavumu Technosol had the lowest increase of 1.1 units. The amendments further increased the pH by 0.1-0.8 units in comparison with the non-amended samples. Goat manure produced the highest pH change. Among the sites, the native forest soil increased with a larger margin of pH units relative to both original soil pH and non-amended samples whereas the cultivated Technosols increased with a lower margin. After four weeks, the soil pH increased by 1.1-1.8 units relative to the original soil pH. The eucalyptus Technosol had the highest pH increase relative to the original soil pH, whereas the lowest was observed in the Kavumu Technosol. Here, the amendments increased the pH by 0.07-0.9 additional units relative to the non-amended samples. Again, goat manure had the largest effect on the pH for all the sites except the Kavumu Technosol.

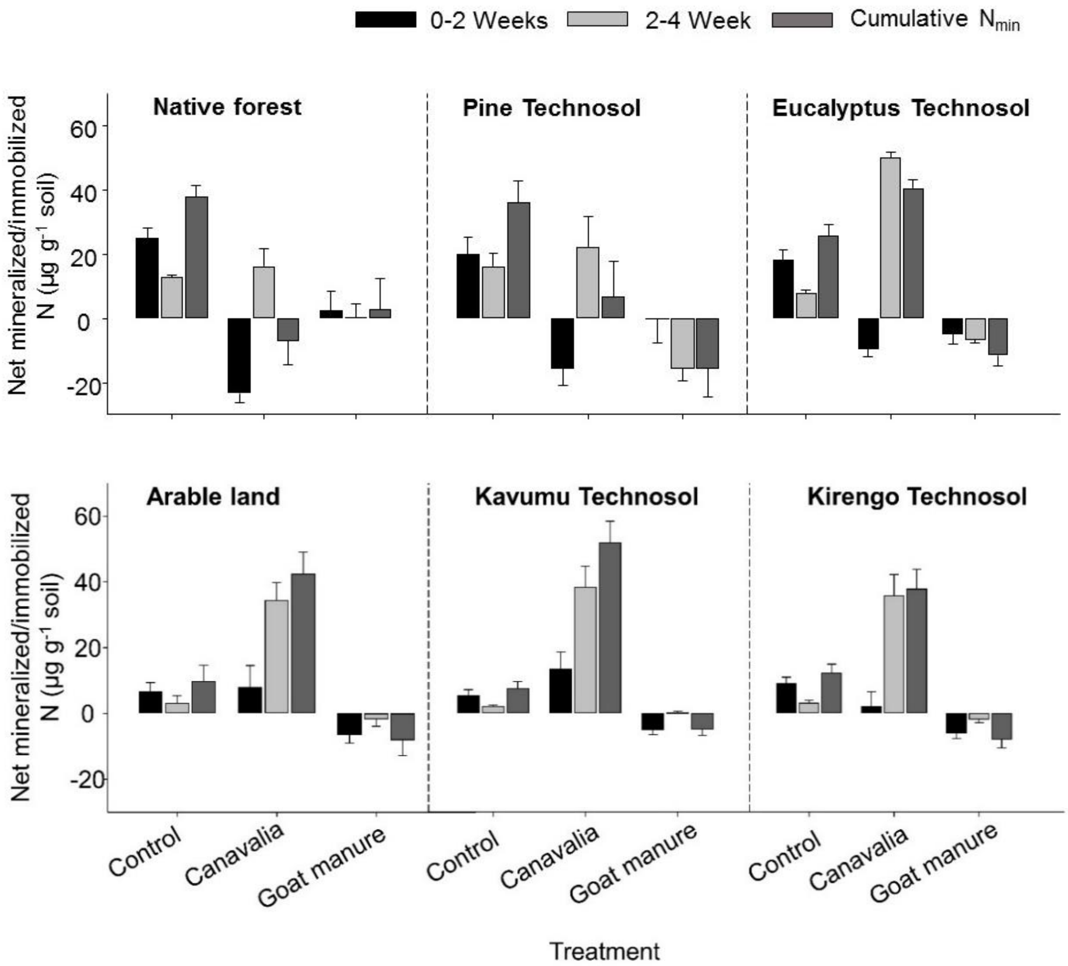

Figure 14. Net N mineralization and immobilization during a four-week incubation of soils from the native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda amended with *Canavalia* biomass and goat manure. Bars (n = 5 ± one standard error of the mean) are exclusive of inorganic N in non-amended soils before and after incubation.

4.4.3 P mineralization

About 94% ± 0.88 of spiked KH_2PO_4 was recovered from the resins, implying that 94% of amendment P sorbed on the resins was eluted. The P mineralization reflected the biochemical properties of goat manure and *Tithonia* (Table 9), varying significantly ($P < 0.01$) among treatments during the incubation period. After two weeks of incubation, goat manure mineralized the largest amount of P (Table 11), which was three to four-fold that of *Tithonia* treatment and about 13 to 30 times that of non-amended samples. This constituted roughly 67% and 35% of total P in goat manure and in *Tithonia* applied to the soils with no significant differences among the sites. By week four, P mineralization decreased slightly in all amended samples except the native forest soil, suggesting a shift in the equilibrium reaction. This was slightly pronounced in the *Tithonia* treatment compared with the goat manure, and in the "cultivated soils" compared with the "forest soils". The application of goat manure increased mineralized P four-fold compared with *Tithonia* and 12- to 25-fold compared with non-amended samples, constituting roughly 66% of applied P. The fraction of applied P mineralized from *Tithonia* treatment was roughly 20% and differed ($P < 0.01$) among sites (Table 11). The native forest soil and the Kavumu Technosol had the highest mineralized *Tithonia* P. Phosphorus mineralization correlated strongly with amendment properties (r = 0.84-0.87, $P < 0.01$). The pH of the soil-resin mixture had a weak and negative correlation with P mineralization (Figure 17) for *Tithonia* biomass ($r = -0.35$, $P < 0.01$) and for goat manure ($r = -0.27$, $P = 0.02$).

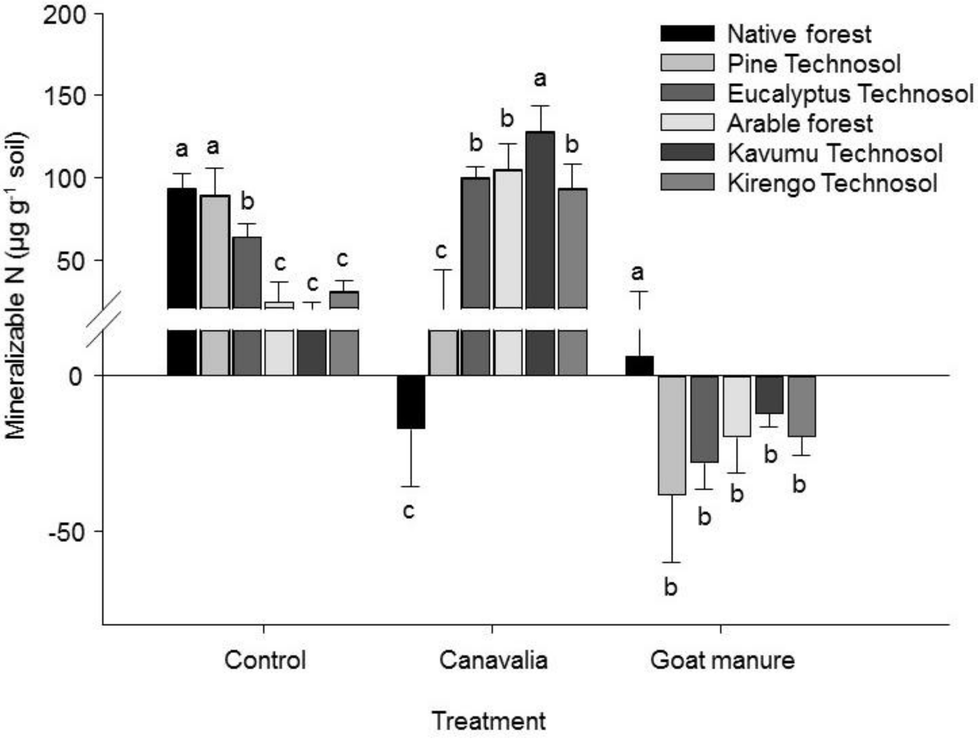

Figure 15. Potential mineralizable N of *Canavalia* biomass and goat manure in soils of the native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda during four weeks of incubation. Grouped bars (n = 5 ± standard error of the mean) with the same letter are not significantly different (Scheffe and Mann Whitney, $P < 0.05$).

Due to a possible ionic competition with phosphate, we measured the sulfate content of the P mineralization extracts. Like P mineralization, the sulfate content also decreased slightly with time and did not differ among the sites except for Tithonia ($P < 0.01$), which had the largest and the smallest sulfate content in the native forest soil and the Kirengo Technosol (Table 11), respectively. The sulfate content of Tithonia was twice that of goat manure and about threefold that of non-amended samples. The Pearson correlation showed a weak negative relation between pH of the soil-resin mixture and sulfate content for both Tithonia biomass ($r = -0.56$, $P < 0.001$) and goat manure ($r = -0.25$, $P > 0.05$).

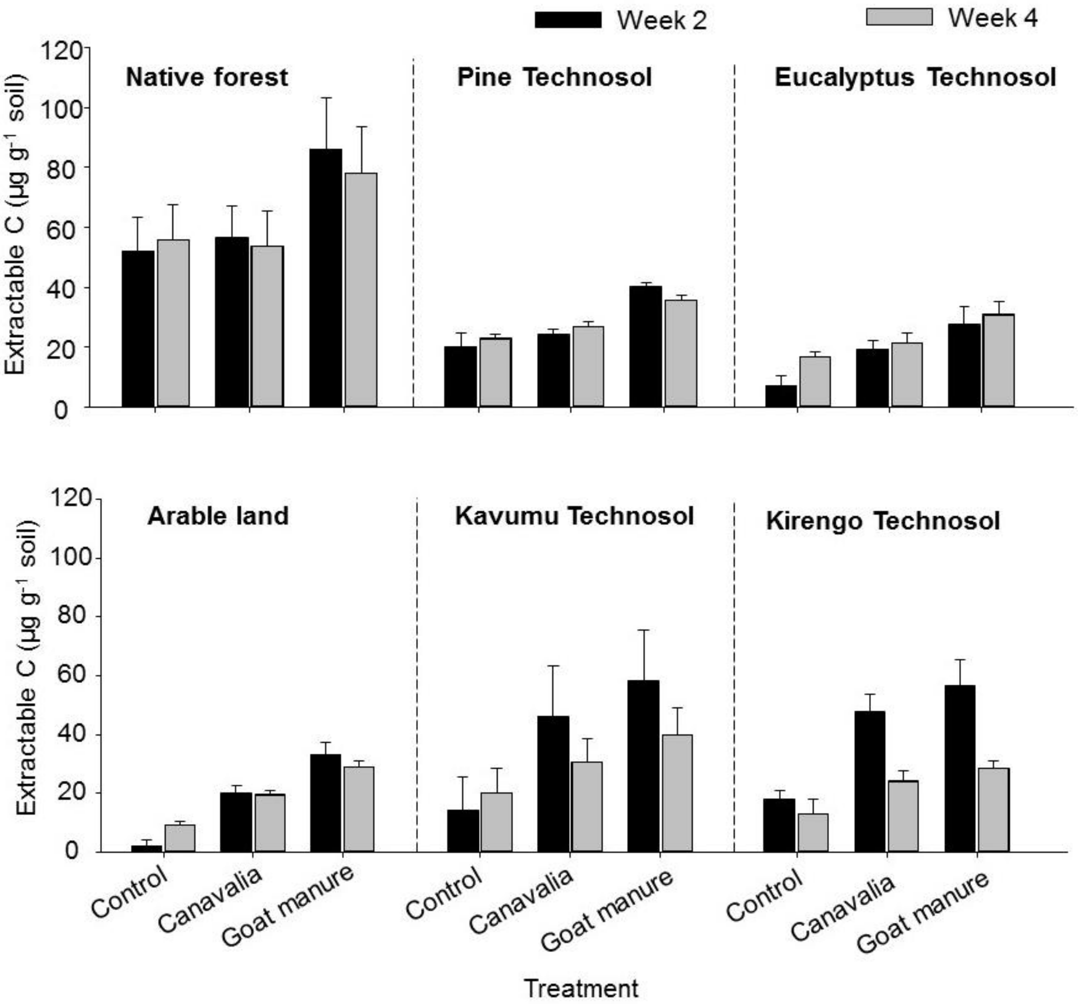

Figure 16. Extractable C during a four-week incubation of soils from the native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda amended with *Canavalia* biomass and goat manure. Two-way ANOVA revealed treatment × site interaction effects ($P < 0.001$) and effects of amendment and site on extractable C.

4.5 Discussion

4.5.1 Potential mineralizable N

Our study confirmed striking differences in the potential mineralizable N of *Canavalia* and goat manure in the tantalite mine soils. The potential mineralizable N is influenced by factors that control N mineralization and auto-correlates with first order decay ($r = 1$, $P < 0.001$). In a study to determine the biochemical characteristics of several organic materials that predict potential mineralizable N, Lashermes et al. (2010) identified cellulose, lignin, initial N content, and soluble organic fraction as the main determinants; the initial N content alone predicted 52% of the potential mineralizable N of the organic materials applied to a wide range of soils. Other factors such as preliminary soil incubation, soil microbial activity (Stanford and Smith, 1972; Stanford et al., 1974), moisture content, incubation temperature

(Stanford et al., 1974; Curtin et al., 1998), and soil pH (Curtin et al., 1998) affect potential mineralizable N.

Table 10. Soil pH of incubated soil-resin mixture of soils (0-20 cm) from the native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda amended with *Tithonia* biomass and goat manure. Interaction effects of site and repeated measures in each amendment were detected using two-way ANOVA. One-way ANOVA and Kruskal-Wallis test were used to compare means within sites for each treatment.

Factor	**Soil pH**			
	Original[*]	**Control**	***Tithonia***	**Goat manure**
Site				
Native forest	3.7 c	5.0 c	5.7 c	5.7 c
Pine Technosol	4.4 b	6.0 b	6.3 b	6.5 b
Eucalyptus Technosol	4.5 b	6.2 b	6.3 b	6.6 b
Arable land	4.9 a	6.3 b	6.8 a	7.0 a
Kavumu Technosol	5.6 a	6.7 a	6.9 a	7.2 a
Kirengo Technosol	5.2 a	6.5 a	6.9 a	7.1 a
Time				
Week 2		6.2	6.5	6.8
Week 4		6.1	6.5	6.7
Probability values				
Repeated measures		*NS*	*NS*	*NS*
Site		*<0.001*	*<0.001*	*<0.001*
Site × repeated measures		*NS*	*NS*	*NS*
CV (±%)		*6.5*	*3.5*	*3.5*

CV = pooled coefficient of variation between replicate field samples (n = 5); means in columns followed by different letters indicate significant differences (Scheffe and Mann Whitney, $P < 0.05$); [*]Data from Chapter 2

In this study, three main factors appear to control the N mineralization of the amendments in our study soils; amendment biochemistry, soil microbial population, and soil properties (Figure 14). The effect of amendment biochemistry lies the role of initial N and lignin, particularly in the *Canavalia* treatment as reflected in high cumulative net N mineralization (Figure 14). This observation supports the results of a previous study where high C and N mineralization was observed in *Canavalia* biomass along with increased and sustained microbial biomass in amended soils compared with non-amended ones (Chapter 3). It also corroborates the findings of Cobo et al. (2002), Carvalho et al. (2009) and Carvalho et al. (2013) who attributed high C and N mineralization to low lignin content (Table 9), high labile C fractions (Carvalho et al., 2009) coupled with high N content as well as hemicellulose content (Chapter 3). A switch in soil microbial preference for more the labile C in *Canavalia* (Wang et al., 2015) might have stimulated a microbial burst and high N requirement for both *Canavalia* and SOM mineralization and re-partition N into the microbial biomass (Tejada et al., 2006). This is driven by co-metabolism as microbial activity and C mineralization reaches a maximum following the addition of fresh labile C (Kuzyakov et al., 2000; Fontaine et al., 2003). Such a trend is further supported by the findings of a previous study where C mineralization of *Canavalia* peaked in the second week of incubation with a resultant increase in microbial biomass (Chapter 3). This may have caused a lag in N mineralization, which led to net immobilization of *Canavalia* N in the forest soils during week two. Also, the extent of net immobilization of *Canavalia* N in each forest soil reflects C mineralization observed earlier (Chapter 3). It conforms to the results of the study of Paterson and Sim (2013), where glucose addition led to net N immobilization and an increase in microbial biomass on a Scottish Podzol. The increased microbial biomass then has a rapid turnover for subsequent N release (Paterson and Sim, 2013). In contrast to *Canavalia*, more lignified goat manure with a C/N ratio of 27 immobilized N in all soils except for the native forest soil. The net immobilization of goat manure N agrees with the findings of Mafongoya et al. (2000) and Azeez and van Averbeke (2010b). Meanwhile, the extent to which *Canavalia* and goat manure treatments switched from net N immobilization to net N mineralization conforms to the findings of Wang et al. (2015) that high quality fresh organic matter switched faster from negative to positive priming effect than low quality fresh organic matter.

Beside the amendment biochemistry, the soil fertility status and C/N ratio of the active SOM pool control the direction of SOM mineralization (Kuzyakov et al., 2000). This presupposes that, when high-quality organic materials are added to C and N-rich soils, N is initially immobilized as a reflection of a microbial burst for subsequent mineralization of the applied substrates, and to some extent, native SOM. This is further determined by the dynamics of SOM-degrading populations whose increase can easily be accelerated by high energy input from fresh organic matter (Fontaine et al., 2003; Wang et al., 2015) and soil nutrients (Wang et al., 2015). Thus, C and N-rich soils have larger co-metabolism and may readily exhibit N immobilization than C and N-poor soils (Kuzyakov et al., 2000) as observed in the sharp contrast of net N immobilization in the “forest soils” on one hand, and the “cultivated soils” on the other. Interestingly, the immobilization of goat manure N in the “cultivated soils” was less (30-70%) severe than in the “forest soils”, signifying an imminent recovery of the amended soils from N immobilization. This has a significant implication for goat manure N supply in these soils compared with their forest counterparts. Hence, for sole goat manure application, the timing of application may be set such that recovery from N immobilization synchronizes with critical plant N uptake stages. The remarkable potential mineralizable N of Canavalia in the Kavumu Technosol also explains the effect of soil properties and is supported by the view of Larney and Angers (2012) that degraded soils exhibit the greatest benefit from amendments.

Table 11. Mineralized P and sulfate content in incubated soil-resin mixture of soils from native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda amended with Tithonia and goat manure. Interaction effects of Site and Repeated measures in each amendment were detected using two-way ANOVA. Means in columns followed the same letter are not significantly different (Scheffe, Mann Whitney, $P < 0.05$).

Factor	**Mineralized P**			**Sulfate content**		
	Control	*Tithonia*	Goat manure	Control	*Tithonia*	Goat manure
			(μg g^{-1} soil)			
Site						
Native forest	8.2	46.1 a	120.3	57.0	161.6 ac	62.0
Pine Technosol	5.6	37.7 b	123.0	52.9	153.6 bc	75.9
Eucalyptus Technosol	4.2	36.4 b	117.8	44.1	127.7 b	61.6
Arable land	6.9	36.0 b	117.1	54.7	112.3 b	43.0
Kavumu Technosol	8.2	42.1 a	115.7	61.0	101.6 b	59.5
Kirengo Technosol	5.6	37.3 b	117.5	30.4	83.6 d	36.4
Repeated measures						
Week 2	5.8	42.0 a	118.6	51.7	125.1	60.3
Week 4	7.1	36.5 b	118.8	48.2	122.3	52.0
Probability values						
Repeated measures	*NS*	*<0.001*	*NS*	*NS*	*NS*	*NS*
Site	*NS*	*<0.001*	*NS*	*NS*	*<0.001*	*NS*
Site × Repeated measures	*NS*	*0.009*	*NS*	*NS*	*NS*	*NS*
CV (±%)	*42.5*	*13.3*	*4.5*	*55.5*	*28.9*	*44.7*

CV = pooled coefficient of variation between replicate field samples (n = 5).

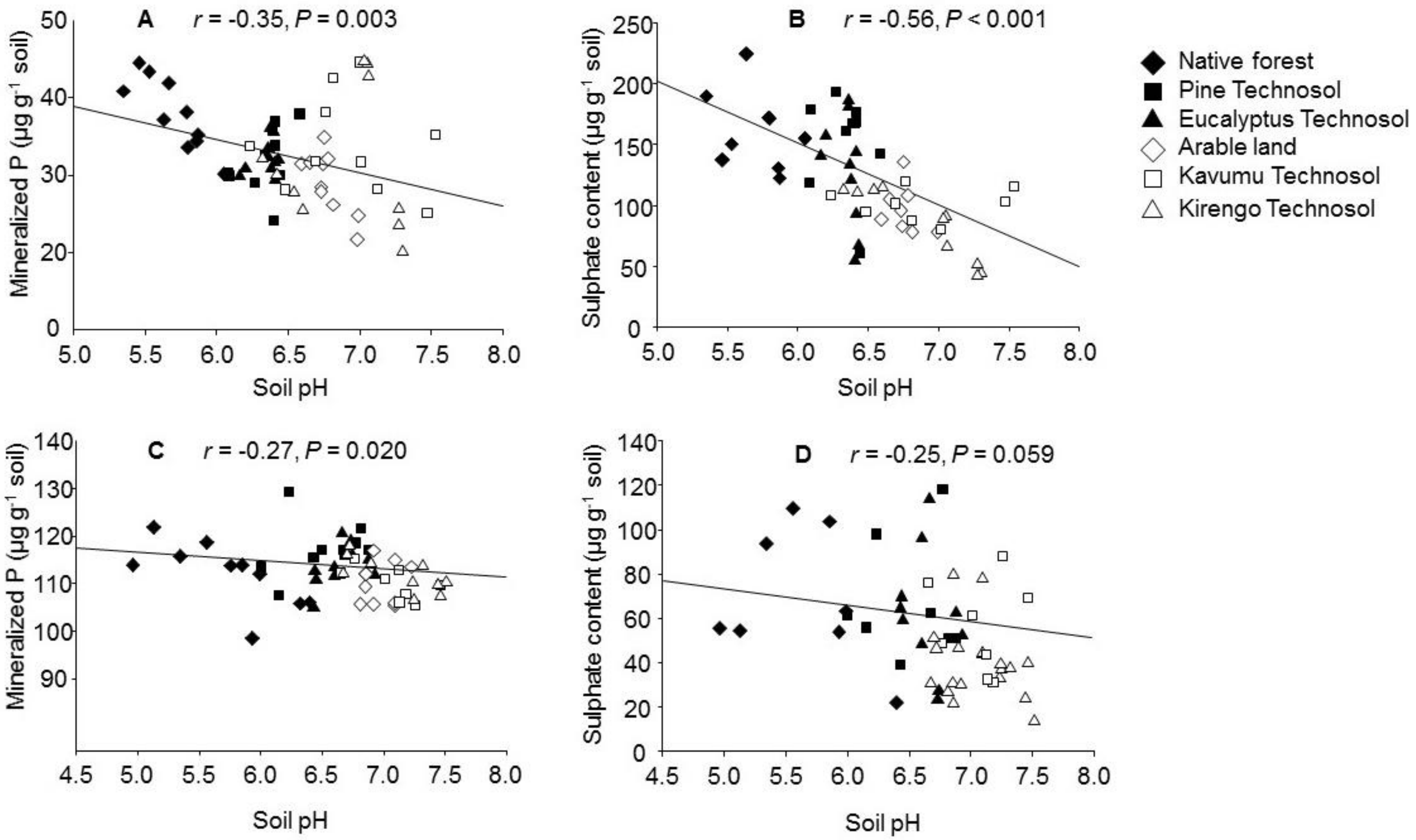

Figure 17. Pearson correlation showing linear relationships of pH change in soil-resin mixtures and P mineralized from *Tithonia* biomass (A), goat manure (C) as well as sulfate content of *Tithonia* biomass (B) and goat manure (D) treatments of soils from the native forest, pine and eucalyptus Technosols, arable land, and Kavumu and Kirengo Technosols from the Gatumba Mining District, western Rwanda during a four-week incubation experiment.

A huge contrast was observed between the P content of *Tithonia* and goat manure and its mineralization, suggesting the importance of animal manure as a P source compared with plant biomass (Mafongoya et al., 2000; Eghball et al., 2002). This has been attributed to large P contents secreted by animals coupled with digestive consumption of C, resulting in low C/P ratios (Mafongoya et al., 2000). The quality of goat manure is dictated by the age and sex of the animal, feed quality, water intake, and management system (Azeez and van Averbeke, 2010a). Consequently, a wide range of C/P ratios are often reported (Mafongoya et al., 2000; Azeez and van Averbeke, 2010a; Azeez and van Averbeke, 2010b; Willich, 2014). High P content, particularly the inorganic fraction, greatly contributed to high P mineralization of goat manure. This corroborates high water-soluble P content of goat manure and high P release after 10 days of incubation in a South African Ultisol (Azeez and van Averbeke, 2010a).

In contrast to goat manure, the *Tithonia* biomass used in our study was of inferior quality, having a total P content, water-soluble P fraction of total P, and C/P ratio of 1.8 mg g^{-1}, 28%, and 251, respectively. Conversely, the *Tithonia* biomass used by Kwabia et al. (2003) had a total P content of 3.2 mg g^{-1}, a 50% water-soluble P fraction of total P, and C/P ratio of 140. These amendment properties influenced P mineralization as shown by a strong positive correlation. Positive correlations between total and water-soluble P and P mineralization, and a negative correlation between C/P ratio of added substrates and P mineralization are in accordance with the findings of Mafongoya et al. (2000). This caused the remarkable differences in the percentage of mineralized P in *Tithonia* and goat manure. In addition, applied organic P increases microbial proliferation (Tarafdar and Claassen, 1988 Gichangi et al., 2009) and is dependent on labile organic P molecules present in the amendments (Tarafdar and Claassen, 1988). Diester phosphates and teichoic acid, for instance, are more readily mineralized than monoester phosphates (Addiscott and Thomas, 2000).

An interesting observation in this study was the slight decrease in P mineralization by week four, particularly in the *Tithonia* treatment, suggesting a shift in the equilibrium reaction. We attribute it to competition from ions with high selectivity coefficients and diffusivity, which may have reversed the ion exchange reaction process. Sulfate is one of such ions, having 17 times the selectivity coefficient of phosphate on the Type I anion exchanger (Table 8). This high affinity is similar to high sulfate adsorption on crystalline iron oxides (Stanko-Golden et al., 1994). Beside sulfate, nitrate, which also has 11 times the selectivity coefficient of phosphate (Table 8) could have contributed to a shift in the exchange equilibrium. However, we discount nitrate because *Tithonia* and goat manure treatments immobilized N in previous N mineralization experiments (Chapter 3). Thus, nitrate was not detected in the extracts. Nitrate is an indifferent anion, which does not enter into specific adsorption and can be completely desorbed by eliminating the positive charges on the exchanger. Sulfate and phosphate, however, can be involved in both mechanisms of anion adsorption, i.e., non-specific and specific adsorption (ligand exchange) (Johnson et al., 1979; Stanko-Golden et al., 1994), but sulfate is thought to be dominantly involved in specific adsorption (Stanko-Golden et al., 1994). Since P sorption is governed by clay type and content, SOM, and the number of phosphate groups in the monoesters (Frossard et al., 1995), it is assumed that humic substances in the organic amendments, especially *Tithonia,* formed metal ligands that favored sulfate adsorption over and/or displacement of phosphate. Records also show that ion exchange by resins is sensitive to competition from soil microbes (Binkley, 1984; Lajtha, 1988).

Variable sulfate content has been reported in many parts of tropical Africa and is said to be related to soil pH (Safo and Sekou, 1976). It has been argued that organic C can block adsorption sites and reduce anion adsorption, resulting in a positive relationship (Johnson et al., 1979; Stanko-Golden et al., 1994) observed in sulfate adsorption potentials (Stanko-Golden et al., 1994). This contradicts other findings, which show a negative influence of organic C on sulfate adsorption, leading to high retention at low pH and the vice versa (Safo and Sekou, 1976; Stanko-Golden et al., 1994). However, this dichotomy seems to be determined, to a large extent, by the biochemical composition and the amount of organic C present in the soils and the interactive effects of reactions between the organic and mineral components of the soil. Therefore, the relation between soil pH and sulfate adsorption found by Stanko-Golden et al. (1994) may be attributed to a remarkably high C content (35.7%) coupled with low soil pH. Overall, the sulfate adsorption capacity of a soil is determined by the amount of sulfate already adsorbed (Johnson et al., 1980; Stanko-Golden et al., 1994).

4.6 Conclusions

Canavalia application led to higher potential mineralizable N than goat manure which was most pronounced in the Kavumu Technosol. Although goat manure immobilized N, the extent of N immobilization in the “cultivated soils” was less severe compared with the “forest soils”, suggesting an imminent recovery from N immobilization. Despite the N immobilization, goat manure played a major role in P mineralization compared with *Tithonia.* Owing to the slight decrease in P mineralization at the end of the incubation period, its fate beyond four weeks is uncertain. Each amendment had a unique contribution to soil N and P supply. Yet, the combined application of these amendments in specific ratios, or co-composting prior to application may yield optimum benefit of N and nutrient release as well as SOM buildup.

Acknowledgments

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5 General discussion and conclusions

5.1 Critical soil properties of the study sites

Soil microbial organisms are essential decomposers of organic materials for soil organic matter (SOM) turnover and determine the rate and type of ecological succession on mine lands (Brenner et al., 1984). For this reason, the first study of this Ph.D. research assessed the effects of land use on microbial indices of the afforested and cultivated Technosols compared with a native forest and an arable soil. The study also identified soil properties that posed constraints to the microbial indices. Like many soils of the humid tropics, the major constraint was acidity (pH 4.4-5.2) linked with amorphous Al (0.2-0.53%) and low P availability (0.5-1.2 mg kg^{-1}) (Chapter 2). Previous studies on the Rwandan soils revealed that soils with pH of 5.0 already contained 20% Al saturation (Vander Zaag et al., 1984). The acidity, coupled with low P availability, accounted for 74% ($P < 0.01$) and 83% ($P < 0.01$) variability in basal respiration and net N mineralization, respectively and enhanced fungi ergosterol content in the mine soils (Chapter 2). For most forest soils, acidity persists due to the decomposition of continuous litter inputs and may not be easily ameliorated. For arable soils, however, liming is required to adjust soil pH, improve P availability, and enhance microbial properties. Previous studies recommended lime application rates between 1.4-6.9 ton $CaCO_3$ ha^{-1} for the Rwandan soils (Vander Zaag et al., 1984; Yamoah et al., 1990; Verdoodt and van Ranst, 2003). Rwanda has some deposits of travertine (Figure 18) comprising 40% CaO and 3% MgO (Nduwumuremyi et al., 2013). The deposits are found mainly in the Karongi and Rusizi districts of the Western Province and in Musanze, Gakenke, and Rulindo districts of the Northern Province. Unfortunately, these deposits are inadequate to neutralize all of the acidity in Rwandan soils because over two million tons of lime would be required to be applied at regular intervals (Nduwumuremyi et al., 2013). For the GMD, the deposits in Karongi district are in close proximity, but the cost of transport is still a challenge for poor smallholder farmers. Although Nduwumuremyi et al. (2013) reported that lime is being provided to some Rwandan farmers through the district offices, it may not suffice for all farmers.

Wood ash is one important but un-utilized liming material and nutrient amendment (Figure 19) from biomass energy that can be obtained in the GMD. About 92% of the rural population in Rwanda depends on firewood and charcoal for cooking (Ndayambaje and Mohren, 2011). Its effective use on even small surfaces would nevertheless require systematic collection by smallholder farmers, particularly in the GMD. For instance, in three weeks we obtained as much as 20 kg fine fraction (< 2 mm) of wood ash (Figure 19) from one secondary school with a student population of 416. An interview with the chief cook of the school revealed that they had difficulties discarding the wood ash on a daily basis. Chemical analysis of the school's wood ash and the author's domestic wood ash showed a total P content of 11.4 and 11.8 g kg^{-1}, respectively with a pH of around 11.9 (Neina, unpublished data). Apart from schools, food vendors and households are additional sources of wood ash in the GMD. Wood ash is a good source of alkali (Onyegbado et al., 2002) containing about 50% calcium carbonate equivalent (Demeyer et al., 2001) and is enriched with P, Ca, Mg, K, and Na (Patterson et al., 2004). The elemental composition depends on the type and source of biomass used as well as the combustion type and temperature (Misra et al., 1993; Demeyer et al., 2001). These properties of wood ash offer a cheap option to ameliorate the existing soil acidity, enhance basic cation contents (Neina and Dowuona, 2013), and improve P nutrition (Lopez et al., 2009) in the soils. Meanwhile, there are concerns over the presence of heavy metals in wood ash, but the actual contents are still linked to the factors that determine its composition and will differ with wood type and location. Further research on the Rwandan wood ash will be required to establish the best rate of application, risks and fate of heavy metals in the food chain.

Figure 18. Travertine deposits located in the Musanze district of the Northern Province of Rwanda.

5.1 Choice of tree species for mine soil restoration

The first study (Chapter 2) showed higher SOC and total N contents as well as microbial biomass and activity in the afforested than the cultivated Technosols and the arable soil. It further revealed a distinction between the pine and eucalyptus Technosols suggesting the effects of specific tree species. In a study on “ecosystem development and natural succession in surface coal mine reclamation”, Brenner et al. (1984) observed higher bacterial activity in deciduous species than in conifers and attributed it to high labile C from the latter for microbial growth. The pine and eucalyptus tree species were among the exotic tree species introduced in Rwanda as part of afforestation and reforestation programs intended to rehabilitate degraded lands, control erosion, create buffer zones, natural reserves, and meet escalating demands for wood products (Biggelaar and Gold, 1996; Roose and Ndayizigiye, 1997; Nsabimana et al., 2008). Interestingly, eucalyptus adapted well to the Rwandan environment and accounts for about 26% of Rwandan forest cover against 5% of pine forests (NISR, 2008). Eucalyptus is greatly appreciated by the Rwandan populace for delivery of wood products. Despite this, eucalyptus has a poor reputation for soil degradation, although there are site-specific effects (Nsabimana et al., 2004; Nsabimana et al., 2008). *Grevillea robusta* is often the preferred exotic on-farm tree species by most Rwandan farmers because of its positive interaction with crops, adaptation to poor soils, and suitability for local use (GMD farmers, personal communication, May 2013; Bucagu, 2013).

Recently, the Rwandan government realized the need to convert the eucalyptus forests into forest types adapted to specific ecological zones and soil types (REMA, 2009). The challenge is the choice between fast-growing tree species for wood products and tree species for sound tree-soil interactions. It is obvious that eucalyptus remains the ultimate choice for wood supply in Rwanda because of its rapid growth and adaptation to the local environment. But for optimum and multiple benefits, mixed-species plantations are the best choice. Studies have shown that mixed plantations of eucalyptus and legume trees are more productive and have higher rates of N and P cycling than monocultures (Forrester et al., 2006). Generally, mixed plantations are more productive when positive interactions dominate competition (Forrester et al., 2006). Aside legume trees, indigenous tree species such as *Makhamia lutea*, *Ficus thonningii*, *Albizia gummifera* (J.F.Gmel.) C. A. Sm., *Chrysophyllum gorungosanum,* and *Erythrina abyssinica* Lam. ex DC. could be incorporated into eucalyptus plantations for mine land restoration since native plant species enhance the density and stability of plant communities on mine lands (Brenner et al., 1984).

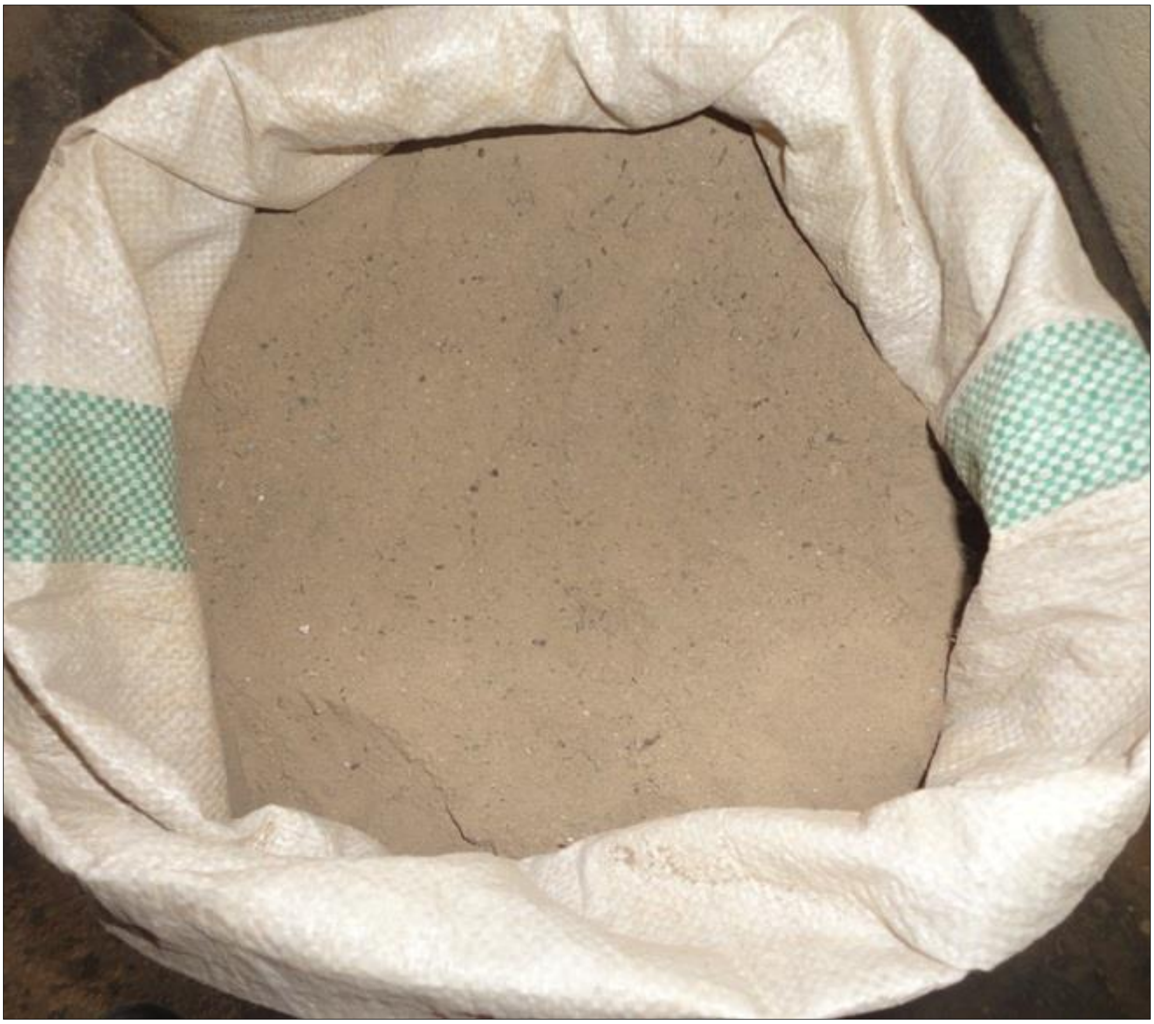

Figure 19. Sieved fraction of wood ash collected in the GMD of western Rwanda.

5.2 Standard mine soil restoration practices

In mine land restoration, standard procedures are laid down as a guide to achieve successful implementation depending on the specific end land uses. Before the enactment of reclamation laws and the Surface Mining Control and Reclamation Act (SMCRA) in 1977 (Torbert and

Burger, 2000), mine lands were either abandoned or restored without topsoil application. The SMCRA requires the salvage of top- and subsoils prior to mining for subsequent replacement and plant establishment. Such mine soils are termed *ungraded* while those replaced with topsoil are *graded* mine soils (Akala and Lal, 2001). *Graded* mine soils are beneficial over *ungraded* ones because they contain SOM and microbes for nutrient cycling (Larney and Angers, 2012), which permit quick plant establishment and colonization. Research shows that topsoiling increases SOC and humus quality of mine soil, available P, and improves the starting quality of the developing soils (Borůvka et al., 2012). Brenner et al. (1984) identified SOM and soil moisture content as the most vital factors that determine tree establishment and growth on mine lands. Therefore, they recommended that all mine land restoration efforts should aim at creating conditions that enhance these factors.

Fundamentally, the second study (Chapter 3) investigated the ability of a Technosol to mimic the graded mine soil environment through C and N mineralization along with microbial proliferation after amendment with FYM, *Canavalia* and *Tithonia* biomass, and *Markhamia* leaf litter. These forms of amendments were selected in the similitude of regular inputs in the soils. It was assumed that during cultivation under low nutrient input and nutrient mining through crop harvests, the Technosol re-gained microbial properties similar to *graded* mine soils and have prospects for quick restoration under intensive organic agriculture. This basis was established in the first study (Chapter 2) and needed to be validated for organic amendments. Unlike most mine soils which are often overwhelmed by metal toxicity and acid mine drainage, the Technosol of GMD in western Rwanda have no such hazards but are enriched with cations, particularly K from feldspars and muscovite (Dewaele et al., 2011; Lehmann et al., 2014; Nieder et al., 2014). Thus, the restorative process in the Technosol was assumed to differ from those of metalliferous mine wastes and waste rock dominated mine soils. The Technosol used in our studies showed a greater overall response to the amendments than the arable soil. As the incubation proceeded, the microbial biomass declined more strongly in the Technosol than the arable soil. It was concluded that the Technosol used has a high potential for quick restoration. But based on the steeper decline in microbial biomass, the Technosol showed, however, the need for repeated amendment at short intervals to reach a reference state.

5.3 Performance of the amendments and prospects for future use

In studies two (Chapter 3) and three (Chapter 4), the contributions of the biomass of *Canavalia* and *Tithonia, Markhamia* leaf litter, and goat manure to C, N, and P mineralization, mineralizable N, as well as microbial biomass were explored. Both studies showed a major potential of *Canavalia* in C and N mineralization and as well as remarkable increases in microbial biomass and fungal ergostrol contents. The results revealed that *Canavalia* and FYM dominated N release while *Canavalia* increased microbial biomass and sustained it over a reasonable period. *Tithonia* contributed considerably to fungal ergostrol content. Moreover, *Tithonia* and goat manure played a key role in P mineralization but led to net N immobilization in the soils.

The reverse may also occur for FYM and goat manure due to the influence of soil and animal factors on the biochemical characteristics. For instance, a mineralization experiment in South Africa showed that goat manure was of high-quality in terms of N release than cattle manure (Azeez and van Averbeke, 2010b) but of low-quality for P mineralization (Azeez and van Averbeke, 2010a). In this study (Chapter 3), a high-quality FYM was used. However, the Rwandan FYM originates from cattle largely fed with Napier grass (*Pennisetum purpureum)* and occasionally, small quantities of sweet potato vines (Mutimura and Everson, 2011), banana leaves, and crop residues (Bucagu, 2013). The quality of Napier grass is influenced by age, height at harvest, watering regime, and soil quality. The recommended height and age for harvesting Napier grass is 60-100 cm and 6-10 weeks depending on watering regime (Kariuki et al., 1998; Muia et al., 1999). At this stage, the crude protein ranges from 7.5-12% depending on the management system, i.e., farm level or experimental field (Kariuki et al., 1998; Muia et al., 1999). The crude protein ultimately affects manure N contents (Cole et al., 2005). The quality of FYM manure in the GMD is further affected by the mode of handling as it is often collected and thrown in the open space until use. This can cause a huge loss of N through ammonia volatilization. *Markhamia, Tithonia,* and goat manure contained high C/N ratios and may contribute to C storage although this potential was not explored. Thus, the integrated use of the amendments is highly recommended as synergistic effects are expected. This has been demonstrated in many studies involving organic amendments and inorganic fertilizers (Ghacengo et al., 1999; Ndoli et al., 2013). Like FYM, the goat manure from GMD may also be of poor quality since the ruminants are given the same feed but both manure types may contribute to P release and SOM accrual.

A field research in the highlands of Rwanda (Yamoah and Mayfield, 1990) revealed large variations in nutrient composition and decomposition rates of herbaceous legumes. Consequently, they recommended legumes that could rapidly cover soil during fallow periods and also release nutrients. *Canavalia* fits in this description for the Technosols. This was also observed during an experiment conducted by the Coltan Environmental Management Project, and through a *Canavalia* fallow established before a cassava experiment. Within two months, *Canavalia* biomass covered over 50% of the ground surface. Such features of *Canavalia* could be utilized in fallows during the short dry periods and subsequently plowed as a green manure before the main growing seasons. This kind of fallow may be suitable for soil erosion control on the steep slopes. Meanwhile, the quality of *Tithonia* used in the study was inferior to those used by Ghacengo et al. (1999), Kwabia et al. (2003), and Ndoli et al. (2013). Apart from the effect of soil fertility, seasonal effects may have reduced the quality of *Tithonia* biomass because it was sampled at the end of the minor season in Rwanda. According to Nieder et al. (2014), *Tithonia* showed a phytoremediation potential by accumulating a substantial amount of Pb and Zn in excess of FAO/WHO guidelines. The fate of these heavy metals in *Tithonia* biomass amended to soils is uncertain and will thus require further research.

5.4 Practical issues with the P mineralization method

The advantage of the soil-resin mixture method of P mineralization (Chapter 4) is that, it eliminates the need to grind soil into fine particles (Sibbesen, 1978) which destroys the natural soil aggregates. The method of separation is simple and energy-saving. However, the main challenge was the estimation of exact recovery of the AERs after separation from the soil compared with the method of Thien and Myers (1991). This suggests that, in terms of resin recovery from the soil, the method is either more suitable for soils with low SOM or soils with highly humified SOM. In subsequent research on nutrient dynamics involving soil-resin mixture, the exact recovery of resins from the soils amended with least decomposed organic materials may require an additional separation step. Presently, the soil-resin mixture method requires improvements. Also, P mineralization decreased slightly by the fourth week of incubation and was attributed to competition from sulfate, particularly in the *Tithonia* treatment. Therefore, the fate of P mineralization beyond four weeks is uncertain.

Another aspect of IERs that may require attention is the type of resins under consideration. In this study, the strong base Type I IERs, containing trimethyl ammonium as the functional group (The Dow Chemical Company, 2015), were used. This type is recommended for P studies (Skogley and Dobermann, 1996) although a previous study reported higher P extracted from the Type II IERs (Sibbesen, 1978), which contain dimethyl-β-hydroxyethylamine as the functional group (Skogley and Dobermann, 1996). The discrepancy has been attributed to the amount of divinylbenzene (DVB) present in the resins. For instance, IERs with a lower DVB content reportedly extracted more P (Sibbesen, 1978). These aspects need be considered in future research to improve the soil-resin mixture method of P mineralization.

5.5 General conclusions

Study I showed that the afforested and cultivated Technosols differ in microbial biomass and activity (Chapter 2). The major constraint of the soils was acidity linked with substantial amounts of amorphous Al, and Fe oxides and hydroxides with low P availability; a source of stress for the soil microorganisms. A cost-effective solution is suggested for smallholder farmers in section 5.6. This study underscored the importance of post-mining land use in the GMD by distinguishing between "SOC builders" and "SOC breakers" in terms of afforestation and cultivation. It also revealed the importance of specific tree species in biological mine soil quality improvement. The second study (Chapter 3) affirmed that the amendments (Table 6 and Table 9) are a feasible option to intiate restoration of the functional integrity of the Technosols by enhancing the biological quality. The amendments used exhibited unique responses according to their biochemical characteristics, and to some extent, the properties of the soil to which they were applied. There was evidence that the Technosols holds sufficient microbial biomass required for the cycling of the amendments and to release plant nutrients as a starting point of restoration. *Canavalia* was identified as a good soil amendment that can produce multiple benefits of sustaining soil microbes and nutrient release. In contrast, the application of *Markhamia* and *Tithonia* led to net N immobilization, which raises concern for their use in the field. In study three (Chapter 4), the N and P supply potential of the organic materials applied to the tantalite mine soils was estimated after four weeks. *Canavalia* had a higher potential mineralizable N and the highest occurred in the Kavumu Technosol. Goat manure led to negative mineralizable N (immobilization) in all soils except for the native forest soil. Interestingly, the N immobilization of goat manure in the arable soil and Technosols was less severe indicating an imminent recovery which suggests that a careful strategy is required to schedule applications such that the recovery from N immobilization will synchronize with critical plant N requirement stages. In the P mineralization experiment, the bicarbonate resins and the amendments increased the pH of the soil-resin mixture and the original soil pH of each site by approximately 1-2 units. Phosphorus mineralization of goat manure was three times as high as that of *Tithonia* with no difference among sites. After four weeks of incubation, it decreased slightly and was attributed to sulfate competition as reflected in a negative correlation, which was steeper in the *Tithonia* treatment.

Each amendment used in the study played a unique role in C, N, and P mineralization as well as contributions to microbial biomass in the tantalite mine soils. Interestingly, the "N immobilizers" exhibited potentials for P release and SOC building. Generally, the combined application of the amendments in specific ratios, or co-composting prior to application is highly recommended for sustainable benefits of nutrient release, sustained microbial biomass, and SOM accumulation. In this case, wood ash could be incorporated in the applications. Considering the constraints associated with the transport of large quantities of organic amendments to fields, smallholder farmers are at a certain advantage since they will only need to cart smaller quantities.

It is emphasized that a greater part of the study was a conducted under controlled and relatively stable laboratory conditions, which exclude the interactive effects of environmental variables. It also employed fine fractions of the amendments compared with the usual practice of applying coarse fractions. Although these findings are indicative of the biogeochemical processes of "real world" situations, it is acknowledged that the dynamics may not exactly reflect those of field conditions.

5.6 General recommendations and outlook

Based on the results of this Ph.D. research and constraints identified in the study soils, the following recommendations can be made:

I. The tantalite mine wastelands should be restored by afforestation with mixed tree species such as a mix of fast-growing eucalyptus and indigenous species or exotic legume trees.
II. At the small scale and provided a systematic collection system is established, the prevalent soil acidity can be ameliorated with wood ash, which can act as a liming material and a soil amendment to improve soil P and cation content (Table 12).
III. There are prospects for sustainable agriculture under continuous and integrated use of the local organic amendments (Table 12).

Further research is needed to:

a. Compare the specific microbial responses to *Tithonia* grown on a soil fertility gradient in both mined and un-mined soils.
b. Investigate the fate of heavy metals in crops grown on soils amended with *Tithonia* biomass due to its phytoremediation potential.

c. Determine the microbial response of the Technosols to combined applications of the amendments.
d. Investigate the fate of P mineralization involving soil-resin mixtures incubated beyond four weeks.
e. Develop an alternative to the fatigue associated with the successive elution of resins, probably using a leaching unit similar to the one used for mineralizable N experiment (Figure 12) involving a drip flow rate through the resins with subsequent suction aided by suction after substantial contact time. This can reduce the loss of resin beads and time during the successive extraction regimes.
f. Assess the recovery of standard P spiked to bicarbonate AERs using extraction solutions such as NaCl, HCl, KCl, $NaHCO_3^-$, and H_2SO_4.
g. Test whether the plant-specific rhizosphere bicarbonate content can be used as a basis to convert resins from other forms to bicarbonate form to determine the relative effects of the various fractions of plant-specific bicarbonate on P mineralization.
h. Investigate the performance of mixed anion-cation resins of different types (Section 5.4) and from different manufacturers using different soil-resin ratios.

Table 12. Proposed integrated nutrient management scheme for the Gatumba Mining District, western Rwanda.

Amendment	Preparation	Mode of usage
Canavalia	▪ Scarify seeds and sow soon after crop harvest: i. Wrap seeds in a piece of cloth and dip in boiled water for 30 min ii. Sow seeds (germinate within two weeks) ▪ Plowed in biomass after two months[1] (ground cover can be over 50%)	Legume cover and green manure
Tithonia and FYM	[2]Co-composting: this can be done in small pits by placing FYM and *Tithonia* biomass in alternating layers.	Organic fertilizer
Wood ash	▪ Pending further research to ascertain the amount required to reach target soil pH for crops, the recommended lime application rate may be used. ▪ Wood ash may be applied either by placement of by plowing into the soils	Lime and fertilizer

[1]Continuous seed supply will be required to sustain this practice; [2]Immediate co-composting may reduce ammonia volatilization

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