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List of abbreviations

°C	Degree celsius
µM	Micromolar
a.s.l.	Above sea level
A.U.	Absorbance units
AfSIS	Africa soil information service
AIC	Akaike information criterion
amoA	Ammonia monoxygenase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
B	Bushumba
BMZ	Bundesministerium fur wirtschaftliche Zusammenarbeit un Entwicklung
BNF	Biological nitrogen fixation
C	Carbon
C.calothyrus	*Calliandra calothyrsus*
C/N ratio	Carbon to nitrogen ratio
$CaCl_2$	Calcium chloride
Ca_{ex}	Excheangeable calcium
Ch^2	Chi-squared
CIALCA	Consortium of improving agriculture-based livelihoods in Central Africa
cm	Centimeter

CUE	Carbon Use Efficiency
D	Dega
DAAD	Deutscher Akademischer Austausch Dienst
DRC	Democratic Republic of Congo
DNA	Desoxyribonucleic acids
DRC	Democratic Republic of Congo
DSS	Decision support system
EthioSIS	Ethiopan soil information system
FAO	Food and Agriculture Organization of the United Nations
FSC	Food Security Center
GmbH	Geselleschaft mit beschänkter Haftung
H	Hydrogen
ha	hectare
HD	High Dega
ICIPE	International Center of Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
ISFM	Integrated soil fertility management
K	Kola
K_2SO_4	Pottasium sulfate
K_{av}	Available potassium
KBr	Potassium bromide
km	Kilometer
L	Lignin

M	Mushinga
Mg_{ex}	Exchangeable magnesium
midDRIFS	Mid-infrared diffuse reflectance Fourier transformation spectroscopy
MLR	Multiple linear-regression
MO	Organic matter
MIRS	Mid-infrared spectroscopy
N	Nitrogen
n.s.	Not significant
N2Africa	Nitrogen for Africa
N_2O	Nitrous oxide
NH_3^-	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
npr	Neutral metaloproteases
O	oxygen
OC	Organic Carbon
Org. C	Organic carbon
P_{av}	Available phosphorus
PCR	Polymerase chain reaction
PLFA	Phospholipid Fatty Acids
PLSR	Partial least square regression
PP	plolyphenols

qPCR	Quantitative polymerase chain reaction
R^2	R squared
RNA	Rubonicleic acids
RNA	Ribonucleic acids
RwaSIS	Rwanda soil information services
SDGs	Sustainable Development Goals
SOC	Soil organic carbon
SSA	Sub-saharan Africa
SYBER Green	An asymmetrical cyanine (Fluorescent DNA binding dye)
T4 gene 32 protein	A nucleic acid binding protein that destabilizes the DNA helical structure
TC	Total Carbon
TN	Total nitrogen
UNDP	United Nations Development Programme
USA	United States of America
VN	Vector normalization
WD	Weina Dega

CHAPTER 1

General introduction

1. General introduction

1.1 Overview

Declining soil fertility has become a major constraint for food security and economic performance in small-holder farmers in Sub-Saharan Africa (SSA) (Sanchez, 2002; Giller et al., 2019). These soils, in their current status, are not able to provide adequate agricultural productivity therefore unable to feed growing human population estimated to be 1.8 billion by 2050 (Batiano et al., 2007; van Ittersum et a., 2016; Jones et al., 2004; Andriesse and Giller, 2015). In developing countries, smallholder farmers contribute the largest proportion of agricultural production and projections suggest that this will remain unchanged for the next 30 years (Thornton and Herrero, 2001; Otsuka and Muraoka, 2017). The current data suggest that size of land holding is less than 2 ha (Samberg et al., 2016; Lowder et al., 2016; Makate et al., 2019), due to land shrinkage as a result of high population density. Meanwhile, agricultural activity remains the main source of livelihoods, where majority of smallholder farmers continue to live in poverty (Gebremedhin et al., 2009; Lim et al., 2020). Low agricultural productivity has led to insufficient total food production (Sanchez et al., 2002; Turmel et al., 2015; FAO, 2019). The low productivity is often associated with the loss of organic matter and soil erosion (Mabit et al., 2008; Pimentel and Burgess, 2013). Hence, the result of soil fertility reduction from continuous cropping and as well long process of high weathering known as causes of nutrient deficiency for tropical soils (Jones et al., 2004; Maranguit et al., 2017).

Particularly, deficiencies of nitrogen (N) and phosphorus (P) have been documented as the major biophysical constrain of crop production in SSA (Sanchez et al., 1997; Verde and Matusso, 2014; Nziguheba et al., 2016), making it difficult for small-holder farmers to meet the required yield. Although these nutrient elements can be supplied to the soil through mineral fertilizers (Chianu et al., 2012; Bindraban et al., 2015), the actual physical characteristic of the soil are seriously degraded (Andriesse and Giller, 2015). In addition, the continent in general still has poor access (Bationo et al., 2007; Sommer et al., 2013; Sheahan et al., 2013) and knowledge when it comes to utilization of mineral fertilizers (Vanlauwe and Giller, 2006; Sommer et al., 2014). Moreover, the cost of purchasing fertilizers remain unfordable for small-holder farmers (Crawford et al., 2003; Patt et al., 2010), leading them to be relying on organic inputs as the only one option for replenishing fertility.

Nitrogen is of special interest as most of this element is held in the soil as stable organic matter with up to 95% of N in some soils (Bingham and Cutrofu, 2016). Its transformation goes through microbial mediated processes crucial in ecosystem functioning. However, the processes are affected not only by farm management practices, but also biotic and abiotic factors (St. Luce et al., 2011). Understanding N transformations and their respective soil microbial functions would be essential in soil fertility management towards increasing agricultural productivity for improving household consumption.

For agriculture to benefit from N transformation, organic material should first satisfy soil microbial demand prior to N being released for root uptake (Seneviratne, 2000; Cassity-Duffey et al., 2020). This can be achieved through residue management. However, organic material should be of good quality to supply substantial nutrient to the soil (Palm et al., 2001). In contrast, low quality residues that release insufficient amount of N, microbes are intended to scavenge inorganic N from the surroundings to satisfy their demand, leading to immobilization of N in microbial biomass. To overcome this, supplement of mineral fertilizers may be added to speedup mineralization process. For instance, organic resource of plant materials has advantage of enriching the SOM pools, this maintain soil fertility as well improve efficiencies of mineral fertilizers. (Vanlauwe et al., 2002; Chivenge et al., 2011). Their direct effects on nutrient availability as influenced by its biochemical composition such as C/N ratio as well as polyphenol and lignin content (Nicolardot et al., 2001; Rasche et al., 2014).

1.2 Soil fertility status under smallholder farmers' fields in tropical agroecosystem of East and Central Africa

Soil fertility a characteristics of physical, chemical and biological process that control plant nutrient availability, is generally decreasing in smallholder farmers in SSA (Stewart et al., 2020). It has been described as fundamental biophysical root cause of declining per capita food production (Sanchezet al., 1997). Tropical agroecosystems are known for their poor nutrient resources stock hence, there is need for improving the nutrient resources in tropical soils (Harcombe, 1989; Tittonell and Giller, 2013). Including East and Central Africa, where a widespread inherent poor soil fertility is feasible. Chemical fertilizers that could supply crops directly with the required rate of nutrients are scarce, affecting negatively farmers' yield in all range of crops (Bekunda et al., 2002; Kintché et al., 2017). Often, mineral fertilizers are not only simply unavailable at local markets but also the purchasing price is high and not recovered by the cost of agricultural produce once sold on the market.

This situation is not different from smallholder farmers living in South-Kivu, DRC and Ethiopia where majority of household food security still relys on small scale-farming (Cox, 2008; Okumu et al., 2011). Agricultural production systems of these regions are characterized by traditional farming methods with low inputs that have led to severe nutrient depletion where only a limited amount of manure or composted crop residues are applied (Pypers et al., 2011). Consequently, soil loss from erosion, acidification and low organic matter stock have been amplified by deforestation leaving most soil uncovered (Singh and Breman, 2008). In these areas, farmers exploit multiple field plots of smaller sizes scattered along landscape which may have different land management history consequently leading to soil fertility gradients (Tittonell et al., 2006; Tittonell et al., 2013). For majority of smallholder farmers, fertilization is allocated to preferred plots from which the main food security crop is grown and often close to the homestead (Tittonell et al., 2013). Such management decisions culminate over time in favoring of gradients of fertility between the remote and homestead farms (Vanlauwe et al., 2002b). Additionally, lack of knowledge and understanding of specific nutrient limitations in these soils are the basis of poor fertilizer management practices (Lambrecht et al., 2014).

Particularly, the majority of the farm plots are located on steep slopes making farming vulnerable to landslides caused by heavy rainfall (Bagalwa, 2010; Heri-Kazi and Bielders, 2020). To date, a soil survey work in South-Kivu has been limited to only a few areas i.e. Kabamba and Burhale (Beart et al., 2013; CIALCA, 2014; Woomer et al., 2014), leaving larger parts of cultivated lands unknown from their soil properties. Only very limited information is available on the soil fertility status that could play a key role in planning land use management decisions (Baert al., 2013). Therefore, more frequent surveys will increase precision on agricultural interventions in South-Kivu region. However, sustainable land management remains a challenge for most of smallholder farmers that prioritize cassava as staple crops for their food security (Munyahali et al., 2017; Kintché et al., 2017). However, this crop is cultivated continuously on the same land year in year out ignoring management for soil fertility replenishment. Despite promising result from improved varieties, a number of studies reported declining level of crop productivity that pose serious food security concerns for the region (Pypers, et al., 2011; Ouma et al., 2011). Land degradation does not only negatively impact the future of smallholder farmers, but also economic growth of agricultural sector as a whole. Thus, soil fertility depletion in smallholder farms will continue to be the major biophysical root cause of reduced food production if farmers do not implement best agricultural practices (Vlek et al., 2008; Giller, 2020).

1.3 Socioeconomic and biophysical factors affecting soil fertility

This doctoral study will discuss agroecology, farm typology and market access as socio-economic and biophysical factors that contribute to degradation and low soil fertility variability in smallholder farming systems.

1.3.1 Agro-ecology define by environmental conditions

Smallholder farmers in SSA are still highly dependent on rain-fed agriculture making this sector interconnected between small farmers' productivity and the state of food security. The large variability in soil fertility conditions is a result of variation in biophysical factors that characterize smallholder farming systems and have profound effects on crop productivity. Tackling the fundamental question of local adaptation into agricultural interventions requires understanding of the complex processes occurring in soil. In particular, for smallholder farmers of the tropics, site-specific information require consideration to alleviate soil fertility gradients dependent on agro-ecological conditions (Masvaya et al., 2010; Diarisso et al., 2016). Despite variation of soil fertility due to farmers' management practices, soil fertility may also vary as result of inherent conditions of the landscape. As known for agro-ecology to be mainly influenced on one hand by climate (i.e., altitude, elevation, rainfall and temperature) that features seasonality during cropping seasons (Sebastian, 2009). On the other hand, biophysical factors such as geological characteristic of parent materials and catena position that are inherent contributing to soil fertility variability that have been observed across farms (Bennett, 1980; Erens et al., 2014).

As known so far, soils are in continuous development process exposed to a series of weathering conditions. In the case of South-Kivu, Eastern DRC soils originate from basalt rocks from which one portion has been rejuvenated by recent volcanism activities (Beernaert, 1999), contributing to differences in both soil type and texture. The province is located at minimum 1000 m above sea level and is dominated by Albertine Rift Mountains. According to the Köppen climate classification, the region faces more of tropical monsoon climate (Chen and Chen, 2013). Although the climate still remains more diverse inside the region as influenced by wind speed and rainfall pattern from lake Kivu and Kahuzi Biega forest. Moreover, South-Kivu is characterized by a bimodal pattern of two rainy seasons, long (September to January) and short (from March to June) rain seasons. The average rainfall amount falls between 1600- 2500 mm annually and monthly average temperatures of 21-23ºC (Hijmans et al., 2005). However, in the last years, climate variability has been observed due to high variability of the rainy seasons and increasing drought events throughout the rainy season. The province has diverse agro-ecological zones including Mountainious savannah in Kabare and Walungu territories (Munyuli et al., 2017). The dominant soil types are mainly ferralsols and nitisols (IUSS Working Group WRB, 2014). Landscape is also dominated by steep topography from hillsides often cultivated with no soil erosion precaution measures and characterized by landslides during rainy seasons (Karamage et al., 2016; Ocimati et al., 2020).

The local farming systems is suitable for a wide range of food crops cultivated in stallholder farmers with economic, social and nutritional importance varying from one agro-ecological zone to another. Generally, roots and tubers (cassava, sweetpatato and yams), fruits (banana), cereals (maize, sorghum, and rice), and grain legumes (common bean and soybean) are the most important food crops. As key principle of agro-ecology, cropping diversification of farming through practices such as, crop rotation, intercropping, agroforestry and livestock integration have been recently introduced to farmers (Reyes et al., 2010; Vanlauwe et al., 2012). In particular, the highland growing environments of Bushumba is considered as the growing baskets of Bukavu city. This site has large potential for agricultural diversification due to favorable soil and climatic conditions found in place. Agricultural productivity is of greater profit as there have been attempts to invest in erosion control measures in the past to limit nutrient losses (CIALCA, 2014; Bagalwa et al., 2016). The midland of Mushinga site with lower rainfall amount throughout the rainy seasons have been facing high degree of soil degradation, the main causes of lower agricultural productivity. This has led to increasing off farm activities such small mining business to rise household income. However, these agroecosystems are still limited to a larger extent with capacity of farmers to adopt the use of mineral fertilizers and pest control technologies.

In Ethiopia, however, the agro-ecology is more diverse offering varieties of farming systems. From highlands to low-land, production systems are diversified and cope with temperatures, annual rainfall and soil types. In these areas, agro-climatic conditions offer to farmers an array of decisions and agronomic capacity to invest in management practices. In highlands, for example farmers are more market oriented, this leads to predominance in investing on chemical fertilizers. While the lowlands farms known for their larger size of lands and large number of livestock are in a better position of investing in ISFM (e.g. chemical fertilizers and organic manure application). While in semi-arid and arid conditions predominate, crop livestock based systems are prevalent, such as the mixed barley, tree crop farming systems. In highland and lowland systems, rainfall induced crop failure is less of a concern than in arid and semi-arid areas (Haileslassie et al., 2005).

1.3.2 Farm typology

In smallholder farms, typology or resource endowment has proven to be a useful tool to agricultural technologies between farmers' classes of different production capacities (Vanclay, 2005; Kumar et al., 2019). Indeed, it assists farmers and researchers in understanding the wide diversity among smallholder farms with focus on targeting of crop production intensification strategies. But also provide understanding on how such strategies may be affected by resource allocation (Tittonell et al., 2005b; Zingore et al., 2007). This tool categorizes farmers into groups according to their main production objectives, orientation and resource constraints (Chikowo et al., 2014). Different approaches including using wealth have been used so far by researchers in generating farm typology classes (Tittonell et al., 2005b; Zingore et al., 2007). Information in regard to farm management practices coupled with household assets provide an indication on understanding the wide diversity of smallholder farmers (Soule, 2001; Tittonell et al., 2005b).

In order to improve farm productivy while reducing the effect of soil variability, a variety of nutrient management strategies have to be developed with focus on farm-specific conditions rather than blanket recommendations across diverse farmers. In the case study of South-Kivu, Eastern DRC, wealthy farmers often own large areas of lands, and to smaller extent livestock that provide them with animal manures entirely dedicated for fertilization (Maass et al., 2012; Ndjadi et al., 2020). On the contrary, poor farmers often have limited land size, with less inputs and labor allocation as the case of Western Kenya in east Africa (Tittonell et al., 2005a; Achard and Banoin, 2003).

In Ethiopia, however, higher application rate of mineral fertilizer and large number of cattle characterize wealthy farmers, while poor farmers have smaller land size and less livestock in addition to the lower rate in mineral fertilizer application (Haileslassie et al., 2005). Besides, previous studies demonstrated large differences in nutrient management between farms, which were linked to differences in resource endowment. Subsequently, these differences become source of soil fertility variability leading to differences in farm productivity and nutrient balances (Zingore et al., 2007).

1.3.3 Market access

Socio-economic constraints including market access and road infrastructure have an effect on the agricultural sector in SSA (Ulimwengu and Funes, 2009; Birachi et al., 2013). For example, many rural producers are facing difficulties in accessing marketplace (Markelova et al., 2009; Jagwe et al., 2010). Farmers' households are heavily dependent on the local markets for selling agricultural products that generate income to the household and meet food need. These agricultural products are subjected to price shocks due to vulnerability of market conditions source of uncertainty in production chain (Dowiya et al., 2009; Jangwe et al., 2010). This situation is aggravated by weakness of fertilizer market, lack of policy and institution to support smallholder farmers in planning agricultural activities. There are also constrains related to poor infrastructure such as transportation systems that affect farmers (Ulimwengu and Funes, 2009; Birachi et al., 2013).

Market access in terms of travelling time varies within region which affect access to mineral fertilizer inputs. This phenomenon is revealed in differences in land productivity between farmers' fields of the remote areas in comparison to those near market centers. Nevertheless, there is a considerable number of smallholder farmers living in remote areas with poor infrastructure, their agricultural produce are often subjected to high transaction costs that significantly reduce their incentives for market participation (Barrett, 2008; Ouma et al., 2010). Increasing farm income stimulate adoption of agricultural inputs, particularly among poor resource farmers (Place et al., 2003b; Mugwe et al., 2009; Hannessy et al., 2019).

1.3.4 Farmers Indigenous knowledge

Farmers indigenous knowledge is an important social asset of soil fertility evaluation in smallholder farmers' communities of SSA (Corbeels et al., 2000). It is used in decisions making, especially to prioritize management strategies based on farmers' perception. The procedure has been recognized to help in understanding soils fertility status (Isaac et al., 2009). However, farmers' understanding may be strengthened with soil laboratory analysis as results of quantification of available nutrients. Often, farmers are aware of their soil fertility status as this is reflected by crop yield performance (Saïdou et al., 2004; Stewart et al., 2020). However, achievement of acceptable soil fertility management can be complex as many factors that determine the extent to which a farmer will invest in fertility need to be continually determined. Such complexity calls for active participation of farmers in designing researcher questions to further generate adapted solutions.

On one hand farmers have better view of the environment production in which cropping systems are being implemented. While on the contrary, researchers understand the fundamental of processes guiding land productivity. Also, to fully exploit production systems local adaptation need to be tested across production scales as new paradigm to support ISFM (Vanlauwe et al., 2015). First, farmers may have very different perceptions of what makes soil "good" compared to researchers. Furthermore, it is an additional knowledge in relation to soil fertility (Gray and Morant, 2003). Hence, the need for designing a participatory research approach that considers farmers' indigenous knowledge in generating agricultural technologies (Desbiez et al., 2004; Osunade, 1992).

In the past, researchers failed to encounter farmers' knowledge that could help in addressing soil fertility related issues (Gowing and Payton, 2004). Today, there is a window for increasing farmers' participation in identifying limitation of soil fertility in smallholder farmers. As known so far, for the past decade farmers have developed knowledge backup about their soils, which is based on what they are able to visualize (Munyuli et al., 2017; Mulimbi et al., 2019). Local indicators such soil color, soil depth, soil texture and yield and crop performances are usually used to characterize plots as either fertile or infertile.

1.4 Nitrogen management in cropping systems for soil fertility improvement

Nitrogen is a critical element determining significantly the performance of crops and yields (Fegeria and Baligar, 2005). This element is known to be essential in providing energy for living organism supporting biological processes in soils (Rütting et al., 2018; Sena et al., 2020). However, land degradation in smallholder farmers have negatively affected this biological processes resulting in nutrient losses (Hengl et al., 2015). It should be noted that N flows and budget play a critical role in agricultural production and may lead to their depletion or accumulation (Kiboi et al., 2019). In addition, majority of smallholder farmers do not adequately replenish N from their fields due to socio-economic constraints (Mafongoya et al., 1997).

Agriculture requires NH_4^+- N and NO_3^-- N in large amount to sustain crop growth (Gao et al., 2020). However, $NO3^-$- N is mobile and can be subjected to losses through leaching particularly during rainy period (Nyamangara et al., 2003; Kotlar et al., 2020). Naturally, leguminous plants have the capacity of fixing atmospheric N through symbiotic process, which allow N addition stocks in the soil environment. By integrating leguminous plants into the local cropping systems, one can contribute to supply N for the subsequent crops as well contribute in maintaining this nutrient in the soil (Dakora and Keya, 1997; Ojiem et al., 2006; Rusinamhodzi, 2020).

In crop nutrition, nitrification is an important pathway for NO_3^--N in supporting plant growth (Galloway et al., 2008). However, due to its high mobility, losses must be minimized in order to improve N use efficiency (Subbarao et al., 2012). In agricultural systems, N from manure, fertilizers, fixation and crop residues are the main sources of this element supplying the soil. However, when added as a single input, this can result in disequilibrium among other soil nutrients and my limit crop growth (Sinclair and Park, 1993; Shen et al., 2019). It is also documented for phosphorus (P) being a second most limiting nutrient often affecting crop growth. When fertilizer is applied to soil, cereal crop takes about 1 unit of P for every 5 unit of N, this variation in nutrient uptake may be accumulated and result in different concertration of N:P ratio in the soil that tend to widen with time in different ecosystems pools (Shen et al., 2019). In tropical agroecosystems, mixing leguminous plants to cereal and tubers may provide additional N but not P element. Hence, there is the need to supplement P to overcome such limitation.

For example, 80% of farms in South Kivu, Eastern DRC are depleted of N (<0.2% N in soil) (Lunze et al., 2012; Pypers et al., 2011). The region has a potential of legume based cropping systems, farmers mix common bean and other leguminous crops with cassava. Agriculture is mainly of subsistence production (CIALCA, 2007). A low fertilization had been observed in the region due to socio-economic limitation that face most farmers (Kumar and Goh, 2003; Yadvinder-Singh et al., 2004; Crews and Peoples, 2005). Even the available ones are of low quality and often result into immobilization of N by soil microbes, leaching and loss through other anthropogenic pathways (Robertson and Groffman, 2006; De Vries et al., 2011).

Applying slower release organic resources and nitrification inhibitors may improve N uptake as well as minimize losses (Shaviv and Mikkelsen, 1993; Ashraf et al., 2019). Incorporation of *Calliandra calothyrsus* leguminous residues has shown to increase availability of N in soils (Zingore et al., 2003). However, the rate and timing of application are critical in determining N availability, hence the degree of uptake and demand to minimize losses (Stanford, 1973; Fageria and Baligar, 2005; Johnston and Bruulsema, 2014). Furthermore, continuous application of organic resource improves the soil organic matter (SOM) and hence N stock of the soil (Giller et al., 2006; Puttaso et al., 2013; Kunlanit et al., 2014). High N agronomic use efficiency is achieved when organic residue are combined with mineral fertilizers in comparison to sole application of organic or mineral fertilizers (Vanlauwe et al., 2011; Chivenge et al., 2011). At this stage, moisture management will be critical in regulation of microbial activities responsible for decomposition. Reduced moisture content may negatively affect N availability, while excess moisture may result to N loss through leaching and denitrification (Fageria and Baligar, 2005; Musyoka et al., 2019). Residue input management applied to the field allows efficient use of organic resources, improves soil structure, that reduce soil erosion and supplying nutrients to crop and living organisms, while building up SOM stock.

1.5 Options for soil fertility management

Maximizing the use efficiency of all inputs at the farm level is one of the underlying principles of the integrated soil fertility management (ISFM). This approach has rapidly become more adopted by development and extension programs in SSA (Vanlauwe et al., 2010). It is expanding farmers' knowledge on different management combination or substitution (Place et al., 2003). Improving soil fertility is key in increasing agricultural productivity in smallholder farmers where fertilizers application is still below the recommended average dose (Vanlauwe et al., 2010). In the past, traditional agricultural practices that included fallow periods and shifting cultivation allowed natural regeneration of fertility (Nandwa and Bekunda, 1998; Tonitto and Ricker-Gilbert, 2016). To date, with increasing population pressure fallow periods have been reduced and no more of shifting cultivation (Carswell, 2002; Josephson et al., 2014). This situation has led to decrease in soil fertility and degradation of natural resources, putting smallholder farmers at high risk of famine (Sanchez and Leakey, 1997; Lal, 2009; Bado and Bationo, 2018).

Research is offering a set of options from physical, chemical to biological solutions to overcome fertility depletion (Vlek, 1990; Smith et al., 1997; Vanlauwe et al., 2014). Different technologies (e.g. crop rotation, mulching, push pull, legume-cereal intercropping) have been developed and tested for this purpose (Smith et al., 1997; Ito et al., 2007; Snapp et al., 2019). However, the choice to invest in a single or combination of technologies results in significant trade-offs with other activities within or beyond the farm boundaries (de Wit et al., 1995; Giller et al., 2011; Valbuena et al., 2015). Eventually, optimizing soil fertility management is highly complex in regions where lack of policy and institutional support are weak (Izac, 1997; Sanginga and Woomer, 2009; Bationo and Waswa, 2011). Improving soil nutrient management is crucial in raising farm productivity (Vanlauwe and Giller, 2006; Bationo et al., 2011; Stewart et al., 2020). Moreover, in SSA the degree of fertility depletion is of such high extent that even application of mineral fertilizer alone may not meet the crop demand (Chianu et al., 2012; Mugizi and Matsumoto, 2020). Therefore, there is need to emphasize in solutions that build up SOM, sink of nutrients and foundation that will maximize benefit to other ecosystem services.

1.5.1 Biochemical quality as indicator of organic residue decomposition

The release of plant available nutrients from organic inputs through decomposition and mineralization processes is performed by various soil microorganism species (Kemmitt et al., 2008; Jacoby et al., 2017; Liu et al., 2020). Biochemical quality of organic inputs is composed of complex material such cellulose, lignin and polyphenols that determine the extent to which plant residue may get decomposed (Palm et al., 2001; Rasche et al., 2014). Additional to C/N ratio that commonly correlate with mineralized C and N (Nicolardot et al., 2001; Jensen et al., 2005). Furthermore, it has been found that N release from decomposing plant materials was mostly affected by initial concentration of lignin and soluble polyphenols (Constantinides and Fownes, 1994).

Although, the amount of residue required to be applied per unit of soil is still under debate (Giller et al., 2011; von Arb et al., 2020), there is evidence that the rate of mineralization is determined by both chemical and physical nature of plant residue material (Trinsoutrot et al., 2000; Marzi et al., 2020). High concentrations of N and low concentrations of lignin and polyphenols are parameters contributing to decomposition and release of N (Mafongoya et al., 1998; Seneviratne, 2000). Plant material of C/N ratios less than 20 are considered desirable for utilization (Taylor et al., 1989; Palm et al., 2001; Zhou et al., 2019). However, in recent years there has been debate on what should be a better indicator for residue decomposability. Lignin/N ratio has been shown as a good predictor of N release; the higher the lignin/N ratio, the slower the decomposition and N release (Baijukya et al., 2006; Talbot et al., 2012; Rahman et al., 2013). Later on, polyphenol/N ratio was included in calculation of pant residue quality index (Tian et al., 1995; Kumar and Goh, 2003). It should be noted that N concentration in the litter material is an indicator of both N mineralization and immobilization (Constantinides and Fownes, 1994; Walela et al., 2014).

Besides, agro-ecosystem characteristics that also affect both N concertration and soluble material result in changing patterns of nutrient release (Palm and Sanchez, 1991; Handayanto et al., 1994).

A review on biochemical quality has been discussed in Decision Support System (DSS) developed for organic residue management (Palm et al., 2001). This decision tree categorizes organic residues inputs into four quality classes based on N, lignin and polyphenol contents, in additional it provided recommendations on whether organic resources should be combined with mineral fertilizer or not. Where, high quality residues (Class I) have high N, low lignin and low polyphenol contents (>2.5% N; <1% lignin; <4% polyphenols). Medium quality residues have high N, high lignin and high polyphenol contents (>2.4% N; >15% lignin; >4% polyphenol) as class II, or low N and low lignin contents (<2.5% N; <15% lignin) as class III. Low quality residues (class IV) have low N and high lignin contents (<2.5% N; >15% lignin).

1.6 Microbial transformation of organic nitrogen

When applying organic residues, proteins and peptides get decomposed into amino acids and NH_4^+-N (Jones et al., 2004; Rousk and Jones, 2010; Hill et al., 2012). This decomposition is performed by extra cellular proteases enzymes secreted by various soil microorganisms including *Pseudomonas* sp., *Bacillus* sp., *Proteus* sp., *Clostridium* sp (Vranova et al., 2013; Singh et al., 2019). These group of enzymes are alkaline and neutral metalloproteases, serine, leucine and alanine amino peptidases (Sharma et al., 2017; Razzaq et al., 2019). The amino acid can be directly utilized by microorganisms as substrate, and also in some limited cases by plants (Owen and Jones, 2001; Henry and Jefferies, 2003). Activities of enzymes are known to be indicators of soil biological processes, responsible of organic matter degradation, mineralization and nutrient cycling (Dick et al., 2000; Marx et al., 2001; Schloter et al., 2018). Enzymes activities are controlling the rate to which organic substrates become available for both microorganisms and plants (Kandeler et al., 1996; Xu et al., 2018; Noll et al., 2019).

Amino acids released during proteolysis are further converted by soil organisms under aerobic conditions into various forms of N including N-NH_4^+ that can be utilized by plants (Ladd and Jackson, 1982; Fuka et al., 2007; Vranova et al., 2013; Rasche et al., 2014). This process is normally followed by nitrification where ammonia gets converted to nitrate, two steps are involved; the oxidation of ammonium ion (NH_4^+) into ammonia nitrites (NO_2^-) mainly performed by *Nitrosomonas* and oxidation of nitrites into nitrates (NO_3^-) through *Nitrobactor* (Hayatsu et al., 2008; Norton, 2015; Wang et al., 2017). Prokaryotic organisms nitrifying bacteria and archaea (AOB, AOA) are dominant groups involved in oxidation of ammonia and nitrite in terrestrial ecosystems, playing a vital role in break down of organic molecules involved in C and N cycles. In recent years, the development of primers has facilitated targeting genes specifically responsible for ammonia monooxygenase (*amoA* gene) (Ratthauwe et al., 1997; Hornek et al., 2006).

1.7 Justification of the study

Tropical agroecosystems are subjected to degradation processes such as loss of C and other soil nutrient depletion that occurr rapidly resulting in a reduction of soil fertility. These challenges are reinforced by the complexity of socio-economic and biophysical factors source of fertility heterogeneity that face agricultural systems (Stoorvogel and Smaling, 1990; Tittonell et al., 2007). The observed spatial variability is the result from inherent soil conditions and land management practices history (Tittonell et al., 2005b), that will require specific response in nutrient requirement (Vanlauwe et al., 2006). Consequently, blanket fertilizer recommendations is no longer suited

(Snapp et al., 2003; Wortmann and Sones, 2017). Therefore, understanding the main drivers of farm heterogeneity will help in designing suitable soil fertility management solutions. Past soil fertility improvement efforts have often focused on inorganic fertilizer use as the primary mechanism for improving soil fertility and improving crop yields (Jayne and Rashid, 2013), ignoring the contribution of organic inputs in sustaining the efficiency of nutrient use that improve the overall negative nutrient balance (Vanlauwe et al., 2002; Chivenge et al., 2010).

Thus, ‘best bet’ and ‘best fit’ technologies that target farm-specific conditions may be appropriate to improve soil fertility. However, management decisions often depend on farmer’s resource endowments and production objective. Still little is known about how farmers’ wealth would affect soil fertility status. As farm typologies may offer opportunities in understanding the wide diversity among farms, this PhD study has investigated spatial fertility variability that exists in smallholder farmers in order to determine fertility status, as a basis to formulate appropriate recommendations. Knowing that to some extent resource endowed farmers have access to fertilizers, will result in nutrient accumulation on wealthier farms over time. While resource constrained farmers will continue mining nutrients from the soil. This situation is one reason of existing soil fertility gradients visible on smallholder farms. Zingore et al. (2007) recognizes farmers’ preference of close to homesteads fields, receiving nutrient application than those far away. This PhD study has gone beyond that, by asking whether access to market could be a source of soil fertility variability, by comparing nutrient status of remote farmers’ fields to those of nearby the market centers. This PhD study has also explored the interaction between market distance and farm typology to provide clear understanding of socio-economic impact on soil fertility variability. Recognizing variability within and among farms and across different locations is an important step in the designing of specific target technology to help poor farmers in overcoming of fertility depletion (Ruben and

Pender, 2004; Tittonell et al., 2007). Furthermore, designing a 'best fit' agricultural technologies for sustainable intensification require a clear understanding of agro-ecological characteristics. Inherent soil fertility causes by a number of factors (parent materials, soil formation processes, farm management history) has resulted in soil variability across the continent. Hence, this PhD study accounted for different sites in DR Congo as well as in Ethiopia in order to assess soil fertility status at both farm to regional level. As innovative aspect, this PhD study based the analysis on mid-infrared spectroscopy (MIRS) to successfully map fertility across large spatial scale (DR Congo and Ethiopia). This approach does not only allow prediction of quantitative soil phyco-chemical properties, but also enables the spectroscopic assessment of soil quality. Soil organic carbon (SOC) quality indicators, i.e. aliphatic and aromatic functional groups, were used to characterize soil fertility (Baes and Bloom, 1989; Shepherd and Walsh, 2002; Demyan et al., 2012). Knowing that SOC pool is an indicator for soil health, this resource has been depleted across tropical agroecosystems, resulting in reduced nutrient use efficiency (Lal et al., 2004).

Besides, previous soil fertility assessments reported the impact of population density and soil types to explain existing fertility gradient in smallholder farmers (Tittionell et al., 2005a; Tittonell et al., 2010; Chikowo et al., 2014). However, the results from these studies were not based on generic and harmonization of soil surveying procedures, able to depict comparisons between different agro-ecologies associated to local farming systems. It should be noted, however, that there have been attempts in mapping soil fertility status by the Africa soil information service (AfSIS) through harmonization of soil sampling approach across Africa (Vågen et al., 2010).

In the context of South-Kivu, Eastern DRC, and Eastern and Central Ethiopia, estimation of soil fertility reduction may be relatively difficult because of fluctuation in soil nutrients in relation to seasonality. This requires long-term observation to understand the declining process of soil fertility. Meanwhile, farmers are day to day experiencing this process as they continuously work their lands. This calls for joint effort in defining soil fertility status (Murage et al., 2000). This PhD study has attempted to reconcile farmers' indigenous knowledge with laboratory measurement in order to reveal similarities between both farmers and researchers across the study agro-ecologies as a proxy for soil fertility surveys.

Furthermore, it should be noted that for so long, researchers have strived to provide sound understanding of soil processes that underline technologies dedicated for fertility restoration. While over the years, farmers have demonstrated capacity to fit agricultural technologies into their local contexts. Therefore, linking research outputs to farmers' indigenous knowledge is more likely to facilitate knowledge transfer to support agricultural systems. Yet, farmers' indigenous knowledge across agro-ecological zones to reflect existing soil fertility variability between farmers' fields have not been considered so far. This PhD study explored such approach starting from farmers' fields expanding to regional scale in order to foster recommendations of local adaptation that require ISFM.

As one option of ISFM, organic residue amendment was tested across different soils of the study region. Leguminous crops have shown a potential niche in improving soil properties in a wide range of smallholder farming systems (Snapp et al., 2002; Kerr et al., 2007; Pretty et al., 2011; Franke et al., 2018). Not only physico-chemical properties, but and also biological activities responsible of nutrient cycling. As N is known for being the major limiting element for agriculture in tropical agroecosystems, processes underlying N transformation need to be explored. Specifically, proteolysis as the initial stage of organic residue input decomposition and nitrification that convert directly the available N form. The fundamental aspect soil microbial functioning, which contributes to the biological fertility of the soil still under-studied also our scientific knowledge of it remains incomplete. Soil microbes play a key role in organic residue input decomposition allowing the release of organically bound nutrients to the soils (Rasche and Cadisch, 2013). It is acknowledged for organic materials from seasonal legumes to release substantial amounts of N to the soil (Koga, 2017; Xiang et al., 2018). However, little is known about the contribution of perennial legumes residues as basis for enhancing soil fertility through proper organic residue management. Biochemical composition of perennial legumes having more complex structures i.e. high amount of recalcitrant (lignin and polyphenols) that protect N substrate to be easily accessed by soil microbes (Prescott, 2005). In such case the release of N and other nutrients is done gradually, allowing long-term supply to the soil. Soil microbes reflect through their activities involved in decomposition and mineralization processes for rapidly degraded organic substrates (Cadwell, 2005; Sinsabaugh et al., 2008), feeding nitrifying bacteria and archaea that are considered as the main drivers in nitrification of N (Van Kessel et al., 2015; Coskun et al., 2017). This PhD study has tested organic residue of *Calliandra calothyrsus* as a model residue of perennial legume type that offer possibility to build up long- term fertility. Because polyphenol-rich plant material decomposes slowly as result of polyphenol-N complexation.

By closing the gap, this PhD study has studied the relationship between soil nitrifying community abundance and functional activities of enzymes, to provide a clear understanding of existing links between microbial community size and function potential. The overall outcome of this PhD thesis aims to provide knowledge for planning in soil fertility management strategies to overcome constrains of low farm productivity that face smallholder farmers of tropical agroecosystems.

1.8 Hypothesis and Objectives

The following hypothesis were addressed in the framework of this dissertation:

1) The market distance was suggested as determinant of agricultural development in DRC, it was hypothesized that with increasing market distance, the soil fertility status of smallholder farming systems decreases since field plots from remote areas, irrespective of the smallholder wealth status, do not have the opportunity to benefit from improved soil fertility management. As the market distance increases, the soil fertility status of smallholder farming systems decreases despite of farmers' wealth since field plots from remote areas do not have opportunity to benefit from market accessibility.
2) Fertility status varied both in agro-ecology and farmers' resource endowments in Ethiopia. Not only individual but also inter-related effects of agro-ecological zones and farm typology affect soil fertility variability.
3) Farmers indiginous knowledge and laboratory assessment result in a similar reflection of on-farm soil fertility across agro-ecologies.
4) High quality organic residues applied to high pH soils have a positive relationship between the functional potential of proteolytic enzymatic activities and abundance of nitrifying communities. This is due to high quality (low (L+PP)/N ratio) that is easily decomposed in high pH soil.

The objectives of the dissertation were:

1) To assess the inter-related influence of market distance and resource endowment classes on soil fertility status of smallholder farming systems of South-Kivu, Eastern DRC (Chapter 2)
2) To assess the inter-related effects of agro-ecology and resource endowment on soil fertility status across crop-livestock system in central and western Ethiopia (Chapter 3)

3) To provide a clearer understanding of the functional linkage between the potential activity of selected proteolytic extracellular enzymes alanine amino peptidase (AAP), Leucine amino peptidase (LAP), Thermolysin-like proteases (TLP) and the abundance of nitrifying populations (i.e. gene copies of *amoA* gene coding ammonia monooxygenase as functional marker for AOB and AOA) in two soils of varying acidity treated with two biochemically different organic residues (Chapter 4) Thermolysin-like proteases

1.9 Review on midDRIFTS and molecular techniques relevant in this study

This section infers to document the choice and justification for specific analysis technique used in this PhD thesis. However, it is not aiming at providing detailed explanation and comparison but a rough introduction to midDRIFTS and molecular methods for studying both soil quality and soil microbial community.

1.9.1 midDRIFTS techniques to assess soil quality

Diffuse reflectance Fourier transformation mid-infrared spectroscopy (midDRIFTS) is a spectroscopic approach referring to the bending and stretching vibrations of organic and inorganic molecules found in the mid- infrared range from 4000 to 400 cm^{-1} (Nguyen et al., 1991; Reeves et al., 2006; Calderón et al., 2011; Soriano-Disla et al., 2014). Generally, the MIRS spectrum is divided in two fundamental regions of vibrations (Bornemann et al., 2010; Lehmann and Solomon, 2010; Parikh et al., 2014). The region 4000-1500 cm^{-1} that includes various bands representing vibrations of different functional groups. The fingerprint region is extended from about 1450 cm^{-1} to 400 cm^{-1} that holds a complex series of peaks (Reeves, 2012; Yang, 2014). The first vibration mode which is the fundamental group region mainly represent the stretching vibrations. While the second vibration mode which is fingerprint region refers to bending vibrations (Vohland et al., 2014; Tinti et al., 2015). Stretching vibration means a continuous change in the interatomic distance along the axis of the bond between two atoms, while bending vibration is the change in angle occurring between two bonds (Sánchez Escribano et al., 2003). Molecular vibration provides information of the structural compound influencing fingerprint region (Stuart, 2005; Demyan et al., 2012; Kunlanit et al., 2014). Vibrations of functional groups corresponding to different peaks suits for studying both composition and dynamics of SOM (Ludwig et al., 2008; Demyan et al., 2012; Calderón et al., 2013; Hansen et al., 2016).

The band from 3400-3300 cm^{-1} is dominated by N-H stretching vibration (Baes and Bloom, 1989). Aliphatic C-H stretch 2930 cm is the one found at 3000-2850 cm^{-1} corresponding to labile organic C pools into the soil (Baes and Bloom, 1989; Janik et al., 2007), and COO^- stretching (Stevenson, 1982). Aromatic C=C stretching vibrations and NH (amide II) bending vibrations belong to the band at 1520 cm^{-1} (1540-1503 cm^{-1}) (Stevenson, 1982), while the band at 1160 cm^{-1} (1172-1140 cm^{-1}) correspond to the C-OH stretching of both aliphatic and alcoholic groups (Senesi et al., 2003). In addition, mineral structures of soil particles reflected by texture and carbonate are also identified by several peaks (Calderon et al., 2013). For instance, the band of 3700-3500 cm^{-1} has two distinct peaks at 3695 cm^{-1} and 3622 cm^{-1} assigned to O-H vibration of clay minerals (Nguyen et al., 1991). Thereafter, several peaks between 2000 cm^{-1} and 1750 cm^{-1} representing non-clay mineral soil, mainly quartz in sand and silt relatively free from interference and overlapping (Nguyen et al., 1991). The peak at 2686-2460 cm^{-1}, 1850-1784 cm^{-1}, 1567-295 cm^{-1}, 889-867 cm^{-1}, 734-719 cm^{-1} and 719-708 cm^{-1} belong to carbonate vibrations (Tatzber et al., 2010; Bruckman and Wriessnig, 2013). To assess SOC stability index, the corrected area will be divided by the sum of the total peaks then multipled by 100 to give the relative peak area. The relative peak areas will be used to assess the relative changes of peak areas in relation to each other (Niemeyer et al, 1992; Demyan et al, 2012). The ratio of relative peak areas at 1620 and 2930 cm^{-1} (1620: 2930), 1530 and 2930 cm^{-1} (1530:2930) and at 1159 and 2930 cm^{-1} (1159:2930) with different hypothesized stabilities will evaluate the distribution of C among assessed factors.

The advantage of midDRIFTS technique is that it requires minimal soil preparation (Nguyen et al., 1991). Soil analysis process starts with water absorption and interference are reduced and resolution of spectrum improved. Then radiation is emanated into the sample surface followed by absorption, refraction, scattering over the sample surface. Bands appeared based on nonlinear scaling of intensity where magnitude of strongly spectral bands will reduce intensity in comparison to low bands and directly resolution of weaker bands will improve in midDRIFTS spectrum (Nguyen et al., 1991). This process is followed by the dilution of potassium bromide (KBr) that is added to the samples in order to avoid soil distortion (Baes and Bloom, 1989; McCarty et al., 2002). After this laboratory measurement, spectroscopic analysis for soil properties predictions is required. Analysis of spectra will need to develop calibration models using appropriate statistical approaches. For that, partial least squares regression (PLSR), a multivariate calibration procedure (Vohland et al., 2011; Rasche et al., 2013) was preferred in this study over multiple linear-regression (MLR) and principal component regression (PCR) as the former is powerful in reducing noise from the data and is able to better handle multi-collinearity (Janik et al., 2007; Janik et al., 2009; Vohland et al., 2011; Nocita et al., 2014). In addition, PLSR is known for reducing the spectral data into a lower dimensional subspace formed by a set of orthogonal latent variables that construct predictive regression models of measured soil properties (Wold et al., 1989; Nocita et al., 2014). For this, there is no need to isolate specific spectral peak before performing PLSR as it is powered of calibrated model even by small spectral variations to be related to the investigated soil properties (Haaland and Thomas, 1988; Tatzber et al., 2010).

However, midDRIFTS-PLSR prediction models need to meet appropriate calibration approaches i.e. independent validation and cross-validation (Demyan et al., 2012; Mirzaeitalarposhti et al., 2015). For independent calibration/validation the spectral dataset should be divided into two separate subsets data, one for model calibration and another data validation (Debaene et al., 2014; Ramirez-Lopez et al., 2014; Mirzaetalarposhi et al., 2015). While in the cross- validation approach named in some cases leave-one out cross-validation, the unique dataset is used for model calibration and validation (Demyan et al., 2012; Mirzaeitalarposhti et al., 2015). Application of midDRIFTS technique is considered as an advanced method that provide an accurate dataset for large scale mapping of variety of soil properties such as TC, OC, particle size, total sulfur, extractable Mn and exchangeable cations (Janik.et al., 1998; McCarty et al., 2002; Shepherd and Walsh, 2007), including soil microbiological population sizes (Rasche et al., 2013). This method is undergoing exponential growth due to it convenience, quickness and relatively low cost (Bellon-Maurel and McBratney, 2011; Mirzaeitalarposhti et al., 2016).

1.9.2 Review of molecular approach implemented in this study

1.9.2.1 Measurement of microbial abundance

Previously, soil microbial studies were based on culturing media techniques to explore microbial diversity in soil (Wolf et al., 1989; Gallego et al., 2001; Hugenholtz, 2002). This technique is outdated due high range of limitation in relation to time consuming and a narrow power of microbial size estimation (Nannipieri et al., 2003; Nihorimbere et al., 2011; Pham and Kim, 2012). To overcome this, techniques such as the analysis of phospholipid fatty acids (PLFA) and community-level physiological profiles were developed with attempt to increase our understanding on soil microbial diversity, activity and functions as able to access and estimate a greater proportion of the soil microbial community (Garland, 1997; Hill et al., 2000; Fierer et al., 2003).

Discovering molecular techniques has revolutionized understanding of soil ecology, it allowed researchers to open the so-called black box of microbial life in soil. Today application of quantitative polymerase chain reaction (qPCR) in combination with the extraction of nucleic acids (DNA and RNA) has been widely recognized as an advanced tool for quantification of soil microbial population (Smith et al., 2006; Deepak et al., 2007). The technique is known for characterizing DNA and RNA from soil organisms. It is based on multiple amplification cycles in which template from both DNA and RNA generates a mixture of microbial genes signatures present in a sample through denaturation referring to real time PCR (Wilhelm and Pingoud, 2003). This step is followed by annealing of two oligonucleotide primers targeting specific sequences and subsequent extension of a complementary standard from each annealing primer by a thermostable DNA polymerase, resulting in an exponential increase in amplicon numbers during PCR (Jarman et al., 2004; Smith and Osborn, 2009). As one of high feature of this technique, the increase in amplicon numbers is recorded in real time during the PCR via SYBR Green I working as detection fluorescent reporter indicating amplicon accumulation during every cycle (Filion et al., 2003; Bustin, 2005; Smith and Osborn, 2009). Primers used for this study and their number of cycles are reported in Table 1.

Table 1. 1 Description of primer sets, PCR ingredients and amplification details used for quantitative PCR.

Target group	Primer set	Therminal cycling profile	References
Bacterial *amoA* gene	amoA-1f	45 cycles	Rotthauwe et al. (1997)
	amoA-2r	95 C 45 s,57 C 60 s, 72 C 60 s	Rotthauwe et al. (1997)
Archaeal *amoA* gene	Arch-amoAF	45 cycles	Francis et al. (2005)
	Arch-amoAR	95 C 45 s,53 C 60 s, 72 C 60 s	Francis et al. (2005)

During this process, SYBR Green I is used as an intercalation dye since it is known to be economical for real-time analysis (Vitzthum et al., 1999; Giglio, 2003; Dragan et al., 2012). When bound to DNA, a fluorescent signal is emitted following light excitation (Zipper et al., 2003; Morozkin et al., 2003; Xiang et al., 2014). However, in its unbound state, SYBR Green I does not fluorescent (Rengarajan et al., 2002; Bourzac et al., 2003; Smith and Osborn, 2009). This step is followed by melting curve analysis known as post PCR dissolution carried out to confirm whether the fluorescence signal is generated only from a target template and not from the formation of nonspecific PCR products (Varga and James, 2005; González-Escalona et al., 2006).

For the quantification of the unknown samples, qPCR amplification from a range of serial dilutions of known concentration of template DNA is used to construct standard curves (Lee et al., 1996; Jansson and Leser, 1996; Smith and Osborn, 2009). Moreover, quantification data generated maybe used to relate gene abundance (in terms of transcript numbers) in comparison with various abiotic or biotic factors and or biological activities and process rates (Sharma et al., 2007; Smith and Osborn, 2009; Rasche and Cadisch, 2013). However, care is needed at all steps to avoid bias as it is difficult to assess abundance of the full microbial community (Feinstein et al., 2009; Lombard et al., 2011; Philippot et al., 2012). Complimenting this techniques with other approaches to achieve a more holistic understanding of microbial functions is thus necessary (Pontes et al., 2007; Adhikari and Kallmeyer, 2010).

1.9.2.2 Method to assess soil enzymatic activities

Enzymes are specialized proteins that combine specific substrate and made with catalytic properties acting in biochemical reactions (Acosta-Martínez and Tabatabai, 2000; Kandeler, 2007). In agroecosystems, extracellular enzymes are associated with proliferating cells bounding to humic colloids and clay minerals. These enzymes have been known as indicators of soil biological processes in organic matter decomposition mineralization and play a major role in recycling of soil nutrients (Marx et al., 2001; Guggenberger, 2005; Das and Varma, 2010). Their activities are essential for energy transformation for nutrient cycling and act as sensors, since they contain information from both microbial status and physico-chemical conditions (Aon and Colaneri, 2001; Marx et al., 2005). Measurement of enzyme activities in soils has been reported to evaluate specific functions in soils (Nannipieri et al., 2012; Talbot et al., 2015).

Most of the enzymes measured are extracellular, intracellular, bound and stabilized enzymes within microhabitat (Sinsabaugh, 1994; Kandeler, 2007; Sakurai et al., 2007). Extracellular enzymes or exoenzymes have been refered to enzymes secreted and performed functions outside the cell (Skujiņscaron; and Burns, 1976; Ai et al., 2012; Stone et al., 2014). These types of enzymes are produced by both prokaryotes and eukaryotes cells and have been shown to be of central importance in many biological processes (Arnosti, 2011; Bach and Munch, 2000; Dash et al., 2013; Vranova et al., 2013). Metabolic reactions of living cells are catalyzed by extracellular enzymes taking place in soil providing a functional component to the molecular techniques (German et al., 2011; Nannipieri et al., 2012).

The principle in measuring activities of extracellular enzymes is based on enzyme reaction with specific substrates (e.g., 4-methylumbellifereryl MUF), following the conversion of product by different methods such as colorimetric, radio-labelled and fluorimetric methods (Marx et al., 2001; Vranova et al., 2013). The fluorimetric method used in this study is the most developed approach so far that study soil enzyme activities (Dick et al., 2013; Deng et al., 2013; Deng et al., 2011). This method is based on utilization of fluorimetrically-labelled substrates. Its main advantage is that the reaction product can be measured directly from the microplate reader without prior extraction and purification of the product unlike many enzyme assays (Marx et al., 2001; Niemi and Vepsäläinen, 2005; Dick et al., 2013). Therefore, the approach saves time and while allowing measurement of a large number of soils and substrates through a small amount of soil sample (Sinsabaugh et al., 2000; Marx et al., 2001; Pritsch et al., 2004; Deng et al., 2011). In addition, due to its high sensitivity it easily allows simultaneous measurement of small quantities of hydrolyzed substrates (Colemna et al., 1976; Wang et al., 2020). Furthermore, a microplate reader is able to measure absorbance or fluorescence of samples in 96 wells which allows to reduce reagent cost that could be much higher if using conventional bench-scale assays. Detailed information on enzymatic analysis carried out in this study is presented in Chapter 4 where it was used to assess the effects of soil pH and residue quality on proteolytic potential enzyme activities from an incubation experiment.

1.10. Outline of the thesis

This PhD thesis was compiled as a cumulative thesis containing three papers, one published (Chapter 2) and two (Chapters 3 and 4) submitted. The thesis contains a general introduction (Chapter 1) concerning socio-economic and biophysical factors affecting the soil fertility status in smallholder farms in tropical environments. It also presents the relevance of soil microbial functions with link to organic residue management as an option for site-adapted soil fertility improvement. Furthermore, the introduction summarizes previous research that attempted to map the soil fertility status in smallholder farmers. As soil is a highly complex system which is influenced by several factors, the manifold sources of soil fertility variability need to be studied across regional scales, including effects on microbially mediated nitrogen (N) cycle (proteolysis and nitrification). Chapter 2 aims at assessing the interrelated effect of market distance and farm typology and site-specific effects in South- Kivu, Eastern DR Congo. It further verifies farmers' indigenous knowledge against lab-based soil physico-chemical assessment on soil fertility status. In Chapter 3, the regional soil fertility assessment has been extended to further understand the effect of agro-ecology and farm typology on soil fertility in crop-livestock systems of Central and Eastern Ethiopia. To further understand the effect of environmental and management factors on soil ecological functioning as key feature of soil fertility, Chapter 4 focused on the validation that potential proteolytic enzyme activities modulate archaeal and bacterial nitrifier abundance in soils differing in acidity and organic residue treatment. The PhD thesis closes with a general discussion (Chapter 5), highlighting outcomes and limitations of this dissertation as well as suggesting future research directions.

CHAPTER 2

Market access and resource endowment define the soil fertility status of smallholder farming systems of South-Kivu, DR Congo

2. Market access and resource endowment define the soil fertility status of smallholder farming systems of South-Kivu, DR Congo

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2.1 Abstract

Integration of the inherent variability in soil fertility conditions along market and agro-ecological gradients remains a key challenge in prioritizing soil fertility management interventions for smallholder farmers in sub-Saharan Africa. To overcome this constraint, the presented study aimed at unraveling the inter-related effect of the factors “market distance”, defined as walking time, “farm typology”, defined as resource endowment, and “site”, defined as geographic location with contrasting agro-ecologies, as well as farmers’ indigenous knowledge on the soil fertility variability in smallholder farming systems in two distinct regions (i.e. Bushumba versus Mushinga) of South-Kivu, Eastern DR Congo. A total of 384 soil samples were randomly selected from representative farmers’ fields and analyzed for soil pH, soil organic carbon (SOC) content and quality, as well as macro-and micro-nutrient contents. To allow an efficient processing of the large sample number, midDRIFTS (mid-infrared diffuse reflectance Fourier transform spectroscopy) coupled to partial least squares regression (PLSR) prediction was employed. MidDRIFTS was also used to calculate SOC stability indexes as proxies of SOC quality. Results revealed that both “market distance” and “farm typology” were key determinants of soil fertility variability, both with contrasting trends in Bushumba and Mushinga. Decreasing soil fertility with increasing market distance was noted across all farm typologies. A significant influence of “farm typology” was found for exchangeable calcium and magnesium ($P<0.01$), while factor “site” resulted in a significant difference of plant available phosphorus between sites (Bushumba (8.8-11.1 mg kg^{-1}) versus Mushinga (7.0-9.6 mg kg^{-1}) ($P<0.05$)). For SOC quality indexes, factor “site” was decisive, as reflected in its interaction with “market distance” (i.e., ratio 1530:2930) ($P<0.01$). However, the effect of “market distance” became obvious in the medium wealthy and poor farms of Mushinga, where an increasing ratio of 1530:2930 with increasing market distance implied a lower SOC quality in remote fields plots. Soil depth and soil color were the most frequently used soil fertility indicators by farmers across sites. In agreement with farmers’ indigenous knowledge, soil fertility levels were higher in deep than shallow soils, which was reflected in higher nutrient stocks in deep soils receiving organic amendments. Our study identified market distance, farm typology and site as factors determining the soil fertility status, providing a vital information for soil fertility variability at special scale in smallholder farming systems of South-Kivu DR Congo.

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2.2 Introduction

In the South-Kivu region of the Democratic Republic of Congo (DRC), the rural population currently counts approximately 3.8 million people (250 inhabitants per km^2) (World Bank, 2018; Muanda et al., 2018). More than 80% of this population are smallholders relying on subsistence agriculture as main activity for generating income (Ministère du Plan RDC/DSRP, 2005). Due to the annual growth rate of the rural population of 3.3% (UNPD, 2017), the region of South-Kivu has been facing low agricultural productivity, a consequence of extraordinarily high levels of soil fertility depletion resulting from intensive cultivation without adequate nutrient replenishment (Pypers et al., 2011; Vanlauwe et al., 2017). A similar trend has been noted in many other regions of sub-Saharan Africa (SSA) (Tully et al., 2015; Tadele, 2017). As a consequence, food insecurity has become a major societal challenge putting people in South-Kivu at severe risk (Murphy et al., 2015; FAO et al., 2018). There is a central demand for intensified food production in the region, while building up and maintaining soil fertility through integrated soil fertility management (ISFM) interventions that include both organic and mineral fertilizers remains challenging (Sanginga and Woomer, 2009; Vanlauwe et al., 2010).

Inadequate infrastructure such as the bad status of roads and transportation systems affects market access, a prerequisite for agricultural development in smallholder farming systems of South-Kivu (Ulimwengu and Funes, 2009). A study in Uganda performed by Yamano and Kijima (2010) revealed positive correlations between household income and soil fertility with adequate road infrastructure. Availability and accessibility of appropriate infrastructure supported the economic development with access to cash and fertilizer inputs that enhance overall soil fertility status. It could be proposed that income of farmers is determined by market access, yet there is no knowledge on how market access (Birachi et al., 2013; Minten and Kyle, 1999; Crawford et al., 2003), especially the distance from the field plots to the market, sets the baseline for smallholder farmers to optimize soil fertility to the extent of their socio-economic capabilities and biophysical contexts. Therefore, prioritization of appropriate ISFM technologies for smallholder farmers remains challenging, as further aggravated by the huge agro-ecological variability across landscapes and the generally limited information on the soil fertility status along market gradients in Central and Eastern Africa (Rahn et al., 2018). Besides, in South-Kivu, rural communities are heterogeneous (Cox, 2012), reflected in highly variable resource endowments for individual households, a similar circumstance reported for Western Kenya (Ojiem et al., 2006; Tittonell et al., 2010) . This has resulted in a large variation in soil fertility levels between farms and even between field plots within a farm, affecting decisions of farmers regarding on-farm soil fertility investment (Tittonell et al., 2005).

There is still a considerable constraint with regard to soil fertility management prioritization as previous assessments of soil fertility in DRC (Dontsop-Nguezet et al., 2016) did not consider the integration of socio-economic and biophysical factors. Socio-economic factors including resource endowment, farmers' decision (i.e. perception), market distance and biophysical factors (e.g., agroecology, landscape heterogeneity) influence soil fertility levels of smallholder farming systems across spatial scales (Crawford et al., 2003; Tittonell and Giller, 2013; Vanlauwe et al., 2016). Assessment of interactions between socio-economic and biophysical factors is difficult since soil type heterogeneity between and within farms, which is further associated with land use and management practices, resulted in obvious soil fertility distinctions at farm level and across farms (Vanlauwe et al., 2006). Currently, both scientists and farmers collaborate intensely to develop applicable solutions through participatory research (Vanlauwe et al., 2017). However, for soil fertility management strategies, it remains vague how farmers' soil fertility assessment aligns with that of scientifically verified quantitative methods, although smallholder farmers have developed the ability to perceive heterogeneity of soil fertility across landscapes (Yeshaneh, 2015). It will be relevant to accompany such process with scientific evidence since incorrect farmers' perception of soil fertility (e.g., knowledge to distinguish fertile and less fertile soils based on local indicators such as soil depth, color or texture) may lead to inappropriate ISFM interventions (Kuria et al., 2019). Science-based approaches, on the other hand, generate a rather general understanding of soil fertility that may not display realistically the local conditions with their complex socio-economic characteristics. Indigenous knowledge of smallholder farmers could thus be a critical complement in guiding agricultural interventions to sustain farm productivity as well as provide support tools for quantitative soil fertility surveys (Dawoe et al., 2012).

To estimate soil fertility levels across spatial scales, midDRIFTS (mid-infrared diffuse reflectance Fourier transform spectroscopy) has been approved as a suitable tool to assess soil fertility variability in and among African agricultural farming systems (Vågen et al., 2006; Shepherd and Walsh, 2007; Cobo et al., 2010). Basically, midDRIFTS employs a non-destructive estimation of physico-chemical soil properties allowing the analysis of spatial variability of soil properties across agro-ecologies (McCarty et al., 2002; Shepherd and Walsh, 2014). Coupled with partial least squares regression (PLSR)-based prediction, midDRIFTS is suited to process large batches of soil samples (Cobo et al., 2010; Rasche et al., 2013). MidDRIFTS also enables the spectroscopic assessment of soil organic carbon (SOC) quality (e.g., functional groups of SOC (such as aliphatic and aromatic compounds), providing a measure of SOC stabilization in agricultural soils (Demyan et al., 2012; Mirzaeittalarposhti et al., 2015).

The first objective of this study was to assess the inter-related influence of market distance and resource endowment classes on soil fertility status of smallholder farming systems of South-Kivu as a case study for DRC. The second objective was to verify, under contrasting socio-economic and agro-ecological contexts, that farmers' indigenous knowledge is a valuable proxy to assess soil fertility status across landscapes complementing a science-based approach. As market access was suggested as a determinant of agricultural development in DRC, it was hypothesized that with increasing market distance, the soil fertility status of smallholder farming systems decreases since field plots from remote areas, irrespective of the smallholder wealth status, do not have the opportunity to benefit from improved soil fertility management. It was further hypothesized that both farmers' indigenous knowledge and a science-based approach result in a similar reflection of on-farm soil fertility across agro-ecologies.

2.3 Material and methods

2.3.1 Study site description

The soil fertility survey was conducted in the "Territoire de Kabare", "groupement" of Bushumba (Site #1, 2º 340'S and 28º 826'E, 1740 m above sea level (m.a.s.l.)), and "Territoire de Walungu", "groupement" of Mushinga (Site #2, 2º 767'S and 28º 681'E, 1604 m.a.s.l.) in South-Kivu in DRC (Fig. 1). At Bushumba, the soil fertility survey was performed in the villages of Mulengeza and Bushumba, while in Mushinga, it was conducted in Madaka and Luduha (Fig. 1). This survey strategy enabled a random distribution of sampling locations to test the effects of the main research factors "market distance", "farm typology", and "site" on the soil fertility status of assayed smallholder farms. Mushinga (1200-1800 mm annual rainfall) is characterized by a slightly drier climate than Bushumba (1500-1800 mm). Soils in Bushumba are classified as Nitisols (IUSS

Working Group WRB, 2014) and characterized by a dominant textural fraction of clay (48-69%) with 25-27% sand, and total carbon ranging from 1.6 to 5.2%, pH ($CaCl_2$) of 5.1, and total nitrogen of approximately 0.45% (Lunze et al., 2012; Muliele et al., 2015). Soils in Mushinga (Ferrasols; (IUSS Working Group WRB, 2014) are characterized by a wide variation in textural fractions of clay (17-70%), with a sand content of 20-29%, pH ($CaCl_2$) of 4.8 (S/W ratio 1:2,5), low base ECEC (6.6 $cmol_{(+)}$ kg^{-1}) and a low total carbon ranging from 1.2 to 3.0% (Pypers et al., 2011). Overall, soils in Bushumba are considered as medium fertile soils since they are developed from recent rejuvenation by volcanic ash depositions (Moeyersons et al., 2004; Baert et al., 2012). Highly weathered soils from Mushinga are characterized as less fertile with low available phosphorus and high aluminum saturation since they developed during Pleistocene eruptions (Pypers et al., 2011).

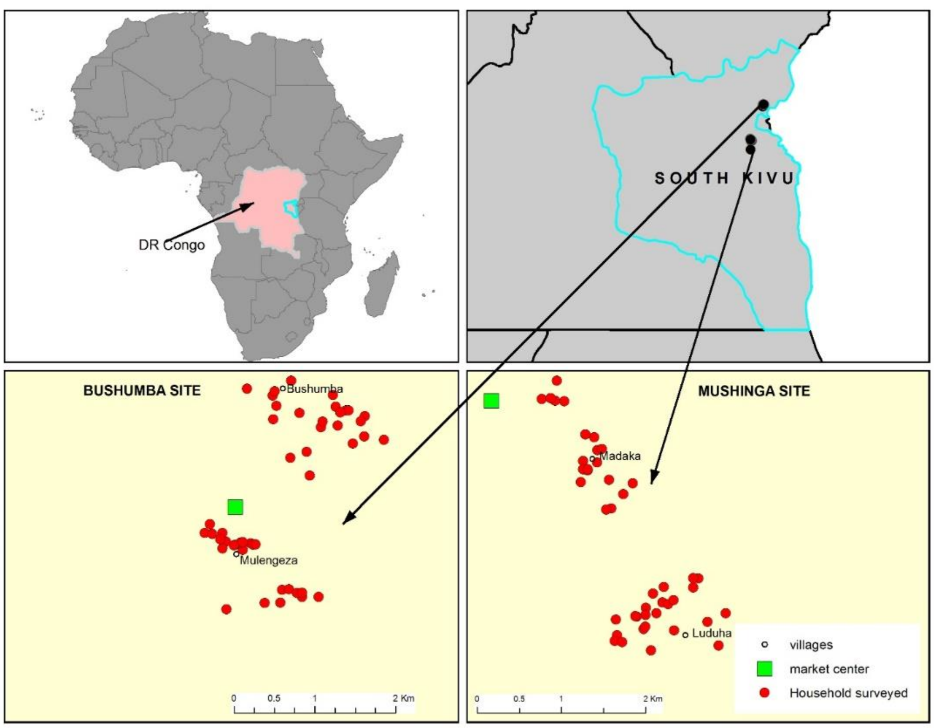

Figure 2. 1: Maps of the two study sites Bushumba (bottom left) and Mushinga (bottom right) in South-Kivu (DR Congo). Soil samples were collected on smallholder farms (red dots) in the four villages Bushumba and Mulengeza (site Bushumba) as well as Madaka and Luduha (site Mushinga) with different distances to the market centers (green squares).

2.3.2 Farm characterization

Villages and households included in this study were selected based on socio-economic indicators, such as market access and population density (Cox, 2012; Barrett, 2008). For population density, villages with more than 500 households and a population density greater than or equal to 100 inhabitants km^{-2} were considered. Walking distance from the field plots to the regional closest market was measured in minutes and ranged from 15 to 200 min. For socio-economic indicators, village meetings and focus group discussions with farmers were conducted to define farm typology classes based on resource endowment. From these discussions, total land area (ha) owned by a household was considered as the prevailing typology indicator (Tittonell et al., 2005; Rusinamhodzi et al., 2012; Chikowo et al., 2014). No additional wealth indicators such as livestock numbers and rates of mineral fertilizer application were used due to their absence or lack of use, respectively. Finally, a total of 96 households (farms) were selected randomly with regard to land holding size: (i) “wealthy” (>2 ha), (ii) “medium wealthy” (1-2 ha), and (iii) “poor” (<1 ha).

To assess farmers’ indigenous knowledge on soil fertility, household heads from selected farms were separated into male and female groups and interviewed. Briefly, focus group discussions and participatory rural appraisals were used through semi-structured interviews (Chambers, 1992). Key information on criteria and indicators used to distinguish “fertile” from “less fertile” field plots was recorded. Interviews were performed with the same farmers invited for the soil fertility survey. In total, 93 farmers were interviewed, while the remaining 3 farmers were not available.

To validate farmers' indigenous knowledge on the fertility status, each household was requested to indicate their most and less fertile field plots to allow a representative survey of soil fertility variability across each farm. Household heads were also interviewed for information regarding the most relevant soil fertility indicators (e.g., soil color, soil depth, soil texture, soil drainage).

2.3.3 Soil sampling and soil analysis

Soil samples were obtained using the Y-shaped scheme technique according to Vågen et al. (2012). The Y-frame with 12.2 meters in diameter was placed in the center of each field to avoid any edge effects and extended 5.64 meters to each sub-plot. During the sampling campaign, samples from the top layer (0-20 cm) and a deeper layer (20-50 cm) of the soils were collected in 4 sub-plots of 0.01 ha. Finally, a total of 384 geo-referenced soil samples on 96 farms for the entire study area were obtained (2 study sites × 2 villages per site × 3 farm typologies per village × 8 farms per typology × 2 plots per farm × 2 soil depths per plot). Out of 384 soil samples collected, 24 soil samples were excluded due to mislabeling during soil sample collection. Remaining soil samples (n = 360) were air-dried, passed through a 2 mm sieve, and shipped for further analysis to University of Hohenheim, Stuttgart (Germany).

Soil organic carbon (Org.C) and total soil nitrogen (TN) content were analyzed by dry combustion. Soil pH ($CaCl_2$) was determined according to Houba et al. (2000). available phosphorus (P_{av}) was measured based on Bray1 extraction (Bray and Kurtz, 1945), and plant available potassium (K_{av}) according to Schüller (1969). Moreover, exchangeable calcium (Ca_{ex}) and magnesium (Mg_{ex}) were measured for all soil samples according to Mehlich (1984).

The midDRIFTS analysis of soil samples was performed according to Rasche et al. (2013), while midDRIFTS coupled with partial least square regression (PLSR)-based prediction of soil chemical properties was done according to Mirzaeitalarposhti et al. (2015). The midDRIFTS-based soil organic carbon (SOC) stability indexes (ratios of aromatic to aliphatic functional groups (1620:2930, 1530:2930, 1159:2930)) were calculated based on the relative peak area of 4 selected spectral peaks (2930 cm^{-1} (aliphatic C-H stretching), 1620 cm^{-1} (aromatic C=C, COO^- stretching), 1530 cm^{-1} (aromatic C=C stretching), 1159 cm^{-1} (C-O bonds of poly-alcoholic and ether groups)) (Table 1) (Demyan et al., 2012). Further information on midDRIFTS-based analysis can be retrieved from the Supplementary Materials of this manuscript.

Table 2. 1: MidDRIFTS peaks representing organic functional groups considered for SOC quality analysis.

Peak name	**Integration limit [cm^{-1}]**	**Assignment of functional group**	**Hypothesized stability**
2930	3010-2800	Aliphatic C-H stretching[a]	Labile
1620	1754-1559	Aromatic C=C, COO^- stretching[a]	Intermediate
1530	1546-1520	Aromatic C=C stretching[a]	Intermediate
1159	1172-1148	C-O bonds of poly-alcoholic and ether groups[b]	Recalcitrant

[a]Baes and Bloom, 1989; [b]Demyan et al., 2012.

2.3.4 Statistical data analysis

The data set was analyzed in a mixed model procedure (Piepho et al., 2003) implemented in R statistical software version 3.6.0, (R Core Team, 2019). Analysis of variance (ANOVA) was performed for market distance, farm typology (resource endowment class), site, and farmers' knowledge as fixed factors, while farm sampling plots entered as random terms for prediction of soil chemical properties using lmerTest package (Kuznetsova et al., 2017). Model selection was based on akaike information criterion AIC. Estimates marginal means comparison and their separation between factors and their interactions were performed according to Searle et al. (1980).

Linear regressions were applied to reveal relationships between soil chemical properties and hypothesized soil fertility determinants (i.e., market distance, farm typology, farmers' indigenous knowledge and site). Linear Pearson correlations were calculated to validate links between Org. C and midDFRIFTS peak data (i.e., relative peak area, SOC stability indexes). The Chi^2 test for independence was applied to determine significant differences within local soil fertility indicators used by smallholder farmers.

2.4 Results

2.4.1 Inter-related effects of market distance, farm typology, and sites on soil fertility properties

There was no clear inter-related effect of market distance and farm typology (i.e., resource endowment) on soil fertility properties, which was only significant for Ca_{ex} ($P < 0.05$) and Mg_{ex} ($P < 0.001$) (Table 2, Fig. 3). The inter-related effect of market distance and sites showed a significant effect for TN ($P < 0.001$) (Table 2, Fig. 3). As a single factor, however, market distance revealed a significant effect for Org. C ($P < 0.01$), TN ($P < 0.001$), and Mg_{ex} ($P < 0.05$) (Table 2, Fig. 3). This was corroborated by linear regression analyses showing negative relations between market distance and Org. C ("wealthy" ($R^2 = 0.20$, $P < 0.01$), "medium wealthy" ($R^2 = 0.42$, $P < 0.001$), "poor" ($R^2 = 0.30$, $P < 0.001$)), and TN ("wealthy" ($R^2 = 0.20$, $P < 0.01$), "medium wealthy" ($R^2 = 0.38$, $P < 0.001$), "poor" ($R^2 = 0.27$, $P < 0.001$)) (Fig. 3 a-b). A significant positive influence of farm typology was found for Ca_{ex} and Mg_{ex} in Bushumba, while a negative correlation was noticed in Mushinga with increasing market distance ($P < 0.01$). Considering factor site only, a significant difference of TN, P_{av}, Ca_{ex} and Mg_{ex} contents was observed ($P < 0.05$) (Table 2).

Table 2. 2: Effects of market distance, farm typology and sites with their interactions on soil chemical properties as predicted by the midDRIFTS-PLSR approach (for data values see Fig. 3 and 4).

Properties	**Factors and interactions**				
	Market distance	**Farm typology**	**Site**	**Market distance × Farm typology**	**Market distance × Site**
Org. C [$g\ kg^{-1}$]	**	ns	ns	ns	*
TN [$g\ kg^{-1}$]	***	ns	***	ns	***
Soil pH [$CaCl_2$]	ns	ns	ns	ns	*
P_{av} [$mg\ kg^{-1}$]	ns	ns	*	ns	ns
K_{av} [$mg\ kg^{-1}$]	ns	ns	ns	ns	ns
Ca_{ex} [$cmol_{(+)}\ kg^{-1}$]	ns	**	***	*	ns
Mg_{ex} [$cmol_{(+)}\ kg^{-1}$]	*	***	*	***	ns
Peak 2930 [cm^{-1}]	ns	ns	***	ns	**
Peak 1620 [cm^{-1}]	***	ns	**	**	ns
Peak 1530 [cm^{-1}]	***	ns	ns	ns	***
Peak 1159 [cm^{-1}]	**	ns	***	ns	ns
Ratio of 1620:2930	ns	ns	***	ns	ns
Ratio of 1530:2930	ns	ns	***	ns	**
Ratio of 1159: 2930	ns	ns	***	ns	ns
Clay (%)	*	ns	*	ns	ns
Sand (%)	**	ns	*	ns	ns
Silt (%)	ns	ns	ns	ns	ns

Significance levels: P<0.001 '***', P<0.01 '**', P<0.05 '*', P>0.05 'ns'.

Farm typology (wealthy, medium wealthy and poor) refers to farmers' wealth class based on farm size.

Sites (Bushumba and Mushinga) located in the region, where the soil fertility survey was conducted.

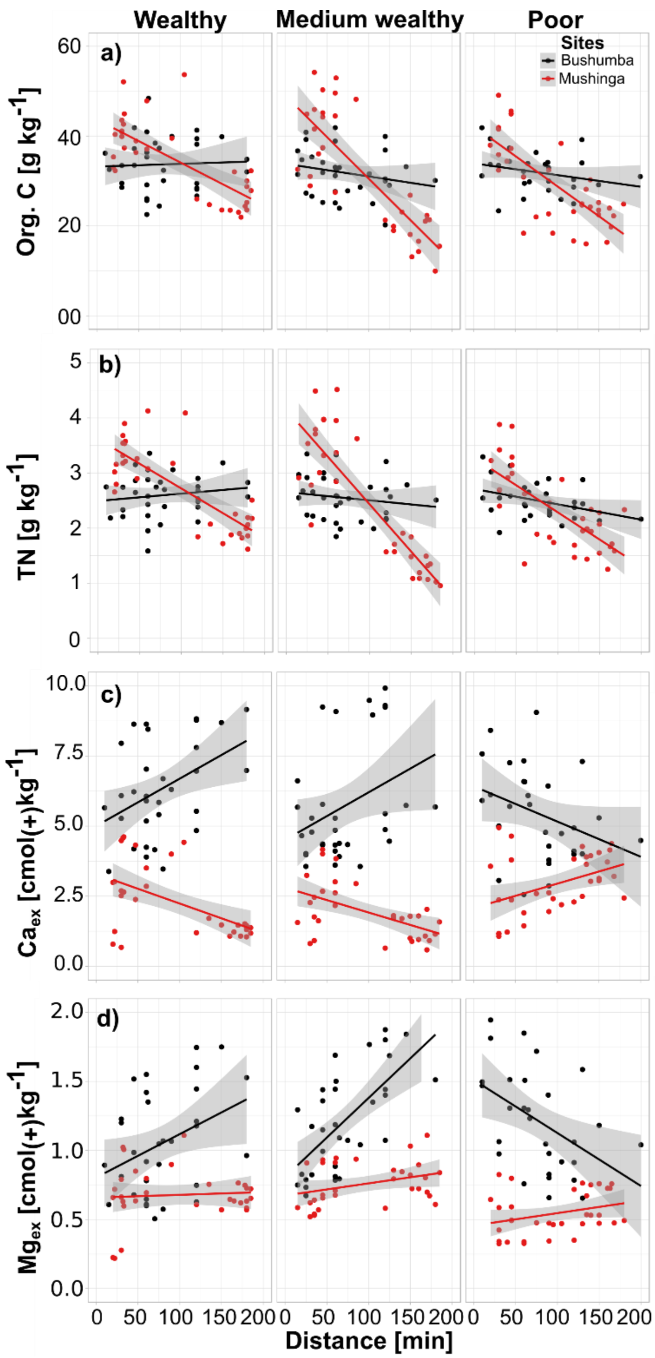

Figure 2. 2: Contents of total carbon (Org. C, P<0.05; a) and total nitrogen (TN, P<0.05; b), as well as exchangeable calcium (Ca_{ex}, P<0.01; c) and magnesium (Mg_{ex}, P<0.01; d) in soils of surveyed smallholder households in the two sites Bushumba (dots and regression line black

colored) and Mushinga (dots and regression line red colored) considering the two factors “farm typology” and “market distance”. Gray color in scatter plots represents the confidence interval

The relative peak areas of 4 representative peaks at 2930 (aliphatic C-H stretching), 1620 (aromatic C=C and COO^- stretching), 1530 (aromatic C=C stretching), 1159 (C-O bonds of poly-alcoholic and ether groups) cm^{-1} and respective stability indexes (i.e., 1620:2930, 1530:2930, 1159:2930) were considered as SOC quality indicators (Table 1). Market distance exposed a significant effect on relative areas of peaks 1620, 1530 and 1159 cm^{-1} ($P < 0.01$) (Table 2, Fig. 4). Its interaction with farm typology was significant for peak 1620, which increased in farm typology “wealthy” with increasing market distance ($P < 0.01$) (Table 2). Factor site had the strongest effect on SOC quality proxies, which was significant for all peak areas, except 1530 cm^{-1} ($P < 0.01$) (Table 2, Fig. 4). Peaks 2930 and 1530 cm^{-1} revealed a significant interaction between market distance and site ($P < 0.01$); as market distance increases, peaks 2930 and 1530 cm^{-1} in Bushumba increased, while they were reduced in Mushinga for the medium wealthy class (Table 2, Fig. 4). Similar results were noticed for 1530 cm^{-1} in Mushinga. Moreover, site had a significant effect on all 3 SOC stability indexes ($P < 0.001$), and for the ratio 1530:2930 showing also a significant interaction with market distance and site ($P < 0.01$) (Table 2, Fig. 4). Except for the ratio 1620:2930, all midDRIFTS-derived SOC quality indicators revealed a significant positive correlation with Org. C content (Table 3).

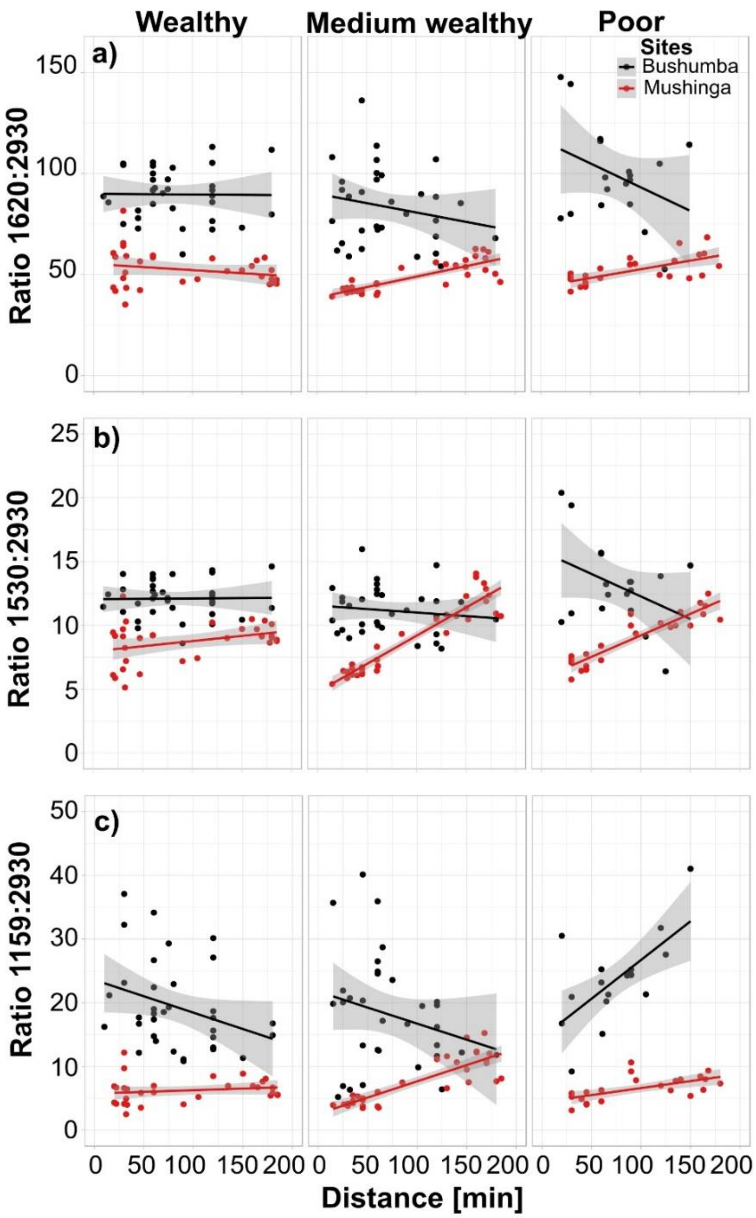

Figure 2. 3: Ratios of midDRIFTS peaks 1620:2930 (a), 1520:2930 (b), and 1159:2930 (c) displaying the SOC quality of soils of surveyed smallholder households in the two sites Bushumba and Mushinga considering the two factors “farm typology” and “market distance”. Gray color in scatter plots represents confidence intervals.

Table 2. 3: Pearson correlation (*r*) between organic carbon (Org. C) content and midDRIFTS peak area analysis derived SOC quality indicators.

Variables	***r***	**F test**
Peak 2930 [cm^{-1}]	0.24	**
Peak 1620 [cm^{-1}]	0.48	***
Peak 1530 [cm^{-1}]	-0.27	***
Peak 1159 [cm^{-1}]	-0.31	***
Ratio 1620:2930	-0.11	ns
Ratio 1530:2930	-0.26	***
Ratio 1159:2930	-0.22	**

Significance levels: P<0.001 '***', P<0.01 '**', P>0.05 'ns'.

2.4.2 Farmers' indigenous knowledge across sites to predict soil fertility variability

Smallholder farmers used different indicators to assess soil fertility, whereby soil depth ("deep" as representative for fertile and "shallow" for less fertile soils) and soil color ("black" as representative for fertile and "red" for less fertile soils) were the main indicators (Table 4).

Table 2. 4: Proportional contribution (%) of farmers to the ranking (Chi^2) of selected soil fertility indicators across sites.

Indicators for soil fertility	**Chi^2**	**Proportion (%)**
Soil depth	22.1 ***	49
Soil color	9.5 *	22
Soil texture	6.9 ns	16
Soil drainage	4.9 ns	11
Distance from homestead	1.0 ns	2

Significance levels: P<0.001 '***', P<0.05 '*', P>0.05 'ns'.

Complementary, laboratory analysis revealed higher concentrations of Org. C and P_{av} in "deep" than "shallow" soils ($P < 0.05$) (Fig. 5 a-b), with similar trends for TN, K_{av}, Ca_{ex}, and Mg_{ex} (Table 5). In agreement with farmers' indigenous knowledge, wet chemistry analyses revealed higher concentrations of P_{av} in "dark" than "red" soils ($P < 0.05$) (Table 5, Fig. 5 d). Org. C, on the other hand, disagreed with farmers' indigenous knowledge, revealing higher values in the "red" than "dark" soils ($P < 0.05$) (Table 5, Fig. 5 c). The same trend was true for TN, while remaining soil chemical properties did not reveal a significant effect between "dark" and "red" soils ($P > 0.05$) (Table 5).

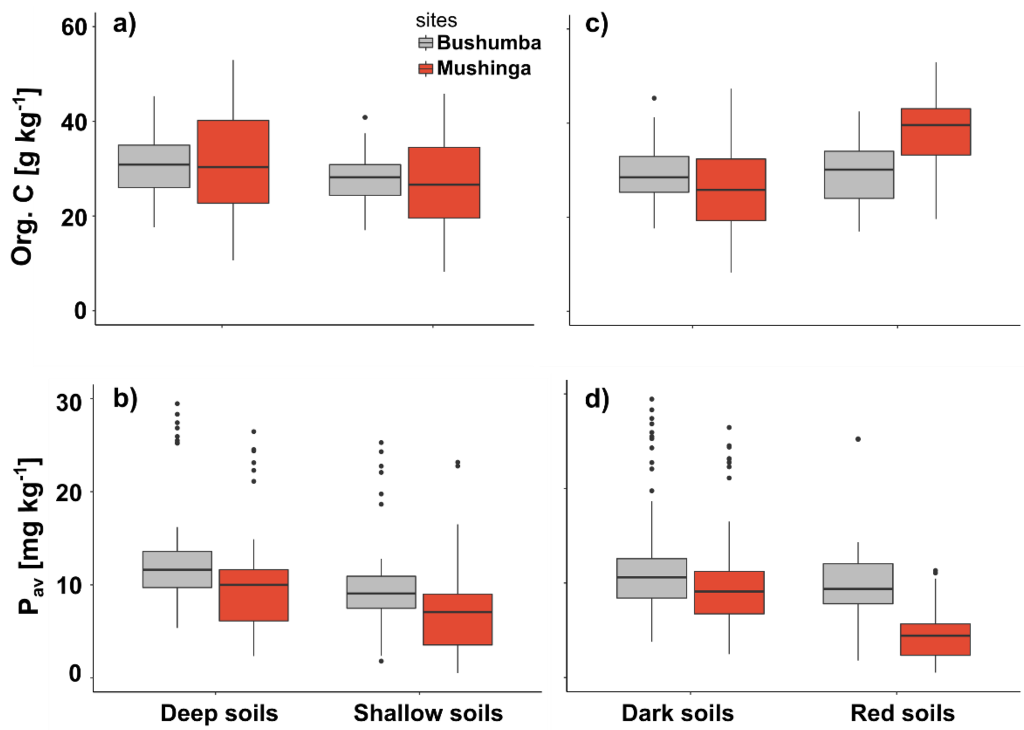

Figure 2. 4: Box plot of farmers' classification of soil fertility according to their local indicators ("soil depth" (deep versus shallow soils) and "soil color" (dark versus red soils)), as exemplified

for determined soil organic carbon (Org. C) and available phosphorus (P_{av}) contents at different sites combining top and subsoil.

Table 2. 5: Averages of selected local soil fertility indicators in soil chemical properties measured across the two sites from top and subsoil (Org. C, TN, soil pH, Ca_{ex}, Mg_{ex}, n = 360), and (P_{av}, K_{av}, n = 96)

Selected indicator		Sites	Soil chemical properties						
			Org. C [%]	TN [%]	Soil pH	P_{av} [mg kg^{-1}]	K_{av} [mg kg^{-1}]	Ca_{ex} [cmol(+)kg^{-1}]	Mg_{ex} [cmol(+)kg^{-1}]
Soil depth	Deep	B	3.05(1.20)^{ab}	0.24(0.11)^{ab}	4.87(0.52)^{b}	12.54(8.53)^{c}	222.07(208.40)^{ab}	5.20(2.40)^{b}	1.04(0.43)^{b}
[0-50 cm]	Shallow	B	2.80(1.12)^{a}	0.22(0.10)^{a}	4.53(0.49)^{a}	9.16(7.99)^{b}	186.77(169.85)^{a}	4.38(2.11)^{b}	0.81(0.36)^{a}
	Deep	M	3.45(1.22)^{b}	0.27(0.11)^{b}	4.70(0.54)^{ab}	8.75(6.20)^{ab}	273.90(191.07)^{b}	2.63(2.36)^{a}	0.77(0.40)^{a}
	Shallow	M	2.98(1.22)^{a}	0.24(0.11)^{ab}	4.60(0.45)^{a}	5.67 (8.63)^{a}	223.64(200.03)^{ab}	2.32(2.36)^{a}	0.71(0.42)^{a}
			**	*	***	***	*	*	***
Soil color	Dark	B	2.90(1.03)^{a}	0.23(0.09)^{a}	4.75(0.45)^{a}	11.26(7.33)^{b}	194.56(174.83)$_{a}$	4.98(2.03)^{b}	0.94(0.37)^{c}
	Red	B	2.95(1.01)^{a}	0.23(0.09)^{a}	4.65(0.44)^{a}	10.44(7.21)^{b}	214.28(156.89)^{a}	4.60(1.93)^{b}	0.91(0.34)^{bc}
	Dark	M	2.60(1.05)^{a}	0.20(0.10)^{a}	4.63(0.47)^{a}	9.32(7.54)^{b}	242.78(170.12)^{a}	2.68(2.05)^{a}	0.77(0..37)^{ab}
	Red	M	3.84(1.05)^{b}	0.31(0.10)^{b}	4.67(048)^{a}	5.10(7.76)^{a}	254.76(175.59)^{a}	2.27(2.09)^{a}	0.71(0.34)^{a}
			***	***	ns	***	ns	*	*

Site: B = Bushumba, M = Mushinga

Standard deviation is given in parentheses.

Superscript letters display statistical differences from the interaction indicator with site.

Significance levels: P<0.001 ‘***’, P<0.01 ‘**’, P<0.05 ‘*’, P>0.05 ‘ns’.

2.5 Discussion

2.5.1 Market distance, farm typology and sites as key determinants of soil fertility variability

Smallholder farming systems in South-Kivu (DR Congo) are influenced by various socio-economic and agro-ecological factors. Our study demonstrated that not only the distance of farmers to markets, but also farm typology were key determinants of soil fertility, both with contrasting trends in the two study regions Mushinga and Bushumba. Specifically, decreasing soil fertility, as exemplified by Org. C and TN, with increasing market distance was noted across all farm typologies, and was most pronounced in Mushinga. This trend was explained by farmers' opportunities to access external inputs available in close proximity to the markets (Soule and Shepherd, 2000). However, P_{av} and K_{av} were more related to site specificity, probably due to the influence of both soil mineralogy and pH levels that differed between sites. Farmers close to markets purchase and transport mineral and organic fertilizers at lower costs than farmers in remote areas exposed to unfavorable road infrastructure and transportation opportunities. Moreover, the proximity to markets provides farmers the opportunity to sell surplus yields of crops. This generates extra income to afford, upon availability, organic fertilizers, irrespective of the wealth status of the farmers. These benefits translate into soil fertility improvement masking partially the hypothesized effect of farm typology. This assumption was corroborated by earlier studies conducted in Kenya and Uganda, observing that the proximity of farms to markets influenced strongly the amount of applied fertilizers across farms regardless of the wealth status (Tittonell et al., 2005; Yamano and Kijima, 2010).

The survey of the Org. C content as a proxy of soil fertility was complemented with SOC stability indexes, as calculated from relative areas of selected midDRIFTS peaks (i.e., 1620:2930, 1530:2930, 1159:2930; Demyan et al., 2012). However, neither distance to market nor farm typology alone exposed a significant effect on the three SOC stability indexes, which was explained by the lack of both, inorganic and organic fertilizers, leading to lower SOC quality. Only the factor site revealed a clear distinction, which was also reflected in its significant interaction with factor market distance (i.e., 1530:2930). A comparable, but non-significant interaction was found for the ratio 1620:2930. The effect of market distance became most obvious in the medium wealthy and poor farms surveyed in Mushinga. For these farm typologies, an increasing ratio of 1530:2930 with increasing market distance was noted, implying a lower SOC quality due to limited or absent organic inputs. This assumption was corroborated by the negative correlation between the ratio of 1530:2930 and Org. C content. A comparable trend was found on the field plots of the poor farmers with remote distance to markets in Bushumba for peaks at 1530 and 1159 cm^{-1}. This corroborated the former argument that primarily wealthy farmers were able to purchase farm yard manure as the only locally available fertilizer (Soule and Shepherd, 2000). However, contrasting trends of respective SOC stability indexes were obtained with increasing market distance. Even though Veum et al. (2013) and Ding et al. (2002) have suggested that the high ratio of poly-alcoholic and ether groups over that of aliphatic compounds (1159:2930) may be related to a lower SOC quality, further research is needed to understand the underlying mechanism of the results obtained in this study. Due to detection limit, no clear effect of tested factors was revealed for peak 2930 cm^{-1}, representing the labile SOC pool (Baes and Bloom, 1989), which was explained by generally low inputs of organic materials (e.g., farm yard manure, crop residues) exposed to high turnover (Demyan et al., 2012).

In contrast to Org. C and TN, contents of exchangeable Ca and Mg were driven by the interaction of both market distance and farm typology. The two sites revealed reverse trends for these cations with increasing market distance. While decreasing soil nutrient stocks with increasing market distance were expected, as noted in Mushinga, Bushumba revealed the opposite for the wealthy and medium wealthy farmers. It was assumed that these farmers with market proximity provided conducive economic opportunities, exerting a considerable production pressure on their land to maximize yield and income (Bationo et al., 2006; Kansiime et al., 2018). Due to such continuously high cultivation pressure, the poor farmers in Mushinga depleted their soils in Ca and Mg, hardly to be replenished by organic inputs alone. Meanwhile in Bushumba, wood ash derived from kitchen waste (Bekunda and Woomer, 1996) is broadcasted on farm plots close to the market center to reach higher soil nutrient contents. The positive effect of this fertilization strategy is more pronounced on farms with small land sizes (<1 ha) than on wealthy and medium wealthy farms that need to manage generally a larger land size (>2 ha), a finding in line with Place et al. (2003). Opposite to farm plots of close distance, remote field plots face low soil nutrient mining, a consequence of low cultivation pressure following market scarcity. Consequently, the soil maintains adequate levels of Ca and Mg stocks.

2.5.2 Indigenous knowledge to validate soil fertility status across market gradients

So far, farmers' knowledge to assess soil fertility has been based mainly on local indicators, including soil color and soil depth (Desbiez et al., 2004; Dawoe et al., 2012). Complementary, the presented study has evaluated the correspondence and discrepancies between farmers' indigenous and scientific knowledge regarding the soil fertility status of contrasting farm typologies, testing whether soils considered fertile or less fertile by farmers show a similar fertility status according to science-based measurements using the midDRIFTS-PLSR approach. In this regard, the laboratory analysis conducted in this study was in agreement with the assessment of soil fertility by smallholder farmers, except for soil color, a finding in line with Yeshaneh (2015) and Murage et al. (2000). A range of soil fertility indicators, such as soil depth, soil color, soil texture and soil drainage, have been developed by smallholder farmers to distinguish between productive (fertile) and non-productive (less fertile) farm plots. Our study found soil depth and soil color as the most common indicators used by the farmers across sites. In agreement with farmers' knowledge, soil fertility levels were higher in deep than shallow soils, which was reflected in generally higher nutrient concentrations in deep soils across surveyed field plots receiving organic amendments. Although soil color was the second most important indicator, a clear correlation to our laboratory measurements was not found. Additionally, Org. C and TN were higher in red than black soils. We assumed that soil color was more related to soil physical properties such as soil texture. This argumentation was supported by Gray and Morant (2003) as well as Dawoe et al. (2012), who found a red soil color to indicate a sandy soil texture, while a grey color is related to a loamy soil texture. In this respect, the Madaka site with a generally high agricultural potential, was dominated by a sandy soil texture with the typical reddish color originating from basaltic rocks (Van Engelen et al., 2006).

2.6 Conclusions

The findings of this study suggested that the inter-related effect of market distance and farm typology was the main driver of soil fertility variability across the studied sites. Soil fertility, as displayed by Org. C and TN concentrations, decreased with increasing market distance, with exception of the wealthy class of Bushumba. This implied that within the market distance gradients (i.e. close, medium, remote), site effects including soil type and climate played a significant role in shaping the soil fertility variability across surveyed farms. It was also evident that farmers' management practices and resource endowment contributed to soil fertility variability, particularly in farms plots remote to markets.

Laboratory measurements of soil chemical parameters agreed with farmers' assessment on soil fertility status. This suggested that farmers' indigenous knowledge is a valuable proxy for soil fertility surveys and may be integrated in prospective science-based soil fertility assessments. However, care should be taken as some indicators used by farmers, such as soil color, may not only relate to soil fertility status, but also reflect soil mineralogy and soil texture.

Our results further inferred that ISFM interventions in smallholder farms must consider various inter-related features to determine soil fertility variability across smallholder farmers. We have complemented these features by the variable market distance to distinguish soil fertility levels across spatial scales. Our assumptions were based primarily on land size, used as key feature to define the wealth status (farm typology) of targeted smallholder farms in the study area. In this regard, prospective soil fertility surveys should not only consider resource endowment (land size) to characterize the wealth status of farmers, but also other socio-economic indicators, including, but not limited to, livestock holding (limited in the discussed study area), availability of labor and use of mineral and organic fertilizers.

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CHAPTER 3

Agro-ecology, resource endowment and indigenous knowledge interactions modulate soil fertility in mixed farming systems in Central andf Wwstern Ethiopia

3. Ageo-ecology, resource endowment and indigenous knowledge interactions modulate soil fertility in mixed farming systems in Central and Western Ethiopia

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3.1 Abstract

The main drivers of soil fertility variability across Sub-Saharan Africa must be understood to develop tailor-made integrated soil fertility management (ISFM) strategies, considering smallholder farmers' resource endowment and their indigenous knowledge of soil fertility. Accordingly, this study verified that soil fertility variability across two model regions in Central and Western Ethiopia is determined by inter-related effects of agro-ecology and farmers' resource endowment ("wealthy" versus "poor" farmers). Using mid-infrared spectroscopy coupled to partial least squares regression analyses (midDRIFTS-PLSR), prediction models were developed to assess soil fertility proxies across a regional scale, including various agro-ecological zones: "high dega" (HD), "dega" (D), "weina-dega" (WD) and "kola" (K). MidDRIFTS peak area analysis of selected spectral frequencies (2930 (aliphatic C-H), 1620 (aromatic C=C), 1159 (C-O poly-alcoholic and ether groups) cm^{-1}) was applied to characterize functional groups of soil organic carbon (SOC) and to calculate the SOC stability index (1620:2930). Total carbon (TC) (coefficient of determination (R^2) = 0.92, residual prediction deviation (RPD) = 3.46), total nitrogen (TN) (R^2 = 0.86, RPD = 2.71) content and pH (R^2 = 0.89, RPD = 3.02) in soils were predicted accurately by midDRIFTS-PLSR. Predictions of available phosphorous (P_{av}) and potassium (K_{av}) were not successful; hence, wet chemistry was used instead. Across the two study regions, higher soil nutrient (e.g., K_{av}, TN) and TC contents were found in fields of wealthy compared to poor farmers. SOC quality of wealthy farms revealed higher and lower peak areas of 2930 and 1620, respectively, than poor farms. Likewise, the SOC stability index was lowest in soils of wealthy compared to poor farms ($P < 0.05$). With regard to farmers' indigenous knowledge across the study regions, fertile and less fertile fields were distinguished by visually observed soil color. Higher pH in K and WD as well as P_{av} in K and HD were found in fertile (brown/black) than less fertile (red) soils. Higher peak areas of 1159 cm^{-1} and SOC stability index were observed in less fertile compared to fertile soils. We conclude that inter-related effects of agro-ecology and farmers' resource endowment determined strongly the observed soil fertility variability across the two study regions. Accordingly, site-specific soil management strategies shall be installed to overcome this constraint. The application of the proposed midDRIFTS-PLSR-based approach was imperative, and shall be translated to other regions across Africa allowing a more comprehensive understanding of inter-related factors of soil fertility variability across larger regions than considered here.

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3.2 Introduction

Integrated soil fertility management (ISFM) is a major intervention strategy to counteract the problem of poor food and feed production of smallholder farming systems in sub-Saharan Africa (SSA) (Vanlauwe et al., 2010). Its adoption across different regions of SSA including Central and Eastern Africa remains, however, a major challenge (Vanlauwe et al., 2015). This is mainly due to critical resource shortcomings (e.g., land size, labor), a strict tenure system prohibiting farmers to invest into their land, and limited access to sufficient fertilizer inputs (Stevenson et al., 2019). These features lead to highly variable soil fertility levels within regions, also magnified by the inherent heterogeneity of agro-ecologies and the wide range of socio-economic status among smallholder farmers (Tittonell et al., 2005a). Heterogeneity of soil fertility does not allow uniform soil management strategies in larger areas, hence there is need to unravel the complex dynamics of soil fertility gradients to develop ISFM strategies adjusted to local contexts.

To tailor demand-oriented ISFM interventions to smallholder conditions under different local contexts, however, it is critical to understand the main drivers of soil fertility variability and to use this knowledge to develop explicit ISFM strategies considering also farmers' resource endowment as well as their indigenous knowledge of soil fertility status (Tittonell et al., 2005b; Vanlauwe et al., 2015). These include, among others, Ethiopia and the Democratic Republic of Congo (DRC), where earlier studies relied solely on interviews on farmers' perception about soil fertility status, which were not validated by laboratory analysis (Corbeels et al., 2000). Others were based on spatially less representative soil chemical surveys (Belachew & Abera, 2010; Pypers et al., 2011; Yeshaneh, 2015). In Ethiopia, for example, a nation-wide soil nutrient map and the Ethiopian Soil Information System (EthioSIS) was developed to provide policy advice on the use of fertilizer at smallholder scale (Amare et al., 2018). However, though these pioneering mapping approaches could initialize site-specific ISFM adaptations, they did not address essential drivers of soil fertility like agro-ecology, resource endowment and farmers' indigenous knowledge. Such efforts shall specifically address those regions of SSA for which only limited or inconsistent data on the main drivers of soil fertility variability are available.

In the regions of interest (Central and East Africa), both Ethiopia (this study) and DRC (parallel study by Balume et al. (in revision)) are characterized by a wide range of socio-ecological and biophysical (geology, soil type, climate) factors, all influencing the process of decision making in soil fertility management among farmers (Ojiem et al., 2014). Previous soil fertility assessments in East (e.g., Kenya) and South Central (e.g., Zimbabwe) Africa revealed the impact of densely populated landscapes, biophysical factors, farmers' resource endowment and distance of cultivated fields from homesteads on soil fertility management options (Nyamangara et al., 2011; Tittonell et al., 2010; Tittonell et al., 2005a). It must be noted, however, that these conclusions were not based on generic and harmonized soil surveying procedures, making direct comparisons of different agro-ecologies and associated farming systems across regions or countries difficult. Although AfSIS (Africa Soil Information Service) and EthioSIS attempted to harmonize soil sampling approaches to representatively map soil fertility status in several African countries (Vågen et al., 2010), including Ethiopia (Amare et al., 2018), there is only limited data available yet that considered inter-related effects of agro-ecology and farmers' resource endowments, considering also farmers' indigenous knowledge on soil fertility variability on a detailed farm scale.

To generate such data, diffuse reflectance Fourier transform mid-infrared spectroscopy (midDRIFTS) has been applied successfully for regional soil fertility mapping (Demyan et al., 2012; Mirzaeitalarposhti et al., 2015; Rasche et al., 2013). MidDRIFTS does not only allow the quantitative prediction of soil chemical properties (e.g., total soil nitrogen and carbon content as conventional soil fertility indicators) across large spatial scales. It also enables the spectroscopic assessment of soil organic carbon (SOC) quality indicators (e.g., functional groups of SOC (i.e., aliphatic (labile) and aromatic (recalcitrant) compounds) as a function of soil fertility (Demyan et al., 2012; Shepherd & Walsh, 2002; Base and Bloom, 1989).

Our objectives were to (i) develop for both target regions Ethiopia (this study) and DRC (Balume et al., in revision) generic and harmonized midDRIFTS-PLSR-based prediction models for selected soil fertility indicators using combined soil physico-chemical data sets of the two countries (Ethiopia, DRC), and (ii) use these models to assess the soil fertility status across a regional scale (Mirzaeitalarposhti et al., 2015). The prediction models were used to test country-specific research hypotheses on drivers of soil fertility variability (this study; Balume et al., in revision). For the Ethiopian case presented here, the first hypothesis was that for the assessment of soil fertility status across a regional scale, not only individual but also interrelated effects of agro-ecology and farmers' resource endowments on soil fertility variability have to be considered. The second hypothesis postulated that farmers' indigenous knowledge on soil fertility status is not driven by inter-related effects of agro-ecology and farm typology. This assumption was based on the continuous knowledge transfer among farmers within and across agro-ecologies (Leta et al, 2018).

3.3 Material and methods

3.3.1 Site selection and farm typology characterization

The survey was conducted in two parallel studies in Eastern (Ethiopia) and Central (DRC) Africa. For site selection in Ethiopia, relevant socio-economic (e.g., distance from the local market, road type and access, farmers' resource endowment) and agro-ecological (e.g., climate, soil type, altitude) characteristics, as well as farming system descriptions (e.g., crop rotation, planting date, type of crops grown) were used to categorize farms into different agro-ecology and farm typology groups. A detailed description about site selection criteria for the parallel study in DRC is provided in Balume et al. (in revision).

For Ethiopia, data on agro-ecologies and farming systems were retrieved from secondary sources before the start of the survey (Table 1). The categorization of sites followed the concept of the traditional agro-ecology classes of the country based on elevation and climate (Mengistu, 2003; Hurni, 1998): (i) "kola" (K) (<1500 meters above sea level (m.a.s.l.), moist hot to warm climate with temperatures of 15 to 27°C and average rainfall of 2037 mm), and (ii) "weina-dega" (WD) (1500-2500 m.a.s.l., sub-humid climate with temperatures of 15 to 27°C and average annual rainfall of 1376 mm) with subsistence maize dominated farming systems, as well as (iii) "dega" (D) (cold) and (iv) "high dega" (HD) (moist cold) (2500-3500 (m.a.s.l.)) with average temperatures of ≤9°C and average annual rainfall of 938 mm, represented market-oriented potato/barley systems. The given agro-climatic differences influence decisions and agronomic capacity of farmers to invest in ISFM practices. In the highlands, for example, due to slow decomposition of organic residues and farmers investment potential on chemical fertilizer market-oriented cropping systems are predominant, while in the lowlands, farms of larger size and a larger number of livestock are found providing opportunities to invest in ISFM (i.e., chemical fertilizer or manure application) (Erkossa et al., 2018).

Sites K and WD were located in Lelisadimtu (36°24’E; 9°02’N) and Fromsa (36°45’E; 9°03’N) sub-locations in Diga District (Western Ethiopia), respectively. Sites D and HD were located in Kolugelan (38°9’E; 9°22’N) and Chilanko (38°11’E; 9°20’N), sub-locations in Jeldu district (Central Ethiopia), respectively (Table 1; Fig. 1). Generally, the selected sites represented a wide range of altitudes from low to highlands (1254-2949 m.a.s.l.) with various lengths of cropping periods (K = 213 days for lowland maize, WD = 219 days for highland maize, D = 210 days for barley, HD = 196 days for wheat) (Amede et al., 2015) (Table 1; Fig. 1). Target sites were selected based on good (Chilanko and Lelisa dimtu) and medium (Kolugelan and Fromsa) market access (T. Temesgen, personal communication).

Table 3. 1: Description of the agro-ecology of the study regions in Central and Western Ethiopia.

Characteristics	**Kola (K)**	**Weina-Dega (WD)**	**Dega (D)**	**High Dega (HD)**
Average elevation (masl)	1281	2177	2784	2911
Mean annual rainfall (mm)	2037	1376	938	938
Mean daily min temperature (°C)	15	15	9	9
Mean daily max temperature (°C)	27	27	27	27
Dominant cropping system	Maize mixed	Maize mixed	Wheat-livestock mixed	Barley/potato-livestock mixed
Soil texture	Clay	Clay	Clay	Clay
Major soil type*	Nitisol	Nitisol/Alisol	Luvisol/Alisol	Luvisol/Alisol

Data sources: Erkossa et al. (2018); Amede et al. (2015); Ogunwale JO. et al., 2014; Berhanu et al. (2013); Deressa et al., 2013; Hurni (1998);.

*WRB classification (FAO, 2014)

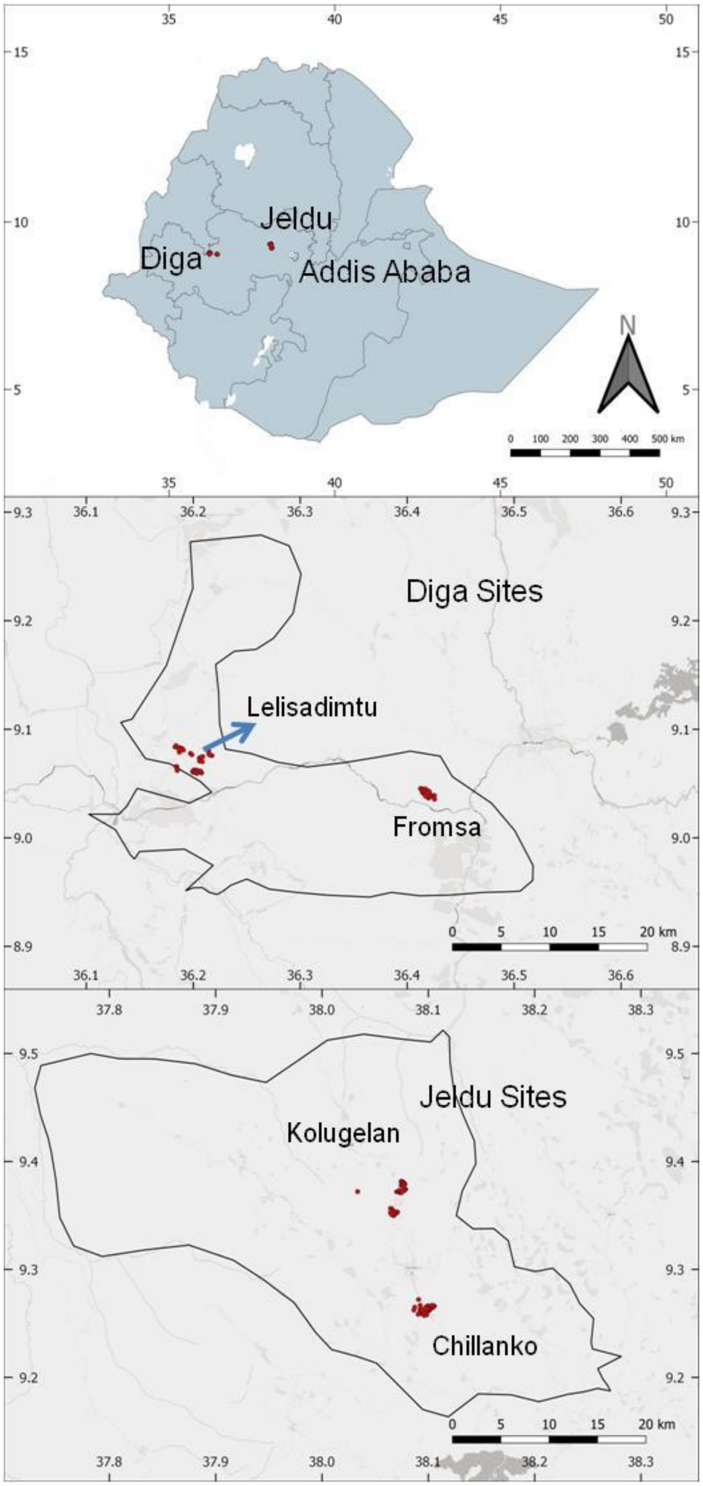

Figure 3. 1: Study regions with respective sampling points located in Central (Dega (D), High Dega (HD)) and Western (Kola (K), Weina-Dega (WD)) Ethiopia.

To select the farm typologies (resource endowment) at the target sites (villages), 2 to 3 village meetings and focus group discussions with an equal share of female and male as well as young and old farmers were conducted at each target site to define farm typology classes and set the threshold for each class based on resource endowment properties. Based on focus group discussions, main farm typology indicators were farm size (land holdings), livestock ownership and level of agricultural inputs (i.e., chemical fertilizer) (Haileslassie et al., 2007). The thresholds set by farmers were < 2 ha of land holding, <7 LTU number of livestock and use of below recommended chemical fertilizer as poor while those who have ≥ 2 ha, ≥ 7 LTU and use full rate or more chemical fertilizer as wealthy farmers. The recommended chemical fertilizer for cereals was 50/50 kg ha^{-1} DAP and Urea. Detailed data on farm typology indicators were collected using a quick baseline survey on 62 households (about 2% of the total population) to characterize socio-economic conditions that may affect soil fertility status. For this study, two major farm typology classes (i.e., wealthy, N=31 and poor N= 31) were identified in each agro-ecology (Table 2) and integrated in the soil fertility assessment.

Table 3. 2: Average values of socio-economic indicators for the different farm typologies in the four study regions (n=62)

Agro-ecology	**Typology[1]**	**Farm size [ha]**	**Livestock holding [TLU[2]]**	**Fertilizer (DAP + Urea) rate [kg ha^{-1}]**
Kola (K)	W	5.7 (1.0)^{ab}	11.7 (1.8)^{a}	117 (25)^{bc}
	P	0.8 (1.0)^{d}	3.2 (1.8)^{d}	64 (35)^{c}
Weina-dega (WD)	W	4.4 (0.9)^{abc}	8.6 (1.59)^{abc}	121 (35)^{abc}
	P	1.1 (1.0)^{d}	4.5 (1.8)^{cd}	72 (35)^{c}
Dega (D)	W	7.0 (1.0)^{a}	9.5 (1.7)^{ab}	198 (27)^{a}
	P	1.8 (1.0)^{cd}	5.4 (1.7)^{bcd}	135 (20)^{abc}
High dega (HD)	W	4.9 (0.8)^{ab}	9.0 (1.5)^{abc}	192 (46)^{ab}
	P	1.8 (1.1)^{cd}	5.5 (1.9)^{bcd}	180 (30)^{ab}
P-level (agro-ecology)		Ns	Ns	***
P-level (typology)		***	**	Ns
P-level (agro-ecology × typology)		Ns	Ns	Ns

Significance levels: NS, not significant at $P < 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. [1]Definition of farm typology based on resource endowment: W = wealthy farmers; P = poor farmers.

[2]TLU = Tropical livestock unit.

3.3.2 Soil sampling

In each agro-ecology (n = 4), 14 households (7 wealthy, 7 poor) per typology class were randomly selected (Dawoe et al., 2012; Nyamangara et al., 2011). On each farm, the head of the household was requested to indicate the most and least fertile field plots based on their individual indigenous knowledge about soil fertility status. Hence two field plots per household (fertile and poor) were subjected for soil sample collection. Farmers used soil color as the main indicator for soil fertility, where black and brown soils were considered as fertile and red soils as less fertile. During soil sample collection the head of the household requested to indicate the color of the specified soil of the field plot.

Soil samples were obtained using the Y-shaped scheme (Vågen et al., 2012). The Y-frame with 12.2 meters in diameter was placed in the center of each field and extended 5.64 meters to each sub-plot within the field. Top (0-20 cm) and sub- (20-50 cm) soil samples were collected using a soil auger with 5.3 cm inner diameter. Four sub-samples from each soil depth were mixed to make one composite sample. Information on elevation, coordinates and soil color were recorded for each field. According to the sampling procedure, a total number of 608 geo-referenced soil samples were collected: Ethiopia (n = 224; 4 agro-ecologies (K, WD, D, HD) × 2 farm typologies (wealthy, poor) × 7 farms per typology × 2 fields per farm (fertile and less fertile) × 2 soil depths (0-20 cm, 21-50 cm)), and DRC (n = 384; 2 study sites × 2 villages per site × 3 farm typologies × 8 farms per typology × 2 plots per farm × 2 soil depths) (Balume et al., in revision). Out of 224 (Ethiopia) and 384 (DRC) soil samples collected, 9 and 24 soil samples, respectively, were excluded from the sample list due to mislabeling during soil sample collection, thus remaining with 215 samples for Ethiopia and 360 for DRC. Soil samples were air-dried, 2 mm sieved, and shipped to University of Hohenheim (Stuttgart, Germany) for further analysis.

3.3.3 Soil chemical analysis

Keeping a recommended 30% of the total sample set as training data set, for a reliable midDRIFTS-PLSR-based model development (Brown et al., 2005; Rasche et al., 2013), 183 soil samples (Ethiopia (n = 96), DRC (n = 87)) representative for the considered categories (agro-ecology, farm typology, farmers indigenous knowledge) were randomly selected from the entire sample set (n = 575). The soil properties of the remaining samples (n = 392) were predicted using the developed midDRIFTS-PLSR-based prediction models. The 183 soil samples were subjected to wet chemistry analysis of selected soil fertility indicators. Soil pH was measured (inoLab1 Labor-pH-Meter, WTW GmbH, Weilheim, Germany) with 0.01 M calcium chloride ($CaCl_2$) extracting solution with a soil-to-solution ratio of 1:2.5 (Houba et al., 2000). Soil pH results showed values of <5, so that total carbon estimation was regarded as equivalent to total SOC (Bertrand et al., 2007). Total carbon (TC) and nitrogen (TN) were analyzed by dry combustion (vario MAX CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). Plant available phosphorus (P_{av}) was analyzed using the Bray1 method (Bray & Kurtz, 1945), and plant available potassium (K_{av}) was analyzed using the method of Schüller (1969).

3.3.4 MidDRIFTS analysis and PLSR-based prediction of soil chemical properties

For midDRIFTS analysis, we used the combined data set of both countries (Ethiopia n = 215; DRC = 360) to assess the robustness of a harmonized survey protocol applicable across regions. Soil samples were ball-milled and soil spectra were recorded on a Tensor-27 Fourier transform spectrometer (Bruker Optik GmbH, Ettlingen, Germany) (Rasche et al., 2013). Each soil sample was analyzed in triplicate from wavelengths 3950 to 650 cm^{-1}. The midDRIFTS-PLSR-based prediction models for each soil chemical property (i.e., TC, TN, pH, P_{av}, K_{av}) were constructed with the OPUS-QUANT2 package of OPUS version 7.5 (Bruker Optik GmbH) (Rasche et al., 2013). For this, the spectral range was set to exclude the background carbon dioxide region (2300-2400 cm^{-1}) and the edges of the detection limits of the spectrometer (<700 and >3900 cm^{-1}) to reduce noise.

Test set validation was preferred for the combined spectral data set (Ethiopia, DRC) over the commonly used leave-one-out cross-validation as the latter generally provides overoptimistic estimates of model predictive accuracy in larger data sets (Mirzaeitalarposhti et al., 2015). For all chemically analyzed soil samples, we used 70:30 sample ratios for calibration and validation of developed PLSR prediction models for selected chemical properties assessed in the soils obtained in Ethiopia and DRC (Brown et al., 2005; Rasche et al., 2013). Therefore, out of 183 chemically analyzed samples, through random selection, 70% (n = 123) of samples were selected for model calibration, while the remaining 30% (n = 60) were used for prediction model validation (Brown et al., 2005; Rasche et al., 2013). Accuracy of each midDRIFTS-PLSR-based prediction model developed for each individual soil chemical property was evaluated by considering the residual prediction deviation (RPD) value (Pirie et al., 2005), the coefficient of determination (R^2) and the root mean square error of the prediction (RMSEP) (Rasche et al., 2013). Several rankings of RPD values exist to judge midDRIFTS-PLSR-based prediction accuracy. For agricultural applications, RPD values higher than 5 indicate that prediction models are commonly qualified as 'excellent', while RPD values 3 to 5 are considered as 'acceptable' and RPD values smaller than 3 greater than 1.4 indicate a 'moderately successful' prediction power (Pirie et al., 2005). RPD values less than 1.4 denote 'unsuccessful' predictions (Chang et al., 2001). Besides, R^2 values show the percentage of variance present in the measured values as reproduced in the regression (Rasche et al., 2013; Saeys et al., 2005). RMSEP displays the prediction error and was calculated as the root mean squared difference between predictions and reference values in the respective measurement unit of the soil property; the lower the RMSEP value the better the prediction accuracy (Pirie et al., 2005).

The 'developed' midDRIFTS-PLSR based prediction models were optimized using the 'optimization' function of the OPUS-QUANT2 package (Bruker Optik GmbH) (Rasche et al., 2013). For each generated prediction model, the pre-processing method was selected based on the highest R^2 and RPD values and lowest RMSEP. The 'optimization' mode of OPUS-QUANT2 makes use of various mathematical pre-processing methods to improve midDRIFTS-PLSR-based prediction models by consideration of vital spectral frequencies in the assayed spectra. For each generic prediction model developed for each individual soil chemical property, the pre-processing method was selected so that PLSR analysis established the best correlation between spectral and chemical property data. The following mathematical pre-processing treatments were used: 1stD, first derivative; VN, vector normalization; SLS, straight line subtraction and COE, constant offset elimination. The 'optimization' of midDRIFTS-PLSR-based prediction models (Table 3) across both countries was performed and optimized prediction models were later referred as 'ComCount'-prediction models. Accuracy of 'ComCount'-prediction models was assessed as described above. Finally, chemical soil properties of 119 soil samples from Ethiopia were predicted.

3.3.5 Peak area integration in midDRIFTS spectra

Peak area integration by midDRIFTS using OPUS 7.5 software (Bruker Optik GmbH) (Demyan et al., 2012) provided an additional measure of the soil fertility status of smallholder farms in the two countries (Ethiopia, DRC). Three prominent peaks (i.e., 2930, 1620 and 1159 cm^{-1}) with their respective integration limits (3000-2800, 1770-1496, 1180-1126 cm^{-1}) representing different organic functional groups of SOC were used as additional soil fertility indicators (Baes & Bloom, 1989; Demyan et al., 2012; Senesi et al., 2003). Peak 2930 cm^{-1} represents less stable aliphatic C-H groups, components of the active SOC pool (Demyan et al., 2012). Peak 1620 cm^{-1} represents more stable aromatic C=C bonds as part of the recalcitrant SOC pool (Demyan et al., 2012). The third peak at 1159 cm^{-1} represents C-O poly-alcoholic and ether groups, commonly regarded as very stable C compounds (Demyan et al., 2012; Senesi et al., 2003). The ratio of the functional groups 1620 and 1159 versus 2930 cm^{-1} are commonly calculated as SOC stability index, which is used as soil quality indicator; the higher 1620:2930 and 1159: 2930 ratio is the higher SOC stability index (Demyan et al., 2012; Inbar et al., 1989).

3.3.6 Statistical data analysis

For statistical analysis, the data sets of the two study countries (Ethiopia, DRC) were separated and analyzed independently. Prior to analysis of variance (ANOVA) and regression, normality tests of the data were conducted to determine if the data met the assumptions of normality. Except P_{av} and K_{av}, all soil chemical properties met the assumption. For P_{av} and K_{av}, logarithmic and square root transformations were performed. Mean comparisons across agro-ecology, farm typology (resource endowment class), and farmers' indigenous knowledge on soil fertility status were performed using a mixed model. ANOVA for predicted and measured data obtained for Ethiopia (n = 211 for TC and TN, n = 205 for pH, n = 107 for peaks 2930 cm^{-1}, 1620 cm^{-1}, and 1159 cm^{-1}, n = 96 for P_{av} and K_{av}) was conducted using SAS statistical software (version 9.4, SAS Institute, North Carolina, USA). Agro-ecology, farm typology and soil fertility status were considered as fixed effects, while each field and the interaction between individual factors were included as random effects (Piepho et al., 2004). Means separation ($P < 0.05$) was done using pdiff LINES command in GLIMMIX (SAS Institute). Linear regressions were calculated in SigmaPlot (version 10.0, Systat Software Inc., San Jose, CA, USA) to assess the relationship between predicted and measured soil chemical properties.

3.4 Results

3.4.1 MidDRIFTS-PLSR-based generic model prediction

Based on PLSR predictions from the combined data set ('ComCount' model) (Ethiopia, DRC), midDRIFTS-based PLSR values for TC (R^2 = 0.92, RPD = 3.46) and pH (R^2 = 0.89, RPD = 3.02) gave acceptable predictions, while that of TN (R^2 = 0.86, RPD = 2.71) was moderately acceptable (Table 3). Predictions for P_{av} (R^2 = 0.14, RPD = 1.08, RMSEP = 11.5) and K_{av} (R^2 = 0.05, RPD = 1.03, RMSEP = 710) were not successful. Figure 2 shows the relations between measured and predicted values based on the 'ComCount' prediction models described in Table 3. The quality of the 'ComCount' prediction models for TC, TN and pH were further confirmed by significant Pearson's correlation coefficients, which ranged from r = 0.921 to r = 0.956 ($P < 0.001$) (Fig. 2). Although the 'ComCount' prediction models for P_{av} and K_{av} showed limited performance, they provided a significant goodness of fit between measured and predicted values (r = 0.28 to r = 0.34; $P < 0.001$). All generic 'ComCount' prediction models were developed on basis of comparable spectral frequencies (Table 3).

Table 3. 3: Calibration results of midDRIFTS spectra of bulk soils across both countries (Ethiopia, DR Congo), based on independent test validation (n = 183).

Soil chemical properties[1]	Model name	Number of calibrated/ validated samples	Pre-processing method[2]	Spectral frequencies	Model accuracy parameters[3]		
					R^2	RPD	RMSEP
pH	pH ComCount	123/61	1stD +VN	2980-2399,1959-1279	0.89	3.02	0.14
TC [%]	TC ComCount	123/61	SLS	2980-2399,1959-1279	0.92	3.46	2.58
TN [%]	TN ComCount	123/61	1stD	2980-2399,1959-1279, 941-698	0.86	2.71	0.03
P_{av} [mg kg^{-1}]	p_{av} ComCount	123/61	COE	3658-3317,2980-2399,2301-1957	0.14	1.08	11.5
K_{av} [mg kg^{-1}]	K_{av} ComCount	123/61	VN	1620-939	0.05	1.03	710

[1]Soil chemical properties: pH, soil pH; TC, total carbon; TN, total nitrogen, P_{av}, plant-available phosphorous; K_{av}, plant-available potassium.

[2]Pre-processing methods (optimization): 1stD, first derivative; VN, vector normalization; SLS, straight line subtraction; COE, constant offset elimination.

[3]Model accuracy parameters: R^2, coefficient of determination; RPD, residual prediction deviation; RMSEP, root mean square error of prediction.

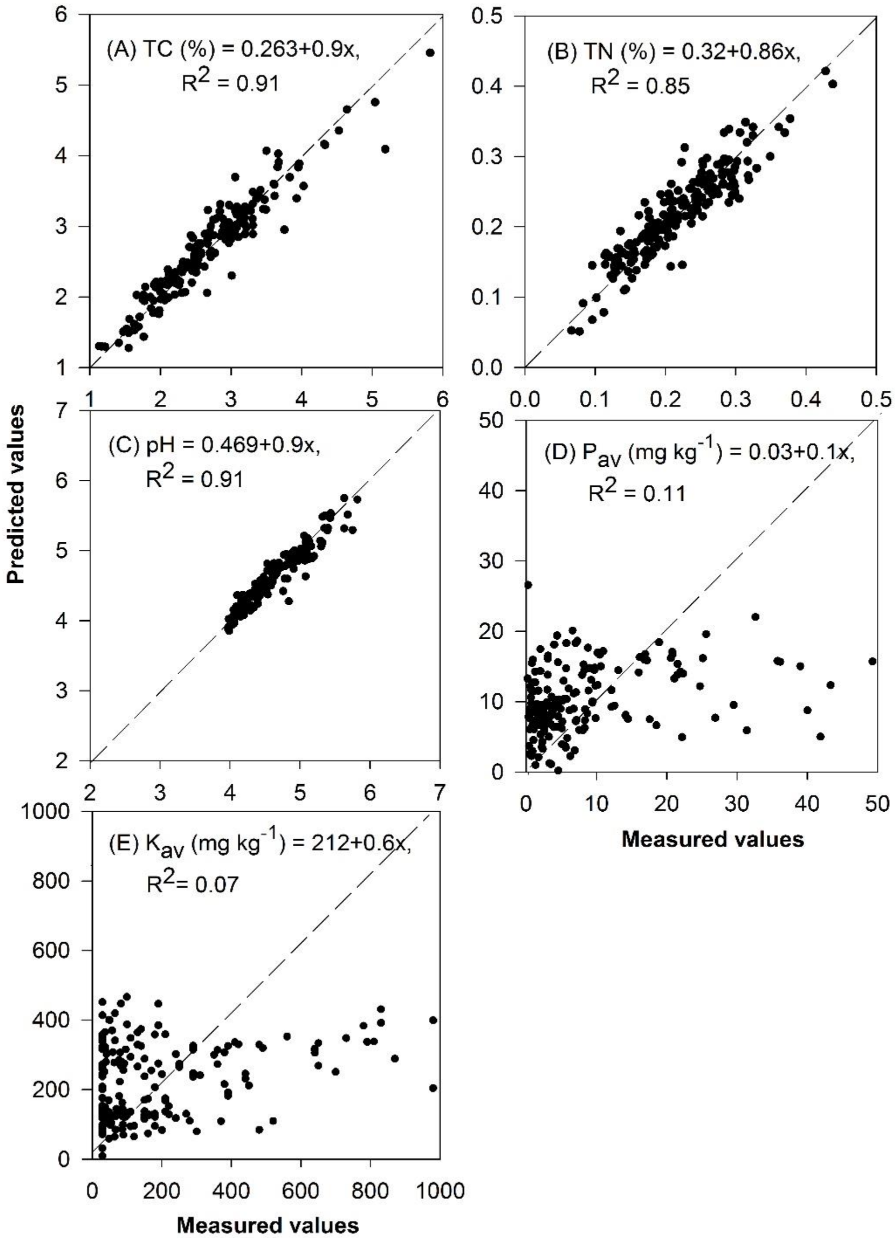

Figure 3. 2: Measured and predicted values of the midDRIFTS-PLSR-based predictions of the selected soil chemical properties (A = total carbon (TC) (%)); B = total nitrogen (TN) (%); C = soil pH; D = available phosphorus (P_{av}) (mg kg^{-1}), E = available potassium (K_{av}) (mg kg^{-1})), using respective 'ComCount' prediction model described in Table 3.

3.4.2 Interrelated effect of agro-ecology and farmers' resource endowment on soil fertility

3.4.2.1 Soil chemical properties

Analysis of variance showed that not only agro-ecology, but also farmers' resource endowment exposed a significant effect on soil chemical properties (i.e., TC, TN, P_{av}, K_{av}, pH; $P < 0.01$) (Fig. 3). In addition, an interaction effect between agro-ecology and resource endowment was observed for K_{av} ($P < 0.01$) (Fig. 3D). Higher K_{av} values (234 mg kg^{-1}) were noted for fields of wealthy farmers in "kola" (K), while lowest K_{av} values (62 mg kg^{-1}) were recorded on wealthy farms in "dega" (D) (Fig. 3D) ($P < 0.01$). Highest values of TC and TN were observed in "weina-dega" (WD) in both farm typologies, while lowest TC was found in the respective fields in D (Fig. 3A) ($P < 0.01$). In "high dega" (HD), higher TC and higher TN contents in K were found in fields of wealthy than less wealthy farmers (Fig. 3A and 3B) ($P < 0.01$). On the other hand, agro-ecology affected soil pH and P_{av} (Fig. 3C and 3E) ($P < 0.001$), where lowest values were observed in WD. No difference was found for factor farm typology for pH and P_{av} (Fig. 3C and 3E) ($P > 0.05$).

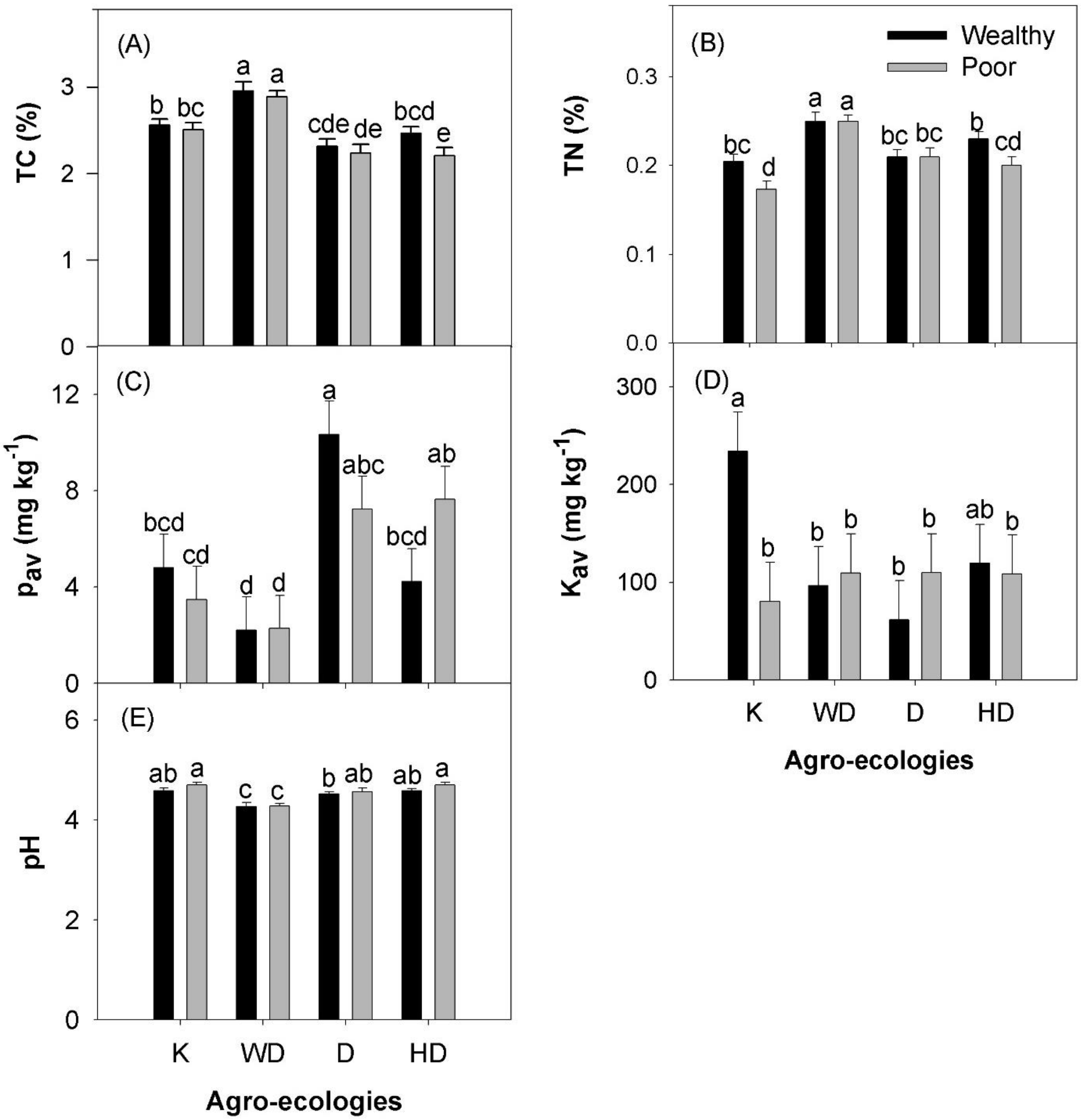

Figure 3. 3: Soil chemical properties (A = total carbon (TC) (%); B = total nitrogen (TN) (%); C = available phosphorus (P_{av}) (mg kg^{-1}), D = available potassium (K_{av}) (mg kg^{-1}); E = soil pH) obtained from soils of fields of wealthy and poor farmers' fields across the four agro-ecologies (K (kola), WD (weina-dega), D (dega), HD (high dega)). Letters on top of standard error bars indicate significant differences at $P < 0.05$.

3.4.2.2 Soil organic carbon functional groups

Three dominant relative peak areas representing SOC functional groups were identified and used as proxies for SOC quality: (i) 2930 cm^{-1} (C-H- aliphatic groups), (ii) 1620 cm^{-1} (C=C- aromatic groups), (iii) 1159 cm^{-1} (C-O poly-alcoholic and ether group) (Fig. 4A to 4C). The relative peak areas of the three SOC functional groups and the SOC stability index, calculated as the ratio of aromatic to aliphatic area (peak 1620 cm^{-1} to 2930 cm^{-1}), varied across agro-ecologies and farmers resource endowment with respective interaction effects (Fig. 4A to 4D) ($P < 0.05$). For example, highest (5.5%) and lowest (3.1%) peaks at 2930 cm^{-1} were noted on fields of poor farmers in K and D, respectively. Similarly, fields of wealthy farmers revealed a larger peak area at 2930 cm^{-1} than those of poor farmers in D (Fig. 4A) ($P < 0.05$). On the contrary, highest (95.2%) and lowest (91.9%) values of relative peak area at 1620 cm^{-1} peak were found in fields of poor farmers in D and K, respectively (Fig. 4B) ($P < 0.05$). The highest relative peak area of 1159 cm^{-1} was observed in K fields of both, wealthy and poor farmers, while the lowest was found in HD for both farm typologies (Fig. 4C) ($P < 0.01$). The highest and lowest SOC stability indexes were calculated for fields of poor farmers in D and K, respectively (Fig. 4D) ($P < 0.001$). In D, a larger index was noted in fields of poor compared to fields of wealthy farmers ($P < 0.05$).

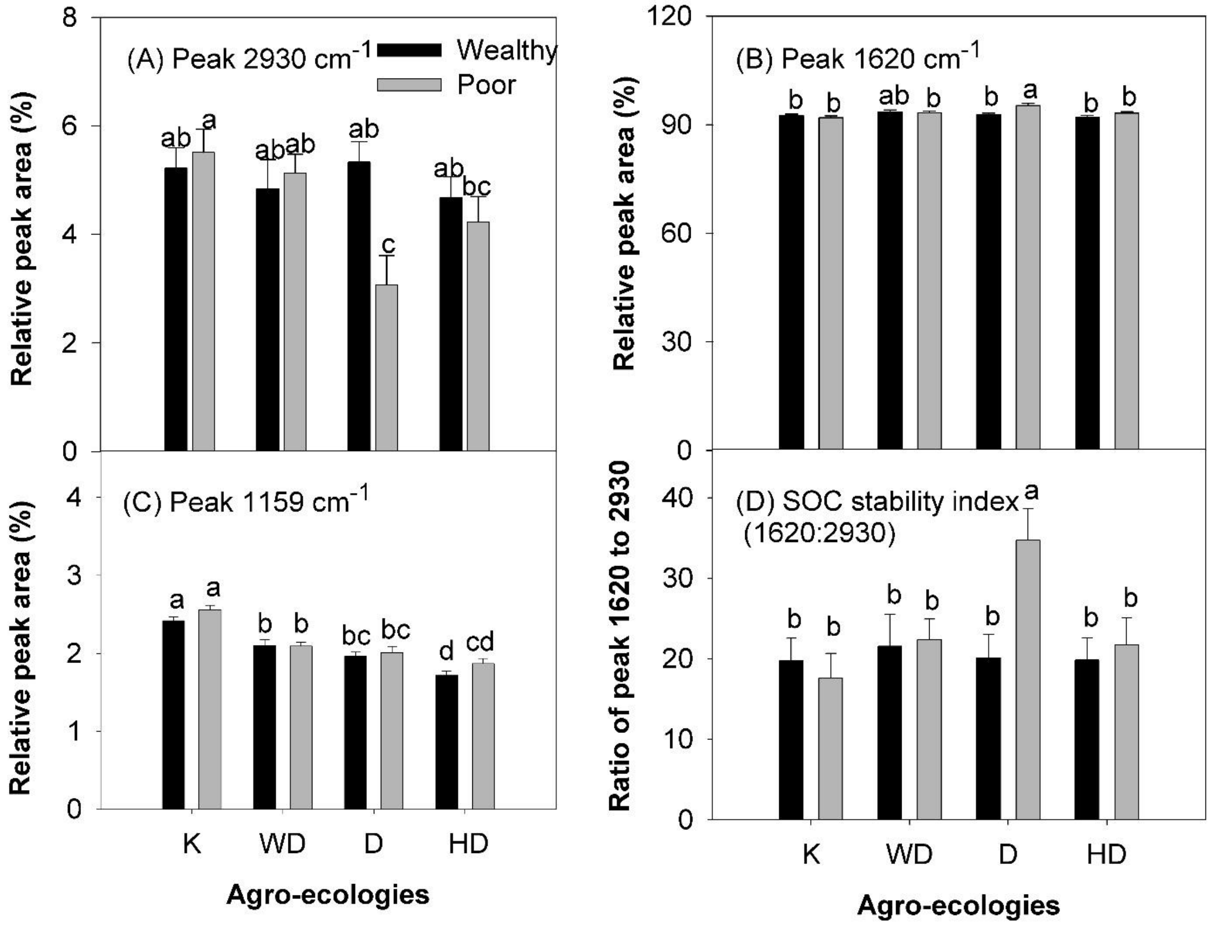

Figure 3. 4: MidDRIFTS relative peak areas ((A) 2930 cm^{-1}, (B) 1620 cm^{-1}, (C) 1159 cm^{-1})) and ratio of 1620:2930 (D) obtained from soils of fields of wealthy and poor farmers' fields across the four agro-ecologies (K (kola), WD (weina-dega), D (dega), HD (high dega)). Letters on top of standard error bars indicate significant differences at $P < 0.05$.

3.4.3 Farmers' indigenous knowledge

In general, when farmers' indigenous knowledge on soil fertility against wet chemistry and midDRIFTS prediction data was evaluated, the pre-defined fertility classes (fertile versus less fertile) were significantly related to P_{av} and pH (Table 4) ($P < 0.05$). An interaction effect between agro-ecology and farmers' indigenous knowledge on soil fertility was noted for pH (Table 4) ($P < 0.05$). Higher pH values were measured in fertile fields in K and HD, whereas higher P_{av} values were recorded in fertile fields in K and WD (Table 4) ($P < 0.01$). However, there was no difference of indigenous knowledge between wealthy and poor farmers towards soil fertility status across agro-ecologies (data not shown). Soil color as soil fertility indicator for farmers indicated that black and brown soils were considered as fertile, while red soils were assigned to less fertile soils. This was confirmed by laboratory analysis, i.e. black and brown soils had generally higher TC, TN, P_{av} and pH than the red soils (Table 5). The indigenous knowledge of farmers towards fertile and less fertile soils was verified by the 1159 cm^{-1} peak area and the SOC stability index (Table 6) ($P < 0.01$). A higher relative peak area of 1159 cm^{-1} was observed in less fertile fields. A similar trend was noted for the SOC stability index.

Table 3. 4: Soil chemical properties: soil pH (pH); total carbon (TC); total nitrogen (TN); available phosphorous (P_{av}); available potassium (K_{av}) in fertile and less fertile fields based on farmers' indigenous knowledge. Standard errors are given in brackets.

Agro-ecology	**Fertility class**	**TC [%]**	**TN [%]**	**P_{av} [mg kg^{-1}]**	**K_{av} [mg kg^{-1}]**	**pH**
Kola (K)	Less fertile	2.49^{bc} (0.08)	0.18^{c} (0.01)	2^{b} (1.37)	158 (40)	4.54^{bc} 0.05)
	Fertile	2.56^{b} (0.07)	0.18^{c} (0.01)	6^{a} (1.37)	158 (40)	4.75^{a} (0.05)
Weina-dega (WD)	Less fertile	2.90^{a} (0.08)	0.24^{a} (0.01)	1^{b} (1.37)	151 (40)	4.13^{d} (0.06)
	Fertile	2.93^{a} (0.08)	0.26^{a} (0.01)	4^{b} (1.37)	78 (40)	4.42^{c} (0.06)
Dega (D)	Less fertile	2.23^{d} (0.08)	0.20^{cd} (0.01)	8^{a} (1.37)	128 (40)	4.55^{bc} (0.06)
	Fertile	2.33^{d} (0.08)	0.21^{c} (0.01)	10^{a} (1.37)	25 (40)	4.54^{bc} (0.06)
High dega (HD)	Less fertile	2.25^{d} (0.09)	0.20^{c} (0.01)	4^{b} (1.37)	78 (40)	4.65^{ab} (0.06)
	Fertile	2.43^{bcd} (0.09)	0.22^{bc} (0.01)	8^{a} (1.37)	158 (40)	4.63^{ab} (0.06)
P-level (fertility class)		NS	NS	***	NS	**
P-level (agro-ecology)		***	***	***	NS	*
P-level (agro-ecology × fertility class)		NS	NS	NS	NS	*

Significance levels: NS, not significant at P < 0.05; *, P < 0.01; ***, P < 0.001.

Table 3. 5: Selected soil chemical properties (TC, total carbon; TN, total nitrogen; P_{av}, available phosphorus ; pH, soil pH) in relation to different soil colors (red, less fertile; black and brown; fertile) across agro-ecologies. Stand errors are given in brackets.

Agro-ecology	Soil color	TC [%]	TN [%]	P_{av} [mg kg^{-1}]	pH
kola (K)	Red	2.89 (0.08)	0.21(0.01)	4.19^{b} (1.25)	4.75^{b}(0.07)
	Black	2.72(0.25)	0.18 (0.03)	15.83^{a} (5.64)	5.13^{a} (0.09)
	P-level	NS	NS	*	*
Weina-dega (WD)	Red	3.00^{b} (0.05)	0.25 (0.03)	1.09^{b}(0.32)	4.12^{b} (0.16)
	Black	3.17^{a} (0.08)	0.28 (0.02)	5.65^{a}(0.91)	4.21^{ab} (0.13
	Brown	3.21^{a} (0.28)	0.28 (0.04)	5.18^{a} (2.8)	4.51^{a}(0.41)
	P-level	*	NS	**	*
High dega (HD)	Red	2.60^{b} (0.45)	0.23^{b} (0.01)	10.33 (6.98)	4.74^{a} (0.29)
	Brown	2.97^{a} (0.41)	0.27^{a}(0.01)	9.44 (7.28)	4.46 ^{b}(0.37)
	P-level	*	*	NS	*

Significance levels: NS, not significant at P < 0.05; *, P < 0.05; **, P < 0.01.

Table 3. 6: Relative peak areas and stability index as indicators of soil organic carbon (SOC) quality with regard to farmers' perception on fertile and less fertile fields. Standard errors are given in brackets.

SOC quality indicators	Fertile	Less fertile	P level
Peak 2930 cm^{-1}	4.95 (0.22)	4.55 (0.22)	NS
Peak 1620 cm^{-1}	92.88 (0.26)	93.18 (0.26)	NS
Peak 1159 cm^{-1}	2.03 (0.03)	2.15 (0.03)	**
SOC stability index (1620:2930)	19.68 (1.57)	24.72 (1.57)	**

SOC quality indicators**:** Peak 2930 cm^{-1}, aliphatic C-H; Peak 1620 cm^{-1}, aromatic C=C; Peak 1159 cm^{-1}, C-O poly-alcoholic and ether groups of SOC functional groups.

Significance levels: NS, not significant at P < 0.05; *, P<0.05; **, P < 0.01.

3.5. Discussion

Heterogeneous soil fertility presents a major challenge to the successful implementation of integrated soil fertility management (ISFM) strategies in Sub-Saharan Africa (SSA), including, but not limited to, Ethiopia and DRC (Vanlauwe et al., 2015). To overcome this constraint, uniform and robust soil monitoring systems that translate into niche-adapted ISFM approaches applicable across regions are required. It was the first aim to develop for two model regions in SSA (i.e., Ethiopia, DRC) generic midDRIFTS-PLSR-based prediction models ('ComCount' models) to predict selected soil fertility indicators, using combined soil chemical data sets of the two countries (Mirzaeitalarposhti et al., 2015). These prediction models were used to survey the soil fertility status in the Ethiopian study region reflecting the main research questions to what extent the hypothesized inter-related effect of agro-ecology and famers' resource endowment determine the inherent soil fertility variability in the study region, and to what extent farmers' indigenous knowledge is suited to distinguish soil fertility status across agro-ecologies and farm typologies. In addition to the survey of selected soil chemical properties, integrated midDRIFTS peak area analysis (i.e., SOC functional groups) was considered as a proxy of SOC quality (Demyan et al., 2012; Senesi et al., 2003).

3.5.1 Inter-related effect of agro-ecology and farmers' resource endowment on soil fertility

It was a key finding that the soil fertility status in the selected Ethiopian study region was determined by an inter-related effect of farmers' resource endowment (farm typology) and agro-ecology. This effect was most pronounced between the wealthy and poor farms located in the lowland (K) and highland (HD) agro-ecologies, as explained by higher TN, SOC and K_{av} in fields of wealthy farms. The farm typologies in the midlands (WD) took an intermediate position with no clear distinction of the soil fertility status with respect to agro-ecology. This finding was in line with Nyamangara et al. (2011) and Masvaya et al. (2010) observing higher TN, SOC, P_{av} and cation exchange capacity (CEC) in wealthy than poor farmers' fields in two different agro-ecologies in Zimbabwe.

The effect of resource endowment in the lowlands was shown by the better soil nutrient status (e.g., TN, K_{av}) in the fields of wealthy farmers than those of poor farmers. It is a main advantage of wealthy farms to have a higher soil fertility status, a result of extended fallowing, organic residue burning and higher livestock numbers (Table 2) a similar finding with Haileslassie eta al., (2006). These features provide sufficient resources to replenish the soil nutrient pool (Haileslassie et al., 2007; Cobo et al, 2010). With this strategy, wealthy farmers also compensate the accelerated decomposition of organic resources by higher temperatures in the lowlands that generally increases the soil nutrient pool (Coûteaux et al., 2002). Apart from the obvious differences in the soil nutrient status in the lowlands, we observed no clear effect of resource endowment on TC content and SOC quality. This was explained with the fast decomposition of active SOC pools, which was, irrespective of the soil fertility management strategy of wealthy farmers, responsible for the pronounced nutrient release. Even though there was no difference between both farm typologies, a higher TC content was found in the warmer lowlands and mild midlands than in the colder highlands, as was earlier reported (Coûteaux et al., 2001; Du et al., 2014; Tian et al., 2016). This increased TC content might have resulted from maize dominated cropping practices in the lowlands and midlands, where the low biochemical quality (high C/N ratio, lignin and poly-phenol content) of respective crop residues enhanced the SOC pool (Wang et al., 2015). Irrespective of the typology classes in the low and medium altitude agro-ecology, it has been shown that the conversion of C derived from crop residues, such as maize, to SOC is generally lower in fields of poor than wealthy farmers due to higher fertilization in the highlands (Wang et al., 2015). This high potential of TC stabilization was corroborated by the presence of recalcitrant SOC pools (i.e., C-O poly-alcoholic and ether groups). In the highlands, contrasting that of low- and midlands, there was a distinct difference of TC content, being higher in the fields of the wealthy than less wealthy farmers. This was explained by the option of wealthy farmers to combine organic and inorganic fertilizer inputs, leading to an increase in C-H aliphatic SOC functional groups, but a decrease of C=C aromatic SOC functional groups. Accordingly, this management option created a higher SOC stability index (i.e., peak area ratio of 1620:2930) in the fields of poor than wealthy farmers. This result contrasted the finding by Balume et al. (in revision), who reported higher C=C aromatic SOC functional groups in fields of wealthy farmers due to less chemical fertilizer use than their counterparts in the Ethiopian highlands. The application of inorganic fertilizer resulted most likely in greater plant biomass production, providing additional resource inputs to accelerate decomposition rate of roots and plant residues to produce more labile SOC pools (Blair et al., 2006). In contrast to the findings in the fields of wealthy farmers, pronounced C=C aromatic SOC functional groups along with a higher SOC stability index were found in the soils of poor farmers in the highland agro-ecology, indicating less organic inputs. Similar results were given by Demyan et al. (2012), who found in plots of the Bad Lauchstädt long-term field experiment (Germany) treated with both chemical and organic fertilizers for more than 100 years higher C-H aliphatic SOC groups than in plots receiving only farm yard manure (FYM). The higher labile SOC pool with lower SOC stability index may be an indicator for high soil fertility as compared to higher C=C aromatic and high stability index because labile pools increased soil aggregate, nutrient supply and can be reserve for microbial energy (Maia et al., 2007; Haynes, 2005; Ghani et al., 2003). This was justified with significant positive correlation of pH and TOC with C-H aliphatic

SOC (r^2=0.39, r^2=0.51) while negative relationships with C=C aromatic SOC (r^2=-0.39, r^2= -0.47) functional groups (P<0.001) (data not shown). On the contrary, C=C aromatic pools increases carbon stabilization and contribute for carbon sequestration (Haynes, 2005).

3.5.2 Validation of farmers' perception using soil fertility indicators across agro-ecologies and farm typologies

This study also tested farmers' indigenous knowledge towards soil fertility status in relation to either the individual factors or inter-related effects of agro-ecologies and farm typology. The identification of soil fertility status based on farmers' indigenous knowledge is often in good agreement with soil chemical properties analyzed in the laboratory (Belachew & Abera, 2010; Haileslassie et al., 2007; Schuler et al., 2006; Yeshaneh, 2015). Irrespective of their wealth status and geographic location, we confirmed that farmers had the capacity to assess soil fertility variability using their indigenous knowledge accumulated through many years of experience and consistent exchange through socio-cultural events (e.g., weddings, funerals) between lowland and highlands (Leta et al., 2018). Such knowledge transfer across agro-ecologies may have been responsible for the homogenously distributed soil fertility perception by smallholder farmers.

Farmers describe and classify their soils using a holistic approach and use relatively homogeneous soil classification indicators across agro-ecologies (Laekemariam et al., 2017). As described by several authors, farmers have been using soil color, soil texture, soil depth, topography and drainage as criteria to categorize their land into fertile and less fertile fields (Belachew & Abera, 2010; Corbeels et al., 2000; Yeshaneh, 2015). In the low- and midlands, a higher variability between fertile and less fertile fields was observed for soil pH and P_{av}. Farmers considered red soils as less fertile and used this as an indicator for soil acidity (soil pH) (Laekemariam et al., 2017). The low P_{av} values might have been a result of P fixation in acidic soils (Agumas et al., 2014). On the contrary, black soils were interpreted as fertile with high SOC and P_{av} contents (Moody et al., 2008). Similarly, we detected higher TC and P_{av} values in black than red soils in the midlands and lowlands, respectively. Higher P_{av} values in black than red soils may have resulted from higher organic P cycling favored by higher SOC and soil moisture content (Corbeels et al., 2000; Moritsuka et al., 2014).

We observed no difference between farm typologies to identify their fertile and less fertile fields based on indigenous knowledge, a likely result of the informal communication channels among social institutions: e.g. 'iddir' (an indigenous and voluntary self-help association in the local community), 'debo' (a collective labor support group to help each other), and 'dado' (a reciprocal labor sharing arrangement among farmers) (Leta et al., 2018). Even though farmers are generally limited to explain on a scientific basis why such differences of soil fertility exist, both wealthy and poor farmers have comparable indigenous knowledge to identify fertile and less fertile fields.

The indigenous knowledge is generally used to design management strategies for site-specific soil fertility problems. Farmers in the lowlands, for example, fallow, burn organic residues and apply higher FYM on fields perceived as fertile. Similarly, farmers in the highlands invest more inorganic fertilizer on their fertile fields than on those with lower fertility. This corroborates the fact that farmers are aware of the soil fertility status, whereby their indigenous knowledge can guide site-adapted ISFM interventions (Tittonell et al., 2005b).

3.6. Conclusions

In the presented study, we found that the inter-related effect of agro-ecology and farmers' resource endowment (farm typology) was a stronger determinant of the soil fertility variability in the studied farming systems than the individual factors. It was inferred that prospective ISFM strategies must be niche-based considering contrasting agro-ecologies and farm typologies to reduce the inherent depletion of soil fertility across smallholder farms in the study region of Ethiopia. Moreover, in all agro-ecologies, farmers identified fertile and less fertile fields based on their indigenous knowledge, which was corroborated by the laboratory-based soil fertility survey.

Our conclusions were based on the development and validation of generic midDRIFTS-PLSR-based models (‘ComCount’ models), using a combined data set retrieved from field surveys in Ethiopia and DRC. For this approach, a well-designed and comparable study site selection was considered allowing a representative farm typology inclusion and homogeneous soil sampling procedure in both countries. Similarly, the wet chemistry analyses and midDRIFTS measurements were uniform for both country data sets. For prospective soil fertility surveys, however, we suggest to extend midDRIFTS-PLSR-based calibrations and validations to additional soil spectra and associated lab-based data originating from other Central and Eastern African countries. This will complement spatially less detailed soil fertility survey approaches of the AfSIS and EthioSIS platforms, finally translating into a more accurate ISFM intervention at regional scale.

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CHAPTER 4

Potential proteolytic enzyme activities modulate archaeal and bacterial nitrifier abundance in soils contrasting in acidity and organic residue treatment

4. Potential proteolytic enzyme activities modulate archaeal and bacterial nitrifier abundance in soils contrasting in acidity and organic residue treatment

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4.1 Abstract

Abstract

Current mechanistic knowledge of soil nitrogen (N) cycling mediated by microorganisms lacks in understanding of the functional links between activities of proteolytic extracellular enzymes that provide substrate for nitrifying populations. This relates specifically to soils of different acidity and organic residue treatments. Our hypothesis was that organic residues of high decomposability applied to less acidic soils promote proteolytic enzyme activities modulating the abundance of nitrifiers. This was justified by the presumed benefit of available substrates to microorganisms under less acidic soil conditions. Organic inputs of high (HQR) and medium (MQR) quality differing in decomposability ((Lignin+Polyphenol)/N ratio of 5.1 (HQR) versus 8.1 (MQR)) were

incubated in less acidic (S5.1) and more acidic (S4.3) soils for 60 days. Soil samples were obtained at defined time intervals and analyzed for potential activities of alanine aminopeptidases (AAP), leucine aminopeptidases (LAP), and thermolysin-like proteases (TLP), along with the abundance of nitrifying bacteria (AOB) and archaea (AOA). Analysis of covariance (ANCOVA) revealed a significant positive relationship of proteolytic enzyme activities with abundance of AOB and AOA, even though the extent of this relationship was more dependent on soil pH and time than organic residue quality. Notably, the positive relationships were pronounced at the later stages of the incubation period. Within the course of the incubation, AOB benefitted from the release of N substrates (NH_4^+, NO_3^-, DON) spurred by proteolysis in S5.1. For MQR and HQR, AOA showed comparable dynamics in S4.3, indicating a niche specialization between AOB and AOA depending on soil acidity and resource availability.

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4.2 Introduction

Nitrogen (N) cycling has been linked to ecosystem functioning, capable of transforming various N forms through microbially mediated processes, including proteolysis and nitrification (Isobe and Ohte, 2014; Prosser, 2007). While recent literature emphasized nitrification and proteolysis mainly in isolation (Rasche et al., 2017; Muema et al., 2016; Musyoki et al., 2015; Rasche et al., 2014), the ecological importance of their functional interaction has so far been neglected. Elucidating the link between these two processes would provide an advanced mechanistic understanding of soil N cycling mediated by soil microorganisms.

Both, proteolytic and nitrifying microorganisms metabolize N as resource substrate (Turner et al., 2017; Robertson and Groffman, 2006). Proteolysis, the initial step of soil organic N cycling, is considered to be rate limiting (Rasche et al., 2017; Weintraub and Schimel, 2005). Organic N compounds (e.g. proteins, peptides) are decomposed by a broad range of extracellular proteases secreted by microorganisms (i.e. *Bacillus* sp., *Pseudomonas* sp.) (Vranova et al., 2013; Bach and Munch, 2000). These include, among others, various metalloproteases (Vranova et al., 2013; Wu and Chen, 2011; Fuka et al., 2007) and aminopeptidases (Steinweg et al., 2018; Štursová and Baldrian, 2011; Poll et al., 2006). Proteolytic cleavage of proteins and peptides releases amino acids as part of the dissolved organic N (DON) pool, which become available for microbial uptake and assimilation to primarily fulfil the carbon (C) requirement of microorganisms (Huygens et al., 2016; Farrell et al., 2014). Ammonification completes the N cycle involving an organic compound, where ammonium (NH_4^+) is released from amine or amide groups mineralization (Romillac, 2019; Strock, 2008). Thereafter, bacterial and archaeal nitrification is spurred by oxidation of NH_4^+ (Geisseler et al., 2010; Leininger et al., 2006; Nicol and Schleper, 2006).

Environmental factors, including soil acidity, as well as availability and biochemical quality of organic residues, determine the composition and functional potential of soil microbial communities (He et al., 2012; Cookson et al., 2007; Bending et al., 2002), including proteolytic and nitrifying microorganisms (Dodds et al., 2017). Low soil pH ($pH < 5.5$) was shown to favor nitrification by archaea (AOA), a contradicting picture was provided for nitrifying bacteria (AOB) (Li et al., 2018; Jiang et al., 2015; Gubry-Rangin et al., 2010). While Muema et al. (2015) revealed a flexible response of AOB to soil pH changes, Zhao et al. (2018) showed that increasing soil pH (4.6 to 5.7) translated into increasing nitrification rates of AOB, further confirming their preference for available NH_4^+ as substrate. Soil pH was reported to also control the synthesis of proteolytic enzymes (Ai et al., 2015; Sinsabaugh et al., 2008; Acosta-Martínez and Tabatabai, 2000). Leucine (LAP) and alanine (AAP) aminopeptidase activities reach their optimum at a soil pH of 7.2, similar to that of thermolysin-like proteases (TLP) and other metalloproteases (Sinsabaugh et al., 2008; Sousa et al., 2007 Niemi and Vepsäläinen, 2005). Generally, the use of optimum pH in enzyme activity measurements provides a measure of the maximum potential activity of a selected enzyme under natural conditions (Talley and Alexov, 2010; Niemi and Vepsäläinen, 2005), although the optimum activity of a particular enzyme may vary in response to contrasting soil physico-chemical conditions (Niemi and Vepsäläinen, 2005; Stemmer, 2004). This circumstance may influence the functional relationship of proteolytic and nitrifying communities.

The magnitude of extracellular enzyme activities has been related to organic residue quality and availability (Sinsabaugh et al., 2002; Sinsabaugh and Moorhead, 1994). Generally, decomposition of organic residues releases N (proteins) from both microbial cells and N protected in complex polymers, such as lignin (L), polyphenols (PP) and cellulose (CL) (Shindo and Nishio, 2005; Mafongoya et al., 1997). Low concentrations of L and PP relative to N ((L+PP)/N ratio) have been acknowledged to facilitate decomposition, hence, stimulate the abundance of proteolytic communities (Rasche et al., 2014). A similar effect was reported for AOB, while abundance of AOA was suppressed (Muema et al., 2015). It could be deduced that a high level of soil acidity rather than alkaline soil conditions along with organic residue quality benefits the discussed relationship among proteolytic and nitrifying soil microorganisms. Accordingly, a stimulation of proteolytic microorganisms may modulate the abundance of nitrifying communities under low soil pH, with respective feedbacks on the soil mineral N pool (Wild et al., 2019; Fiorentino et al., 2016).

The aim of this study was to provide a clearer understanding of the functional linkage between the potential activity of selected proteolytic extracellular enzymes (AAP, LAP, TLP) and the abundance of nitrifying populations (i.e. gene copies of the *amoA* gene coding ammonia monooxygenase as functional marker for AOB and AOA) in two soils of varying acidity treated with two biochemically different organic residues. Our hypothesis was that organic residues of high quality (low (L+PP)/N ratio) applied to less rather than more acidic soils will result in a positive relationship between the functional potential of proteolytic enzymes and abundance of nitrifying communities. This was justified by the presumed higher benefit of available substrates to microorganisms under elevated soil pH status (Saiya-Cork et al., 2002; Chen et al., 2014).

4.3 Material and methods

4.3.1 Soils and organic residue materials

Two soils classified as humic Nitisol (IUSS Working Group WRB, 2014; Erkossa et al., 2018) with similar clay texture were selected for the present study. The two soils differed in soil acidity, whereby a soil pH of 5.1 (noted as S5.1) was collected from a farmers' field in Bushumba in South-Kivu, Eastern Democratic Republic of Congo (DRC) (2º 21'S, 28º 49'E, 1740 m above sea level (a.s.l.)). The second soil with pH of 4.3 (noted as S4.3) was obtained from a farmers' field in Lelissa Dimtu Kebele, an administrative unit of Diga district, Ethiopia (9°02'N, 36°24'E, 1281 m a.s.l.). The sampling locations were selected according to the known differing soil pH levels at both sites. Soil S5.1 was collected from a field with cassava-legume intercropping with low input farm management. Soil S4.3 was collected from a field with maize-livestock mixed farming. Soil samples of the topsoil layer (0-20 cm) were obtained according to the sampling design of Vågen et al. (2012). Soil samples were air-dried, passed through a 2 mm sieve, and transferred to the University of Hohenheim (Stuttgart, Germany) for further processing. Prior to the start of the incubation experiment, soil chemical characteristics were determined using standard procedures: S5.1, total carbon (TC) 35.2 g kg^{-1}, total nitrogen (TN) 2.9 g kg^{-1}, plant available phosphorus (P_{av}) 0.014 g kg^{-1}, plant available potassium (K_{av}) 0.204 g kg^{-1}; S4.3, TC 23.2 g kg^{-1}, TN 1.5 g kg^{-1}, P_{av} 0.007 g kg^{-1}, K_{av} 0.140 g kg^{-1}.

Two types of above-ground residues (leaves, twigs) of the tropical shrub *Calliandra calothyrsus* were collected, a medium quality residue (MQR) from Kenya and a high quality residue (HQR) from DRC. Biochemical quality of organic residues was determined according to VDLUFA (2012) and presented in g kg^{-1}: total nitrogen (TN) (MQR: 19.9; HQR: 22.5), total extractable polyphenol (PP) (MQR: 63.4; HQR: 46.2), acid detergent lignin (L) (MQR: 102; HQR: 68). TC (MQR: 426,4; HQR: 408,8), Cellulose (MQR: 208; HQR: 170) Hemicellulose (MQR: 143; HQR: 101). Decomposability of plant residues was mainly defined by their (L+PP)/N ratios (Rasche et al., 2014), which was 8.1 for MQR and 5.1 for HQR.

4.3.2 Set-up of the incubation experiment

Leaves and twigs (ratio 2:1) of air-dried residues of C. *calothyrsus* were chopped to 5 to 8 mm length and thickness of less than 1 mm diameter. A total of 1500 g dried composite sample of each soil was pre-incubated for 4 days at 60% water holding capacity (WHC) and 25°C. After soil pre-incubation, 33 g sub-samples of the soils were mixed each with 0.33 g of each MQR or HQR. Mixtures were transferred into 50 ml plastic jars as experimental units. In total, 90 samples were arranged in an incubation chamber (60% WHC, 25°C, no light), using a randomized complete block design with 6 treatments (S4.3-MQR, S4.3-HQR, S5.1-MQR, S5.1-HQR, 2 control soils (S4.3, S5.1) without organic residues) × 5 sampling dates (7, 15, 30, 45, 60 days of incubation) × 3 replications. During incubation, WHC of 60% was maintained by adding distilled water, if necessary.

After each sampling, soil pH (Houba et al., 2000), mineral N (NH_4^+, NO_3^-) (Joergensen and Brookes, 1990; Bamminger et al., 2014) and soil moisture were directly determined from fresh soil samples removed from the incubation chamber. The values of soil pH remained constant over the experimental period. A remaining proportion was frozen at -28°C before further processing (e.g. gene abundance (section 2.3), enzyme activities (section 2.4), dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) (Mueller et al., 2016)).

4.3.3 Gene abundance

DNA was isolated from 0.5 g of each frozen soil sample using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Solon, Ohio, USA) following manufacturer's instructions with slight modifications. Two extra washings with 500 µL of guanidine thiocyanate (5 M) were applied before washing with SEWS-M buffer to avoid contamination with humic acids (Cabrol et al., 2010). Quality of extracted DNA was checked on 1.5% (w/v) agarose gels prior to photometrical quantification (Nanodrop ND™-2000/2000c spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). DNA extracts were stored at -28°C until further analysis.

Quantification of target genes (ammonia-oxidizing bacteria (bacterial *amoA* gene; AOB) and archaea (archaeal *amoA* gene; AOA)) was determined according to Rasche et al. (2011). All qPCRs were run on a StepOnePlus™ Real Time PCR System (Applied Biosystems). For quality check, melting curves of amplicons were generated and reaction efficiency determined (AOB 98%, AOA 99%) using StepOne™ software version 2.2.2 (Applied Biosystems).

4.3.4 Potential enzyme activities

Kinetics (nmol of 7-amino-4-methyl coumarin (AMC) hr^{-1} g^{-1} dry soil) of potential activities of leucine-aminopeptidases (LAP); alanyl-alanyl-phenyl aminopeptidase (AAP) and thermolysin-like proteases (TLP) were determined as the rates of fluorescence of an enzymatically hydrolyzed substrate containing the highly fluorescent compound AMC (i.e., L-Leucine-AMC hydrochloride (LAP) and Ala-Ala-Phe-AMC hydrochloride (AAP) (Sigma-Aldrich), Suc-Ala-Ala-Phe-AMC hydrochloride (TLP) (Bachem AG, Bubendorf, Switzerland)) (Agumas et al., 2021; Rasche et al., 2017; Marx et al., 2001).

4.3.5. Statistical analysis

To estimate the effects of factors (soil pH, residue quality, time), analysis of variance (ANOVA) was performed from a linear model (Piepho, 2000), implemented in the software package R (version 3.6.0, R Core Team, 2019). Analysis of covariance (ANCOVA) was followed to evaluate the relationship between the potential activity of proteolytic enzymes (LAP, AAP, TLP) and abundance of nitrifying genes (AOA, AOB). A linear regression model was extracted from the overall fitted ANCOVA to provide significance of the relationship between the potential activity of proteolytic enzymes and abundance of nitrifiers (AOA, AOB), considering the soil pH range, organic residue quality and time of incubation as covariates, by estimating their respective contribution to the total variation from the fitted model (Marill, 2004). Data were checked for normality and homogeneity of variance on model residual using quintile-quintile (Q-Q) plots, histogram and studentized residuals plots (Kozak and Piepho, 2018). Data transformation was performed at log scale to meet model assumptions. Accordingly, models were selected using the Akaike Information Criterion (AIC). Possible pairwise comparisons of least square means and latter display from mixed model procedure were used to separate treatment means. Linear regressions were applied to reveal relationships between gene abundance and potential activities of selected enzymes with soil chemical properties. Graphical representation was performed in R software using *ggplot function()* in the ggplot2 package.

4.4 Results

4.4.1 Descriptive statistics

All measured biological and chemical soil properties were responding significantly to the three factors, except AOB and NH_4^+ for soil pH (Table 1). Similarly, significant interactions of tested factors were found for most of the soil properties, except for "Residue quality × Soil pH" (TLP, AOB) and "Residue quality × Time" (TLP). Except TLP, that was only affected by Time (Fig. 1c), AAP and LAP were shaped by "Soil pH" and "Time" (Fig. 1a-b). AOB responded to the interaction "Soil pH × Time" (Fig. 1d), while for AOA the interaction of "Residue quality × Time" was effective (Fig 1e).

Table 4. 1: Analysis of variance to reveal significant effects and interactions of soil biological and chemical properties.

Factors	Enzymes activities			Gene abundance			Chemical properties		
	AAP	LAP	TLP	AOB	AOA	NH_4^+	NO_3^-	DON	DOC
Residue quality	***	***	*	**	*	**	***	***	***
Soil pH	***	***	***	ns	***	ns	***	***	***
Time	***	***	***	***	***	***	***	***	***
Residue quality × Soil pH	***	***	ns	ns	*	*	***	*	***
Residue quality × Time	***	***	ns	***	***	***	***	***	***
Soil pH × Time	***	***	***	***	***	***	***	***	***

Significance levels: *** = P<0.001; ** = P<0.01; * = P<0.05*; ns, not significant (P>0.05).

Enzymes: AAP: Alanine aminopeptidase; LAP: Leucine aminopeptidase; TLP: Thermolysin-like proteases.

Gene abundance: AOB: Ammonia oxidizing bacteria; AOA: Ammonia oxidizing archaea.

Chemical properties: NH_4^+: Ammonium; NO_3^-: Nitrate, DON: Dissolved organic nitrogen; DOC: Dissolved organic carbon.

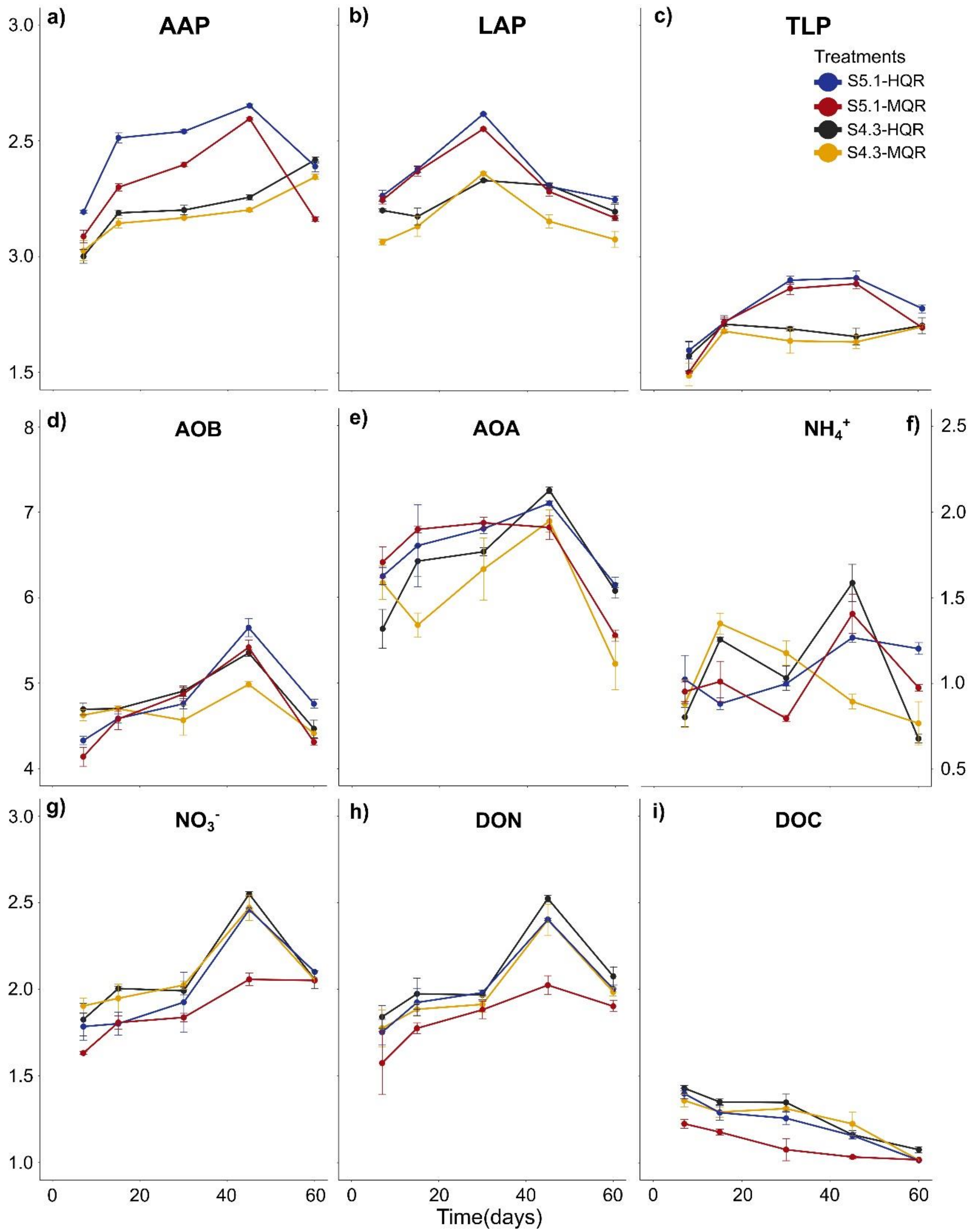

Figure 4. 1: Biological (a-e) and chemical (f-i) soil properties as influenced by organic residue quality (medium (MQR) versus high (HQR) quality) and soil pH (4.3 (S4.3) versus 5.1 (S5.1)) along the incubation period. Enzymes: AAP: Alanine aminopeptidase (nmol AMC h^{-1} g DM^{-1});

LAP: Leucine aminopeptidase (nmol AMC h^{-1} g DM^{-1}); TLP: Thermolysin-like proteases (nmol AMC h^{-1} g DM^{-1}). Gene abundance: AOB: ammonia oxidizing bacteria (gene copies g DM^{-1}); AOA (gene copies g DM^{-1}): ammonia oxidizing archaea. Chemical properties: NH_4^+: Ammonium (mg kg^{-1}); NO_3^-: Nitrate (mg kg^{-1}), DON: Dissolved organic nitrogen (mg kg^{-1}); DOC: Dissolved organic carbon (mg kg^{-1}).

4.4.2 Relationships of potential enzyme activities and gene abundance in response to soil pH, organic residue quality and time

Potential activities of proteolytic enzymes revealed generally a significant relationship with the abundance of AOB and AOA, even though the extent of this relationship was more dependent on soil pH and time, but not organic residue quality (Table 2). For AOB and their relationship with LAP, no influence of any factor and interaction was determined.

Using AAP as predictor, only in the less acidic soil (S5.1), a positive relationship of AAP was found for AOB ($r^2 = 0.752$, $P<0.001$) and in high acidic soil (S4.3) no relationship was observed between AAP and AOB ($r^2 = 0.009$, $P>0.05$) (Fig. 2a). A similar pattern was revealed for the relation of AAP to AOA in less acidic ($r^2 = 0.415$, $P<0.001$) and high acidic ($r = 0.0301$, $P>0.05$) soil (Fig. 2b). When considering LAP as predictor, a relatively low contribution of S4.3 was detected for AOB ($r^2 = 0.164$, $P<0.05$), while S5.1 had no influence ($r^2 = 0.031$, $P>0.05$) (Fig. 2c). For AOA, a relatively higher relationship than AOB was found for both soils (S5.1; $r^2 = 0.335$, $P<0.01$; S4.3, $r^2 = 0.26$, $P<0.01$) (Fig. 2d). TLP showed a positive relationship with AOB ($r^2 = 0.689$, $P<0.001$) (Fig. 2e) and AOA ($r^2 = 0.253$, $P<0.01$) (Fig. 2f) in S5.1, but not in S4.3.

Factor “Time” had a significant influence for most relationships between proteolytic activity and nitrifier abundance, except LAP and AOB as well as TLP and AOA (Table 2; Fig. 2). Incubation time also revealed a significant interaction with soil pH ($P<0.05$), except LAP, while no interaction was found with residue quality. These positive relationships between potential enzyme activities and nitrifier abundance were pronounced at the later stages of the incubation period (Fig. 2). Then, AOB showed positive correlations with AAP (r = 0.81, P<0.01), LAP (0.85, P<0.01) and TLP (r = 0.80, P<0.01) at Day 45, as well as AAP (r = 0.60, P<0.05) and TLP (r = 0.69, P<0.05) at Day 60 (Table S1). For AOA, such relationships were effective at Day 30: AAP (r = 0.72, P<0.01), LAP (0.71, P<0.01) and TLP (r = 0.61, P<0.05). LAP maintained its positive relationship with AOA from Day 15 (r = 0.76, P<0.01) to Day 60 (r = 0.77, P<0.01). Notably, AAP, LAP and TLP revealed strong correlation with AOB and AOA at the later decomposition stage (Table S2).

Table 4. 2: ANCOVA effect model analyses for gene abundance, potential enzyme activities and assayed factors.

Factors	AOB			AOA		
	AAP	LAP	TLP	AAP	LAP	TLP
Residue quality	ns	ns	ns	ns	ns	ns
Soil pH	**	ns	**	*	ns	*
Time	**	ns	**	*	**	ns
Soil pH × Residue quality	**	ns	**	*	ns	*
Soil pH × Time	*	ns	*	*	*	*
Residue quality × Time	ns	ns	ns	ns	*	ns

Significance levels: *** = P<0.001; ** = P<0.01; * = P<0.05*; ns, not significant (P>0.05).

Enzymes: AAP: Alanine aminopeptidase; LAP: Leucine aminopeptidase; TLP: Thermolysin-like proteases.

Gene abundance: AOB: Ammonia oxidizing bacteria; AOB: Ammonia oxidizing archaea.

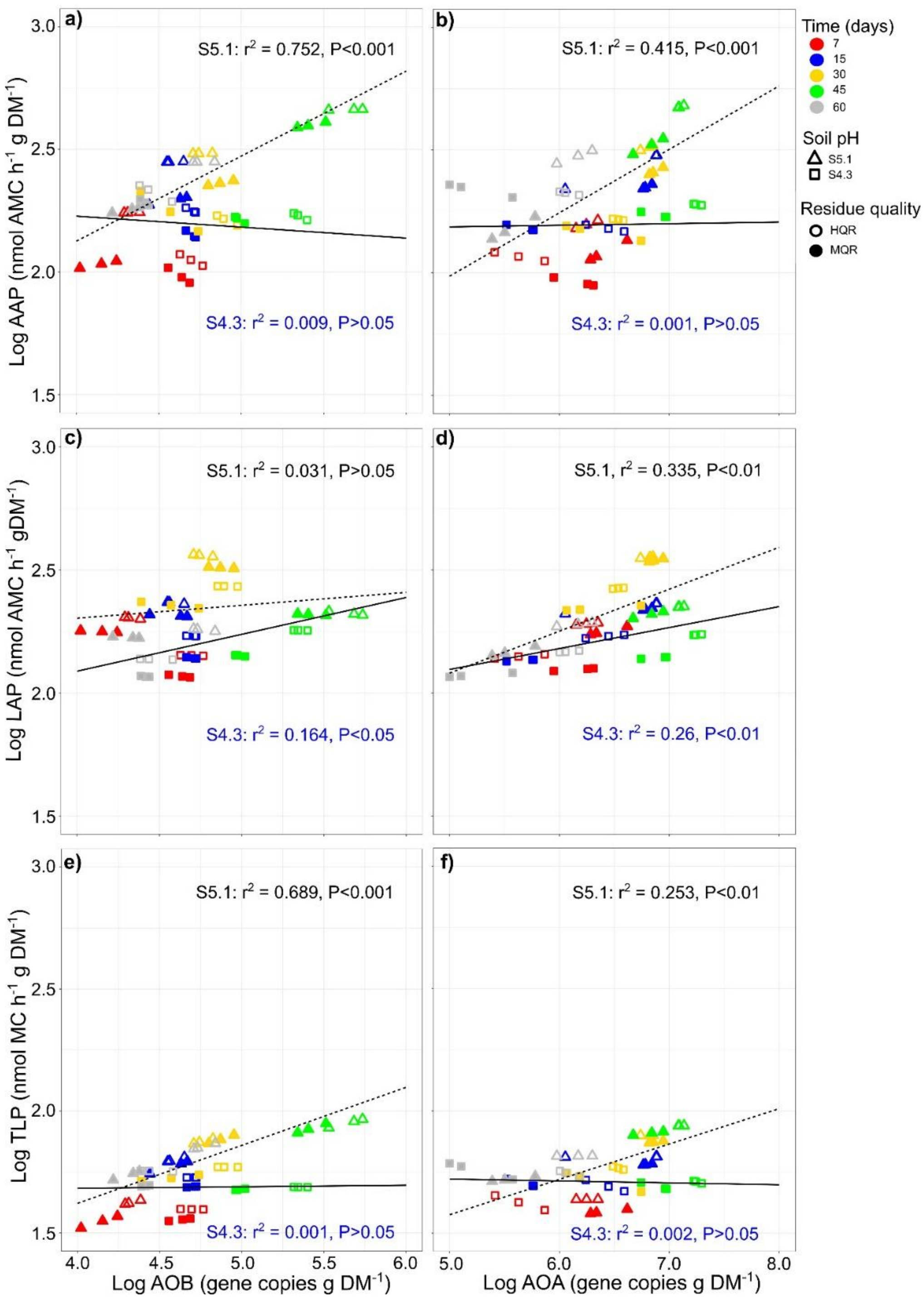

Figure 4. 2: Linear relations between potential proteolytic enzyme activities and *amoA* gene abundance, as a result of a combined soil pH and residue quality effect: a) AAP and AOB, b) AAP and AOA, c) LAP and AOB, d) LAP and AOA, e) TLP and AOB, f) TLP and AOA. Enzymes:

AAP: Alanine aminopeptidase; LAP: Leucine aminopeptidase; TLP: Thermolysin-like proteases. Soil pH: S4.3 = soil pH of 4.3, S5.1 = soil pH of 5.1. Residue quality: HQR = high residue quality, MQR = medium residue quality.

4.4.3 Correlations of biological and chemical soil properties

In S5.1, NH_4^+ showed a positive correlation (r) with AAP (r = 0.50), TLP (r = 0.53) and AOB (r = 0.74), while NO_3^- correlated positively with AAP (r = 0.48), TLP (r = 0.40) and AOB (r = 0.66) (Table 3). DON correlated positively with all soil biological properties, except LAP, while DOC revealed negative correlations with TLP (r = -0.44) and AOB (r = -0.37). In soil S4.3, NH_4^+ showed a positive correlation only with AOA (r = 0.48), while NO_3^- correlated positively with LAP (r = 0.37), AOB (r = 0.53) and AOA (r = 0.49). In the same soil, DON correlated positively with AOA (r = 0.70), while DOC revealed a positive correlation with TLP (r = 0.43) (Table 3). Raw data of chemical soil properties can be retrieved Table S3.

Table 4. 3: Pearson correlations (r) of potential enzyme activities and gene abundance with soil chemical properties.

Chemical properties	Soil pH	Enzymes activities			Gene abundance	
		AAP	LAP	TLP	AOB	AOA
NH_4^+	S5.1	0.50**	ns	0.53**	0.74***	ns
	S4.3	ns	ns	ns	ns	0.48**
NO_3^-	S5.1	0.48**	ns	0.40*	0.66***	ns
	S4.3	ns	0.37*	ns	0.53**	0.49**
DON	S5.1	0.70**	ns	0.62***	0.62**	0.39*
	S4.3	ns	ns	ns	ns	0.70***
DOC	S5.1	ns	ns	-0.44*	-0.37*	ns
	S4.3	ns	ns	0.43*	ns	ns

Significance levels: *** = P<0.001, ** = P<0.01, * = P<0.05*; ns, not significant (P>0.05).

Enzymes: AAP: Alanine aminopeptidase; LAP: Leucine aminopeptidase; TLP: Thermolysin-like proteases.

Gene abundance: AOB: ammonia oxidizing bacteria; AOA: ammonia oxidizing archaea.

Chemical properties: NH_4^+: Ammonium; NO_3^-: Nitrate, DON: Dissolved organic nitrogen; DOC: Dissolved organic carbon.

4.5 Discussion

The hypothesis that a less acidic soil treated with organic inputs of high biochemical quality would increase the potential activity of proteolytic enzymes, and hence, modulate the abundance of nitrifiers was verified by this study. This positive relationship was specifically reflected between the abundance of ammonia oxidizing bacteria (AOB) and the potential activities of alanine aminopeptidase (AAP) and thermolysis-like proteases (TLP). Both proteolytic and nitrifying groups prefer less acidic soils (i.e. pH of 5.1) with easily decomposable organic resources (HQR; C/N ratio: 18.2, PP+L/N ratio: 5.1) to maintain metabolic functions (Noll et al., 2019; Esch et al., 2017; Carey et al., 2016; Zhang et al., 2012). A comparable relationship, but of lower magnitude, was noted for ammonia oxidizing archaea (AOA), corroborating their general ability to cope with a broader soil pH range to sustain their metabolism, including NH_4^+ oxidation (Muema et al., 2016; Hu et al., 2014; Gubry-Rangin et al., 2010). It was thus deduced that AOB rather than AOA benefitted from the proteolysis of HQR, which partially compensated the N limitation under lower pH conditions (Zhang et al., 2012; Sinsabaugh et al., 2008). Furthermore, the acknowledged niche specialization between AOB and AOA (Prosser and Nicol, 2012) was verified. Activities of leucine aminopeptidase (LAP), in contrast to AAP and TLP, showed no obvious relation with neither AOB nor AOA, suggesting no traceable dependence of nitrifying populations on LAP activities to sustain their N demand.

Not only the higher soil pH provided appropriate conditions for proteolytic activities (Leprince and Quiquampoix, 1996; Feng et al., 2016), but also the consequential soil nutrient changes shaped the cascade of N cycling investigated here. The slower release of N (i.e. ammonium (NH_4^+)) in MQR was supposedly responsible of the slight suppression of microbial activity in the more acidic soil (S4.3). Oppositely, N mobilization under less acidic and HQR conditions may have been promoted, as a result of higher NH_4^+ availability (16 ± 2.4 mg kg^{-1} (S5.1) versus 13 ± 3.6 mg kg^{-1} (S4.3)) and higher total soil N content (TN; 29 mg kg^{-1} (S5.1) versus 15 mg kg^{-1} (S4.3)). This enhanced resource availability in the less acidic soil may have favored the metabolism and proliferation of proteolytic and nitrifying groups, as also reflected in reduced investment to scavenge N resources (Sinsabaugh et al., 2008; Mutabaruka et al., 2007). Likewise, the prevalence of HQR in the more acidic soil provided sufficient substrate to benefit proteolytic enzyme activities and hence nitrification, corroborating the functional relationship of both processes under low soil pH.

It was inferred that in the initial decomposition phase, labile residue compounds were decomposed using residual soil nutrient (e.g. N) resources, while in the subsequent phase, generated energy conserved in microbial biomass was invested to decompose more recalcitrant fractions of applied organic residues (Herzog et al., 2019; Poll et al., 2010; Moore et al., 2004). This involved the decomposition of organic residues with high protein-polyphenol complexation (MQR) (Muema et al., 2016). In an analogous study, the dependence of energy demand on the decomposition stage was explained with a higher microbial carbon use efficiency in the less acidic (pH 5.1) soil amended with HQR than in the more acidic (pH 4.3) soils amended with MQR, due to less energy investment in microbial metabolism in the former case (Agumas et al., 2021). Accordingly, AOB rather than AOA profited from the proteolysis of HQR-derived proteins to counteract the N limitation under low pH conditions (Zhang et al., 2012).

LAP activity, on the other hand, was less sensitive to NO_3^- and NH_4^+ availability, with a lower magnitude in S5.1 than S4.3. This fact may have been founded in a change of enzyme kinetic efficiency (Loeppmann et al., 2016; Chen et al., 2018). Proteolytic enzymes, including LAP, have their optimum activity around a neutral pH (Sinsabaugh et al., 2008; De Kreij et al., 2002; Feder and Schuck, 1970), although some studies suggested different pH optima in the context of varying soil mineralogical backgrounds and resource alterations (Moorhead et al., 2016; Turner et al., 2014). However, if this metabolic flexibility explains the discussed absent interrelated effect of soil pH and organic residue quality on LAP activities will be a matter of prospective research.

4.6 Conclusions

This study showed that soils differing in acidity treated with organic residues of different biochemical quality are key factors modulating the functional relationship between selected proteolytic enzyme activities and the abundance of nitrifying prokaryotes (AOB, AOA). The given experimental set-up under controlled incubation conditions provided clear indications that AOB rather than AOA benefitted from N substrate release spurred by proteolysis. This functional relationship was specifically prevalent at low soil pH, suggesting a soil pH and resource-dependent niche distinction between AOB and AOA. To verify the given assumptions about the functional relationships between proteolytic and nitrifying soil communities, it is suggested to extrapolate and substantiate the presented results in field studies considering soils with a broader soil acidity range and organic residues with more distinct biochemical qualities. In addition, future studies should also consider the activities of enzymes involved in the degradation of recalcitrant C compounds (e.g., lignin) including, but not limited to polyphenol oxidase and peroxidase to account for their effect on the studied functional, substrate-dependent relationships of N cycling microorganisms in the later stages of decomposition (He et al., 2019; Muema et al., 2016).

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CHAPTER 5

General discussion

5. General discussion

5.1 Overview

Soil fertility depletion in smallholder farms continues to be a fundamental biophysical threat to agricultural production leading to food shortages and poverty in SSA (Buresh et al., 1997; Kamuru and Jama, 2005). The use of blanket fertilizer recommendation is no longer appropriate for farmers because of the large heterogeneity in crop growth as result of soil fertility variability (Tittonell et al., 2006). This fertility variability has caused crop yield gaps, putting smallholder farmers in a cycle of poverty (Tittonell and Giller, 2013). Within the frame of this PhD thesis, the development of midDRIFTS for mapping soil fertility levels across spatial scale was achieved, suited to assess soil fertility variability in and among African agricultural farming systems across regions (Chapter 2 and 3). The midDRIFTS approach developed was essential as it is robust in harmonized protocol applicable across regions of contrasting agro-ecological and sociological context.

As soil management options, this PhD study has tested organic residue of *Calliandra calothyrsus*, a leguminous shrub to promote build-up of long-term soil fertility. The use of organic residue a component of ISFM is known to favor soil microbial activities through biological processes of soil microorganisms that play a fundamental role in defining soil quality. Although results from the use of *C. calothyrsus* residue treatment from our controlled experiment has led to the successful control activities of proteolytic enzymes independently from nitrifying gene abundance, its contribution in shaping the relationship between potential activities and gene abundance is questionable. The main reason for this is lack of consideration of wide range in biochemical composition of litter material. Previously, many studies on organic residue management focused on C/N ratio as a major biochemical characteristic that influence decomposition, yet little is known for lignin and polyphenlols that are key to play in organic residue decomposition process from which soil microorganisms drives soil nutrient cycling. This PhD study has combined gene abundance and enzymatic activitites to provide understanding of soil processes (proteolysis and nitrification) in the N cycle as regulated by soil pH and organic input quality. Recommendations for best site-suited management options that promote soil health will be discussed.

5.2 Soil fertility gradient, spatial variability in smallholder farms

This PhD study has considered a number of factors including limited access to land, financial capital and inputs, high level of vulnerability in market participation that characterize smallholder farmers in comparison from large-scale, profit-driven enterprises. Our result suggests that economic status in interaction with biophysical factors had influenced soil fertility, implying that access to farm management practices including access to inputs for resource endowed farmers was the reason of high level of soil fertility status in wealthy farmers' field comparing to farms of both medium and poor resource endowment classes. Resource endowment so far documented as useful tool assisting in understanding diversity among smallholder farmers in making decisions for targeting specific management strategies was proven in the case studies from DRC and Ethiopia (Chapters 2 and 3).

However, the threshold for clustering farmers based on their wealth has not been harmonized across countries, questioning regional comparisons. For instance, land ownership has been seen as important source of livelihood and indication of wealth in certain countries (e.g., DRC), while for others both land and livestock are highly interlinked (e.g., Ethiopia) revealing diversity of smallholder farmers. However, access to fertilizers remain challenging in both study regions because of high cost of importation. It should be noted that the Africa continent does not produce mineral fertilizers, a situation that contributes to scarcity of this resource. To cope with this situation, it would be appropriate for those farmers with good economic status i.e., larger land sizes, to locally generate organic inputs from their lands. However, this option does not fit for farmers holding small sizes of land as food production has to be prioritized. Another influential factor could be accessing labor, as this is not a major constraint for wealthy farmers. It could be because of purchasing power to cover related costs that wealthy farmers have, making their lands more productive than those of poor and medium wealthy farmers. This results in soil fertility variability between farms within and outside locations.

Previously, spatial variability has been indexed to agro-ecology alone e.g., diagnosis of nutrient deficiency research implemented in Western Kenya, Zimbabwe and Malawi (Tittonell et al., 2005a; Zingore et al., 2007; Cikowo et al., 2014). However, the results from these studies could not be extrapolated to rest of the continent due to limited number of country samples. Nziguheba et al. (2008) identified beyond N and P limitation, Ca and Mg and S were also severely affecting maize fields in West Africa. Unlikely, some studies revealed variation in soil nutrient losses between and even within agro-ecologies (Giller et al., 2011; Ebabu et al., 2020). For instance, moderate rate of depletion was found in humid forest and wet land of Central Africa, whereas high rate of nutrient mining is reported in the sub-humid savannas of West Africa and the highlands and sub-humid areas of East Africa (Hanao and Baanante, 2006; Tully et al., 2015).

It is obvious that only in tackling both biophysical and socio-economical context in which smallholder farmers live together, will provide a clear understanding of the source of soil fertility variability. African farming system is diverse in space (e.g., resource endowment), variability that goes through time (i.e., dynamism) and often multidimensionality in terms of strategy (production and consumption decisions). As a result, not all farmers are equally fertility constrained, resource-poor or market oriented in their production systems. For this purpose, any effort to understand smallholder farming systems needs to acknowledge this difference.

Evidence from this PhD study has demonstrated the interconnection between socio-economic (market access, resource endowment) and biophysical (site and agro-ecology) factors defining soil fertility status. Meaning that, farmers management decisions are dependent on wealth status. However, when markets opportunity was present, even the poor farmers were embarked in fertility management as shown in Mushinga site (DRC).

Beyond the study region, soil fertility variability has been observed even between countries, questioning the generalization of soil fertility generated through soil information systems (AfSIS, EthioSIS, RwaSIS). The reason for such result is that these surveys remain of high prediction of soil properties partially because of ignoring the contribution of socio-economic aspects (e.g., farm resource endowment) throughout their methodological set-up. It is a parameter that need to be accounted in formulation of management recommendations. As matter of fact, different crop responses to fertilization was revealed within and between farmers' fields in Western Kenya (Tittonell et al., 2007; Vanlauwe et al., 2010). By investigating further in understanding the actual cause of such situation, it became clear that socio-economic conditions in which farmers live were not considered. Tittonell et al. (2008b) revealed resource endowment has been a major influential factor in allocation of farm management resources. On the other hand, biophysical conditons such as topography highly influenced fertility gradient in high and mid-lands. This is because of organic matter accumulation at the bottom of hills, bringing soil fertility variability. Also, soil mineralogy and soil type as well as elevation significantly influence soil fertility status and need further investigation. Becoming clear that both socio-economic and biophysical conditions were causing fertility gradient.

Accounting for market distance to study fertility gradient has been a novel aspect that this PhD study, contributing to the existing body of knowledge for further understanding complexity in soil fertility of smallholder farmers. The gradient of fertility was explained by economic opportunity provided to farms of closer distance, indicating that accessing the market boosted farm income. Because their produces were easily sold in the market, hence allowing farmers to re-invest in management for continually maintaining production. That benefited soil fertility irrespective of farm typology.

This evidence led to the conclusion that market opportunity stimulated investment in soil fertility management for all the farmers, as easier access to improved seeds and fertilizers facilitated such decisions. Whereas, farmer plots belonging to poor farm typology class in the remote had lower soil nutrient stocks than those of wealthy farm typology. This was explained by the lack of proper farm management practices in farm plots of poor typology class that cannot afford the cost of fertilizers inputs aggravated by absence of market opportunity. Even though, accessibility and affordability of agro-inputs in this part of Africa still have a long way to go, as mineral fertilizers remain luxury goods due to their high prices, complicating more the situation of poor typology farming in remote areas with no market opportunity.

5.3 Local adaptation as entry point for targeting site specific management recommendations

Generalization of soil fertility has misleading experts in formulation of recommendations, instead, variation of soil fertility at farm level need to be included in formulation of soil management solutions. This PhD study revealed variation of soil fertility within site, which tended to increase between wealthy and poor resource endowment. Similarly, between sites at few miles away as result of site-specific characteristics. Consequently, this variation in soil fertility was translated into yield performance differences (Vanlauwe et al., 2011; Rahn et al., 2018).

If possible, field specific soil fertility recommendation that fit local conditions would be ideal for targeting specific ISFM recommendations. However, complexity of factors surrounding smallholder farms need to be covered in order to propose effective management strategies. Looking back into previous studies dealing with management of tropical soils, agro-ecology has been neglected in formulation of recommendations. Yet, agro-ecological zones have been established to fit different cropping systems (FAO/IIASA, 2005; FAO/IIASA, 2012). Ignorance of this parameter has resulted partially in failure of most of the agricultural technologies developed in the past (e.g., alley cropping, legume integration, etc.). Furthermore, fertilizers blanket recommendation often 60 Kg of N ha^{-1} was formulated based on long term research trials, implemented without recognizing spatial variation, making it difficult for certain crops to meet their nutrient demand just few miles from the test fields.

With the current condition, the majority of smallholder farmers does not have access to fertilizer inputs. This situation put farming at risk of so called “poverty trap”. On one hand, absence of structured market for agricultural inputs as a major obstacle for increasing farm productivity. One the other, failure in dissemination of proven agricultural technologies that could participate in revolutionizing agriculture sector. For instance, adoption of improved seed varieties and fertilizers have resulted in doubling farm productivity through green revolution in Asia. Today, agriculture in Africa could learn from positive aspects of the Asia green revolution of the 1960s and adapt the approach to actual situation including challenges from natural resource degradation, soil erosion, crises of climate change in addition to financial crisis and Coronavirus pandemic that definitely will have consequence on the food system. It would be important to engage stakeholders involved in food systems to explore their respective role and how these could be linked to others to accelerate transformative actions in support to achieve the sustainable development goals (SDGs).

Generated solutions need to able to respond to dualism that face agriculture in smallholder farmers today. On one hand, growing population density has been a reason for reduction of land sizes, pushing farmers to continually cultivate their small portion of land without fallowing period, a situation that has resulted in nutrient mining. On the other, expansion of new fields (i.e., opening new lands for agricultural purposes) putting pressure on forests, jeopardizing preservation of natural resources. Therefore, this study supports the sustainable intensification (SI) approach as a way forward for developing agriculture production in SSA. But, this cannot be achieved without including fertilizer into farm management package.

As a way forward, since most of African governments have adopted the Abuja declaration that declared fertilizers as a strategic commodity to be subsidized across Africa to meet the Green Revolution, its effects have to be felt by smallholder farmers through easier access to mineral fertilizer inputs. Essential for agricultural production, this commodity has been neglected in the past by African governments leaving farmers in vulnerable conditions. It is clear today that African farming systems suffer from both accessibility and affordability of mineral fertilizers inputs. For instance, farmers of remote areas should only rely on in situ production of organic inputs such as compost, manure and organic residue. The major challenge is that even organic inputs produced locally are of lower quality therefore not able to satisfy crop demand. In addition, lack of knowledge in handling fertilizer for those farmers living closer to urban center with access to mineral fertilizers is limited. This has partially contributed to the lower productivity in the places where extension systems are weak and cannot fully support dissemination of agricultural technologies (Lambrecht et al., 2014).

The fundamental assumption of site-specific fertility management recommendation, is to ensure optimization of fertilizers across agricultural fields toward nutrient use efficiency. Monitoring soil testing need to be affordable to allow farmers to appreciate fertilizer requirements. Promising technologies such as precision-farming technique of micro-dosing allow farmers to reduce spreading mineral fertilizer and enhance fertilizer use efficiency by applying only recommended rates closer to seeds. However, fertilizer amendment is not always beneficial for all soils. Some non-responsive soils continue to be major challenges for increasing crop productivity towards achieving the agronomic efficiency (Sanchez, 2010; Assenga et al., 2015; Kihara et al., 2016). Cost-effective soil and land management techniques that are profitable for farmers' business and minimize risk related to production and soil degradation are required.

Although, for achieving greater response to fertilizer input application, soils need to hold a minimum of fertility serving as nutrient starter to boost fertilizers response. At this point, more research to determine the organic matter stock level will be crucial in order to plan for better fertility rehabilitation. Meanwhile, prioritization of management such as agro-ecological farming may be promoted as this respond to on-farm fertility variability. Additional technologies such of smart farming which consider not only location but also data, context awareness and time. As known so far different soil type and texture influences nutrient dynamics together with the microbiome.

5.4 Organic inputs and N availability in smallholder farming systems

Understanding N dynamic has been challenging because of the spatial and temporal variability characterizing agricultural landscape. This dynamic is often regulated by soil microbes that are negatively affected by biotic and abiotic factors including lack of organic inputs in smallholder farmers of the tropics. By testing the effect of *C. calothyrsus* organic residue, the result from this PhD study revealed greater activities of enzymes in less acidic soil that has received high quality residue [(lignin + polyphenol)-to-N ratio] partially because of N substrate that was easily accessible for soil microbes. As N excess from decomposition of plant litter was available for nitrifiers communities that allows them to perform nitrification process which resulted in niche specialization between AOB and AOA. In this process, depolymerization of N, consisting in breaking down proteins into amino acids, release of compounds and soil nutrient was performed by proteolytic enzymes. At this level, biotic and abiotic factors including residue quality have been reported to influence this step. That is why biochemical quality attributes have to be considered that regulate N release emphasizing not only limited to C/N ratio, but also the (lignin + polyphenol)-to-N ratio.

Within tropical environmental conditions, application of readily degraded residue material of high quality such as leguminous of *C. calothyrsus* favors decomposition and as well as short-term increase in the labile N pool during the growing seasons. Whereas, application of low quality material in the same conditions generally favors immobilization, a process that might result in accumulation of organic matter and promotion of humus formation. The latter increases more the potential for improving soil structure through persistence of recalcitrant (high lignin and polyphelons) that prolong decomposition.

In this PhD study, the medium quality residue treatment [i.e. high (lignin + polyphenol)-to-N ratio] released N was unable to satisfy microbial demand, because of slow decomposition of plant material. This was attributed to the substrate characteristic of medium quality residue (MQR) which had more complex of lignin and polyphenols, not easily degradable by microbial enzymes. At this point, building longer term fertility together with organic matter stock can be manipulated, especially by taking advantage of soil nutrients being released gradually into rhizosphere. That is why it is essential to account for organic residue quality inputs that is suitable management strategies to achieve production objective. As documented C, N, lignin and polyphenols levels in the substrate influence activity of soil microbes responsible to catalyze reactions to release nutrient. Ultimately, recalcitrant materials are broken down and get transformed into humic substances important in formation of soil structure and nutrient storage.

When the organic residue was added to the soils, microbes responsible for decomposition were utilizing both C and N from available organic substrate to build their biomass. Manipulation in building soil fertility will be achieved when plant residues are added to the soil at faster rate than soil organisms convert it to CO_2. Here C will gradually accumulate in the soil to fuel sequestration of this resource. This is more likely to bring back restoration of soil physical, chemical and biological fertility through building SOM. But also nutrient turnover as some of the N in the organic matter is converted to plant-available nitrogen (NO_3^- and NH_4^+), as well as to fulfil other soil functions (Berry et al. 2002).

However, turnover rate has not been studied deeply in relation to soil fertility variability and diversity of smallholder farming systems (Fungo et al., 2019; Purwanto and Alam, 2020). This would open the so-called black box of soil microbial dynamics with environmental characteristics to shape nutrient status. As fundamental knowledge, it is known that organic residue has to pass through microbial decomposition in the soil, where N is getting released and made accessible for both microbes and plant roots. Putting microbial activity at the center of nutrient transformation in agroecosystems should be key for replenishing soil fertility. The challenge is that inorganic N is often available at the earlier stages of fertilizer application, often leading that to lost through leaching and runoff.

Also maintaining ammonium N into the system has been challenging for agriculture since decades, this is due to the short time that roots can take up N. However, several approaches including those that control ammonium substrate availability and those that inhibit ammonia oxidizers organisms are proposed, including timing of fertilization to coincide with rapid plant uptake, agricultural practices strategies of controlling ammonium substrate availability. That is why initiatives such as formulation of fertilizers with slow release properties are encouraged as this will enable to continuously provide plant with the exactly N demand to support growth. Another promising strategy is to inhibit nitrifier organisms through direct chemical compounds that slow nitrification of ammonium. Even though, commercial inhibitors are effective but their use is not straightforward. However, the interactions of native nitrifying organisms with plants (e.g., *Brachiaria humidicola*) or microbes producing nitrification inhibitors (e.g., urease, ammonia monooxygenase) and this can be a promising approach, but yet they need to be critically examined. Effective management strategies will need to consider optimization of timing during which N is applied.

Others factors such of geochemical and environmental need to be considered. Because climate, soil types and temperature, rainfall and soil pH influence nutrient availability, therefore the demand to supply N for building microbial biomass is essential. The nature of tropical soils and their fertility remains highly influenced by weathering process. Thus, it has been the primarily source in dropping properties such soil pH, which has a direct consequence in raising aluminum (Al) and iron (Fe) levels to become toxic. This condition contributes negatively to solubility of most nutrients and microbial activities that support crop nutrition. According to Grozier et al. (2010), when soil pH drops below 5.5 level, reduction of N, P, K, Ca, Mg, Su and Mo are more likely to occur from soil solution.

The nature of parent material also plays a major role. For instance, inherent rich material, such basalt is more likely to develop more fertile soils than soil formed from granitic material containing fewer mineral nutrients. Besides, soil texture is another important factor, for example clay mineral surface hold both living and dead biomass. Two mechanisms can explain the increasing of clay content with the increase of SOM. First, the bond between the surface of clay particles and organic matter prolong decomposition process. Second, soil with high clay content increase the potential for aggregate formation.

With numerous options of organic inputs available in smallholder farmers, animal manure has been presented to be promising option, but this has recently attracted more concern in relation to food safety and emission of methane gas becoming a major concern for the globe. Compost could be alternative solution, however, this has relatively low amount of plant nutrients stock and mostly N is not directly available to crop directly right at the time of application rather during the next cropping season. Furthermore, most of organic residues is limited from both quality and quantity needed to satisfy soil demand. The process of cut and curry plant biomasses may result in fertility reduction at the production site by transporting nutrient through organic residue. In addition, land sizes will be another limitation for adoption of such activity as there will be tradeoff between food and biomass production (Kell, 2011).

For better understanding organic residue decomposition processes and how they will be affected by stress from climate change, there is need to study the link between N pools and biochemical quality attributes during the late decomposition stages to further elucidate the role of physical quality of plant material. Coupled with physiological parameters, this will provide information concerning the climate change induced modifications of biochemical quality in organic residue material. Likewise, linking decomposition to humus formation by keeping focus on factors controlling N availability during different stages of decomposition process.

5.5 Soil microbial activity towards feedback between carbon and nitrogen cycles

As a result of this PhD study, the relationship between activity of enzymes and nitrifier gene abundance was revealed towards the end of the incubation. This was due to the fact that N substrate to feed the nitrifier community (AOB and AOA) had been made available ahead by enzymes that perform the first step in decomposing organic material. It should be noted that during this process C and N actively involved at various pathways to balance microbial demand e.g., C is being consumed through microbial respiration and N (i.e. proteins to peptides and peptidases) to inorganic (amino acids) to balance the reaction. In the soil, C resource is stored in microbial biomass constitute of the living C pool and the non-biomass in recalcitrant plant structures (i.e., lignin, cellulose and starch).

However, C cycle does not operate independently from that of N, both are in permanent interaction in order to satisfy microbial metabolisms. Compound of N such as amino acids and proteins are required for growth of microbial biomass. It is only when the needs for microbial demands are satisfied, prerequisite condition to be accomplished ahead of nutrients being released for other uses. However, the extent to which microbial activities are linked to soil C, N remains unclear. We can only relay on data from long-term field experiments that suggest organic input treatments require more time before they start supplying crop demand (Ebhin Masto et al., 2006; Bai et al., 2018). This is due to the level of decomposability of organic materials that depend on biochemical composition as well as environmental factors. As known the product of litter mass loss is the sum of CO_2 released and discharge of various compounds including that of C. Here C is returned into atmosphere as CO_2 through heterotrophic respiration. This shows that in addition to microbial activity, C and N cycles are the backbone of processes occurring in agroecosystem. From a single study interaction between C and N that control decomposition pathways have to be clarified with a special focus on C sequestration and litter decomposition as source of inorganic N.

The C provides substrate and energy to support microbial activity indicator of soil health, even though the amount of microbial biomass which is the easiest biological measured indicator estimated to be less than 35% of the total organic C in the soil (Li et al., 2018). To benefit from this resource, implementation management options that allow the storage of carbon are in need, because this resource is at the center of living organisms in the agro-ecosystem. On the other hand, SOM continues to be a reservoir of nutrient including N and P that supply plant productivity. That is why it will be of great importance to explore how soil microbes participate in SOM content with direct effect to increase aggregate stability. Larger aggregates that hold plant-available water, create aeration and drainage while supporting microbial activity.

According to Agumas et al. (2021), higher carbon use efficiency (CUE) was obtained in less acidic (pH 5.1) soil amended with residue of higher quality than in more acidic (pH 4.3). This was influenced by management regime, as in less acidic soils favorable microbial development, therefore, questioning the biophysical characteristics. If solution is to be provided to such biophysical constraint, management options that contribute to raising soil pH need to be prioritized. Only soils with acceptable range of nutrient status and soil pH tend to respond to proposed management practices. For instance, more acidic soils may induce suppression of microbial activities that reduced decomposition and therefore resulting in lower CUE.

The major challenge for organic management input systems is that timing for N release often does not coincide with the demands of root uptake. This is where most of N in agricultural soil gets lost in the system, as the result of rapidly conversion of ammonium ion (NH_4^+) into nitrate (NO_3^-). Reason for this study to include nitrification process carried out by ammonia oxidizing bacteria

(AOB) and archaea (AOA). These organisms gained energy from NH_4^+ to NO_3^- reaction and their growth by fixing of inorganic C into biomass. To mediate their growth, the energy remains dependent on NH_4^+/ NH_3 and/or NO_2^-. Once C entered the soil, strategies for its storage (i.e., C sequestration) need to be promoted, that is why agricultural practices that increase organic inputs in the system and participate in reviving soil microbial activities are encouraged.

Another biophysical factor is the clay content that have been revealed to influence nutrient dynamics in the soil. However, it impacts on soil microbial community need further studies (Muema et al., 2016). In addition, the extent to which climate will influence on C cycling are confounded with microbial adaptation to certain environmental factors and remain unknown. Six et al. (2006) suggested that the relative abundance of fungi to bacteria is of high importance, because more stable C is being formed in soils with high fungal/bacteria ratios. This might be the explanation for fungi to have a higher CUE than bacteria reflected in more biomass per unit of C utilized.

Previous studies (Nielsen et al., 2011) reported relationships between soil biodiversity and C cycling processes such as respiration, but most of them have only focused on total species richness but not the C specialized species function. For now, is not clear yet how the microbial community are using litter C influencing the status microbial biomass. Furthermore, the full range of geo-chemical factors controlling CUE in a single study have not been characterized. Ultimately, it is not clear to what extent microbial community within agricultural systems can be manipulated for C sequestration playing a significat role in mitigation of climate change. If microbial community is important in C sequestration, the next step should be to understand the functional group specific for that expression and as well the proportion for each. This information may be the capital in building SOM models. It is concluded by pointing that for further research there is need for quantification of the proportion of C from the residue plant material that goes for specific microbial group and with group partitioning its use in different pools (biomass, metabolites and CO_2 production). It is important to remember that microbes catalyze most of the transformations of soil nitrogen into plant-usable forms. Diffrernt soil microbes are involved in various geochemical cycles where they interact. Overall, the processes should promote applied inputs for the benefits of agricultural management.

5.6 Limitations of the study

During soil fertility assessment this PhD study did not assess the amount of fertilizer inputs (organic and inorganic) allocated per ha. That additional information would have reinforced our findings related to diminishing of soil fertility with increasing market distance. It was assumed that farmers' fields near market centers benefited from market participation to easily selling agricultural produce, therefore continue investing in soil fertility management than farmers' fields in the remote. Previously, gradients of fertility have been revealed with increasing distance from the homestead as result of differences in resource allocation in farmer field (Tittonell et al., 2005b). The reason was that home garden fields have accumulated nutrients from compost and animal feeds over time than remote fields. In addition, collecting information on labor allocation would have been essential to reinforce our argumentation explaining soil fertility differences between resource endowment classes. The assumption would be that wealthy resource endowment may have benefited from intensive labor than the poor resource endowment farmers, factor that could result in severe labor shortage on farms of poor resource endowment, therefore, negatively impacting soil fertility of their lands.

This PhD study did not assess mass loss of the *C. calothyrus* residue. This information could have provided the rate of decomposition allowing to reinforce assumptions on biochemical attributes. Previous studies have used such set-up to assesses organic matter breakdown, important functional process in the changes of soil organic resource applied to the soil over time (Knacker et al., 2003). In addition to mass loss, soil texture as an important physical property was missing. This could have reinforced argumentation on differences in initial fertility status between the two soil classes (soil pH 5.1 versus 4.3). The study of Muema et al (2016) revealed modulation of microbial community structure by soil texture. Finally, the lack of a higher range in biochemical quality [(high quality (L+PP)/N = 8) versus (low quality (L+PP)/N = 5)] used for this study may have been a short coming. It was deduced that selection of residue quality of more contrasting biochemical quality could react differently leading to stronger effect on nitrifying community.

5.7 Recommendation for future studies

I recommend to conduct more studies on site specific contribution to fertility in order to evaluate the effects of biophysical, socio-economic on farming systems to fit formulation of management strategies to local adaption. Careful assessment of landscape needs to be considered as this is composed with different geological material influencing the nature of soils. Future studies should explore other biophysical and socio-economic factors that could contribute to fertility variability. There is a need to enlarge the typological indicators to off-farm household income due to the fact that extra-farm activities generate cash that may also be allocated to farm management.

For further understanding of ecological functioning of soil microbes, I recommend for future studies to combine phospholipid fatty acids (PLFA) and DNA-based stable isotope probing. These techniques will contribute in determining specific microbial species active in performing specific processes in the soil. Regarding organic input management, there is a need of including seasonal leguminous of high quality i.e. with easily decomposable attributes in order to increase N supply at earlier stages of decomposition so that seasonal crops can utilize that at reproductive stages. Future decomposition studies need to consider temperature sensitivity to adapt the proposed organic residue management practices to fit dynamic of the C pools stored in SOM, key question in climate change research and fertility rehabilitation of tropical soils. Knowing that the increase in temperature of 1°C could lead to a loss of about 10% of soil organic C, affecting both microbial community structure and functions (Wei et al., 2014). Careful examination of the interaction between soil type and residue quality would be especially useful for improving the efficacy of organic inputs in managing soil fertility. More research is needed to better understand the role of microbial functions in proposed soil management strategies. Finally, future studies should consider age of organic residue inputs, as lignin and polyphenol concentrations accumulate over time in plant cells. Finally, there is a need of extending this PhD study to field-based experiment in order to capture various environmental factors that occurr on farmers' fields.

5.8. Summary

Soil fertility in tropical agroecosystems is often subjected to degradation that leads to nutrient depletion with negative effects on land productivity and food security. This challenge is aggravated by the complexity of socio-economic (market distance, farm typology) and biophysical (agro-ecology, site) conditions causing soil fertility variability. Consequently, blanket fertilizer recommendations cannot be applied in areas of high fertility variability. In this PhD study, methods were harmonized to assess drivers of soil fertility status across regions. Despite being pointed as factors contributing to soil fertility variability, market access, farm typology (resource endowment) and agro-ecology have not been subjected to soil fertility assessment. This PhD study aimed mainly at verifying that these factors have to integrated rather than considered in isolation to enable accurate assessments of soil fertility across spatial scales and socio-economic gradients.

It was hypothesized that market distance and farm typology is a determinant of agricultural development in Democratic Republic of Congo (DRC). As market distance is increasing, the soil fertility status of smallholder farming systems decreases despite farmers' wealth. In a parallel study conducted in Ethiopia, it was complementarily hypothesized that the soil fertility status is also influenced by inter-related effects of agro-ecology and farm typology. As nitrogen (N) is known to be limiting in smallholder farms, conservation and sustainable provision of this nutrient will be essential to achieve niche-based integrated soil fertility management (ISFM) strategies. Therefore, understanding of the ecological processes (proteolysis, nitrification) that control soil N availability through organic residue management in varying soil fertility variability conditions will be essential. Low concentrations of lignin (L) and polyphenols (PP) relative to N have been acknowledged to facilitate decomposition, hence, stimulate the abundance of proteolytic and nitrifying soil microbial communities. Therefore, it was hypothesized that high quality (low (L+PP)/N)) residue applied to high pH soils have a positive relationship between the functional potential of proteolytic enzymatic activities and abundance of nitrifying communities.
The survey studies in DRC and Ethiopia were guided by the following objectives; 1) To determine the inter-related influence of market distance and farm typology on soil fertility status of smallholder farming systems of South-Kivu, Eastern DRC. 2) To assess the inter-related effects of agro-ecology and farm typology on soil fertility status across crop-livestock systems in Western

and Central Ethiopia. Moreover, to better understand the ecological processes (proteolysis, nitrification) that control N through organic residue management in varying soil fertility variability conditions, an incubation study was performed to meet objective 3) To verify that potential proteolytic enzyme activities modulate archaeal and bacterial nitrifier abundance in soils with differing acidity and organic residue treatment.

Results from the soil survey study in DRC revealed a decreasing soil fertility with increasing market distance across all farm typologies. A significant influence of farm typology was found for exchangeable calcium and magnesium, while factor site resulted in a significant difference of plant available phosphorus. Furthermore, factor "site" interacted with market distance for soil organic carbon (SOC) quality indexes. In addition, the interaction of market distance and typology became obvious in the medium wealthy and poor farms. Market distance effects were associated with walking distance, while site effects were attributed to factors such as soil type and climatic conditions. In Ethiopia, inter-related effect of agro-ecology and farm typology was found. Higher total carbon and total nitrogen was found in wealthy farmers' field compared to poor farmers' field in the highlands. As an indication of soil quality, lowest SOC stability indexes were revealed in soils of wealthy compared to that from poor farm typology. These differences in soil fertility were attributed to farm management practices among typology classes and agro-ecological zone distinctions.

The result from the incubation study revealed a significant relationship of proteolytic enzyme activities with the abundance of ammonia oxidizing bacteria and archaea, even though the extent of this relationship was more dependent on soil pH and incubation time, but not residue quality. This suggests that the effect of soil pH is stronger than that of residue quality on enzyme activity and nitrifiers community, reflecting the importance of soil physico-chemical conditions rather than management practices. The incubation study further showed that nitrifying prokaryotes benefitted from the release of N spurred by proteolysis, and indicated a niche specialization between ammonia oxidizing bacteria and archaea depending on soil acidity and resource availability.

Overall, this PhD study showed that market access, typology and agro-ecology were important drivers of soil fertility variability in the study regions of DRC and Ethiopia. However, factor site played a significant role in shaping soil fertility variability, implying that site-specific recommendations could be a way forward for designing soil fertility management in smallholder farmers. It was inferred that prospective niche-based ISFM strategies must consider such contrasting but interrelated factors including, but not limited to agro-ecology, farm typology and market access. This would reduce the effect of soil fertility variability across regions.

This PhD study only considered land size (DRC, Ethiopia), livestock and mineral fertilizers (Ethiopia) as key features to define the wealth status of targeted farms; future studies should consider a wider range of socio-economic and biophysical factors including labor availability, off-farm household income and soil management history for more accuracy of soil fertility variability. This will strengthen the accuracy of prospective soil fertility assessments across socio-economic gradients and spatial scales. Finally, it is suggested to extend the results from the incubation study to field conditions considering soils with a broader soil acidity range and organic residues with more distinct biochemical quality. This will verify the given assumptions about the functional relationships between proteolytic and nitrifying soil communities. Overall, the presented PhD study has contributed to ongoing research on best-fit soil fertility recommendations and knowledge gaps about soil ecological functioning, by providing an advanced understanding of driving factors of soil fertility variability and soil microbial functioning in smallholder farms in tropical environments.

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Zusammenfassung

Die Bodenfruchtbarkeit in tropischen Agrarökosystemen ist häufig einer Degradation unterworfen, die zu einer Verarmung der Nährstoffe führt, was sich negativ auf die Produktivität des Bodens und die Ernährungssicherheit auswirkt. Diese Herausforderung wird durch die Komplexität der sozioökonomischen (Marktentfernung, Betriebstypologie) und biophysikalischen (Agrarökologie, Standort) Bedingungen, die eine Variabilität der Bodenfruchtbarkeit verursachen, noch verschärft. Folglich können pauschale Düngeempfehlungen in Gebieten mit hoher Fruchtbarkeitsvariabilität nicht angewendet werden. In dieser Studie wurden die Methoden harmonisiert, um die Einflussfaktoren auf den Bodenfruchtbarkeitsstatus in verschiedenen Regionen zu bewerten. Obwohl sie als Faktoren genannt wurden, die zur Variabilität der Bodenfruchtbarkeit beitragen, wurden der Marktzugang, die Betriebstypologie (Ressourcenausstattung) und die Agrarökologie noch nicht einer Bewertung der Bodenfruchtbarkeit unterzogen. Diese Studie zielte hauptsächlich darauf ab, zu verifizieren, dass diese Faktoren integriert und nicht isoliert betrachtet werden müssen, um genaue Bewertungen der Bodenfruchtbarkeit über räumliche Skalen und sozioökonomische Gradienten hinweg zu ermöglichen.

Es wurde die Hypothese aufgestellt, dass die Marktdistanz und die Betriebstypologie eine Determinante der landwirtschaftlichen Entwicklung in der Demokratischen Republik Kongo (DRC) ist. Mit zunehmender Marktdistanz sinkt der Bodenfruchtbarkeitsstatus kleinbäuerlicher Anbausysteme trotz des Wohlstands der Bauern. In einer parallel durchgeführten Studie in Äthiopien wurde ergänzend die Hypothese aufgestellt, dass der Bodenfruchtbarkeitsstatus auch durch miteinander verbundene Effekte der Agrarökologie und der Betriebstypologie beeinflusst wird. Da Stickstoff (N) in kleinbäuerlichen Betrieben bekanntermaßen limitierend ist, sind der Erhalt und die nachhaltige Bereitstellung dieses Nährstoffs von entscheidender Bedeutung, um

nischenbasierte Strategien des integrierten Bodenfruchtbarkeitsmanagements (ISFM) zu erreichen. Daher ist das Verständnis der ökologischen Prozesse (Proteolyse, Nitrifikation), die die N-Verfügbarkeit des Bodens durch die Bewirtschaftung organischer Rückstände unter variierenden Bedingungen der Bodenfruchtbarkeit steuern, unerlässlich. Es ist bekannt, dass niedrige Konzentrationen von Lignin (L) und Polyphenolen (PP) im Verhältnis zu N die Zersetzung erleichtern und somit die Abundanz von proteolytischen und nitrifizierenden mikrobiellen Gemeinschaften im Boden stimulieren. Daher wurde die Hypothese aufgestellt, dass qualitativ hochwertige (niedrige (L+PP)/N)) Rückstände, die auf Böden mit hohem pH-Wert ausgebracht werden, eine positive Beziehung zwischen dem funktionellen Potenzial proteolytischer enzymatischer Aktivitäten und der Abundanz nitrifizierender Gemeinschaften aufweisen.

Die Erhebungen in der DRC und in Äthiopien hatten folgende Ziele: 1) Bestimmung des wechselseitigen Einflusses von Marktdistanz und Betriebstypologie auf den Bodenfruchtbarkeitsstatus von kleinbäuerlichen Anbausystemen in Süd-Kivu, im Osten der DRC. 2) Die Bewertung des wechselseitigen Einflusses von Agrarökologie und Betriebstypologie auf den Bodenfruchtbarkeitsstatus von Ackerbau- und Viehhaltungssystemen in West- und Zentraläthiopien. Darüber hinaus wurde eine Inkubationsstudie durchgeführt, um die ökologischen Prozesse (Proteolyse, Nitrifikation) besser zu verstehen, die den N-Gehalt durch die Bewirtschaftung organischer Rückstände unter variablen Bedingungen der Bodenfruchtbarkeit kontrollieren. 3) Es sollte überprüft werden, ob potenzielle proteolytische Enzymaktivitäten die archäische und bakterielle Nitrifikationshäufigkeit in Böden mit unterschiedlichem Säuregehalt und organischer Rückstandsbehandlung modulieren.

Die Ergebnisse der Bodenuntersuchung in der DRC zeigten eine abnehmende Bodenfruchtbarkeit mit zunehmender Marktentfernung über alle Betriebstypologien hinweg. Ein signifikanter Einfluss der Betriebstypologie wurde für austauschbares Kalzium und Magnesium gefunden, während der Faktor "Standort" zu einem signifikanten Unterschied des pflanzenverfügbaren Phosphors führte. Außerdem interagierte der Faktor "Standort" mit der Marktentfernung für die Qualitätsindizes des organischen Kohlenstoffs im Boden (SOC). Darüber hinaus wurde die Interaktion von Marktdistanz und Typologie bei den mittelreichen und armen Betrieben deutlich. Die Effekte der Marktdistanz wurden mit der Laufdistanz in Verbindung gebracht, während die Standorteffekte auf Faktoren wie Bodentyp und klimatische Bedingungen zurückgeführt wurden. In Äthiopien wurde ein wechselseitiger Effekt von Agrarökologie und Betriebstypologie festgestellt. Höherer Gesamtkohlenstoff und Gesamtstickstoff wurde auf den Feldern wohlhabender Bauern im Vergleich zu den Feldern armer Bauern im Hochland gefunden. Als Hinweis auf die Bodenqualität wurden die niedrigsten SOC-Stabilitätsindizes in den Böden der wohlhabenden im Vergleich zu den Böden der armen Farmtypologie festgestellt. Diese Unterschiede in der Bodenfruchtbarkeit wurden den landwirtschaftlichen Bewirtschaftungspraktiken zwischen den Typologieklassen und den Unterschieden in der agro-ökologischen Zone zugeschrieben.

Insgesamt zeigte diese Studie, dass Marktzugang, Typologie und Agrarökologie wichtige Einflussfaktoren für die Variabilität der Bodenfruchtbarkeit in den Untersuchungsregionen der DRK und Äthiopien waren. Der Faktor Standort spielte jedoch eine bedeutende Rolle bei der Gestaltung der Bodenfruchtbarkeitsvariabilität, was bedeutet, dass standortspezifische Empfehlungen ein Weg für die Gestaltung des Bodenfruchtbarkeitsmanagements bei Kleinbauern sein könnten. Daraus wurde gefolgert, dass zukünftige nischenbasierte ISFM-Strategien solche gegensätzlichen, aber miteinander verknüpften Faktoren berücksichtigen müssen, einschließlich, aber nicht beschränkt auf Agrarökologie, Betriebstypologie und Marktzugang. Dies würde die Auswirkungen der Variabilität der Bodenfruchtbarkeit in verschiedenen Regionen reduzieren.

Diese Studie berücksichtigte nur die Landgröße (DRC, Äthiopien), den Viehbestand und Mineraldünger (Äthiopien) als Schlüsselmerkmale, um den Wohlstandsstatus der Zielfarmen zu definieren; zukünftige Studien sollten eine größere Bandbreite an sozioökonomischen und biophysikalischen Faktoren berücksichtigen, einschließlich der Verfügbarkeit von Arbeitskräften, des Haushaltseinkommens außerhalb der Farm und der Geschichte der Bodenbewirtschaftung, um die Variabilität der Bodenfruchtbarkeit genauer zu bestimmen. Dies wird die Genauigkeit der prospektiven Bodenfruchtbarkeitsbewertung über sozioökonomische Gradienten und räumliche Skalen hinweg verbessern. Schließlich wird vorgeschlagen, die Ergebnisse der Inkubationsstudie auf Feldbedingungen auszudehnen, wobei Böden mit einem breiteren Bodensäurebereich und organische Rückstände mit unterschiedlicher biochemischer Qualität berücksichtigt werden. Dies wird die gegebenen Annahmen über die funktionellen Beziehungen zwischen proteolytischen und nitrifizierenden Bodengemeinschaften verifizieren. Insgesamt hat die vorgestellte Studie einen Beitrag zur laufenden Forschung über bestmögliche Bodenfruchtbarkeitsempfehlungen und Wissenslücken über die ökologische Funktionsweise des Bodens geleistet, indem sie ein fortgeschrittenes Verständnis der treibenden Faktoren für die Variabilität der Bodenfruchtbarkeit und der mikrobiellen Funktionsweise des Bodens in kleinbäuerlichen Betrieben in tropischer Umgebung liefert.

Appendices

Appendix 1: Supplementary material for chapter 2.

MidDRIFTS analysis

Spectra of ball-milled soil samples were recorded on a Tensor-27 Fourier transform spectrometer (Bruker Optik GmbH, Ettlingen, Germany) (Rasche et al., 2013). Each soil sample was analyzed in triplicate from wavelengths 3950 to 650 cm^{-1} (Fig. S1).

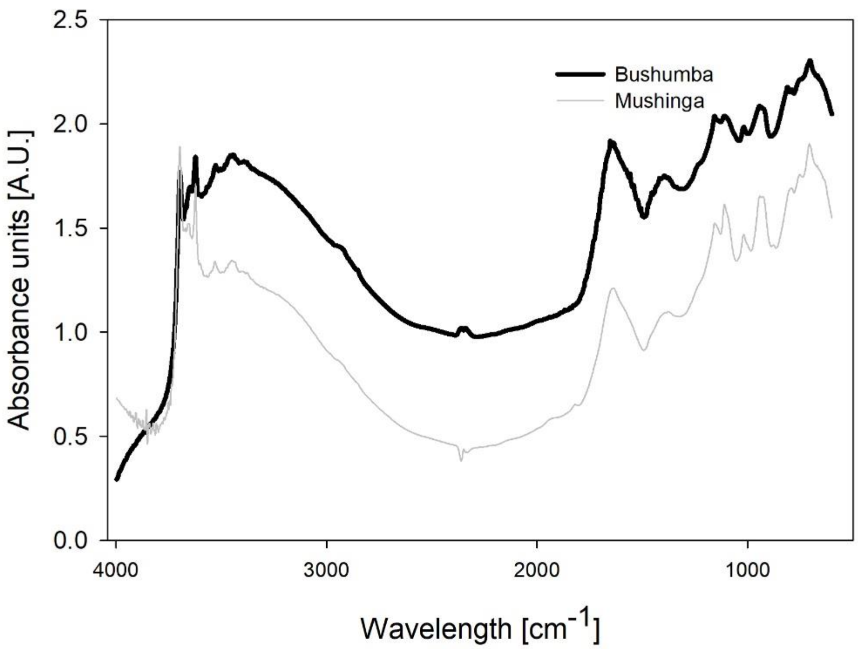

Figure S1. Visualization of midDRIFTS patterns obtained from the soils of the two sites Bushumba and Mushinga. The presented midDRIFTS spectra are averaged data calculated on the basis of the soil samples collected in the two villages of each site.

Appendix 2: Supplementary material for chapter 4

S: Table 1. Pearson correlation between proteolytic enzyme activities and nitrifier gene abundance as affected by time

Nitrifier abundance	Time (days)	Enzymes activities AAP	LAP	TLP
AOB	7	- 0.60 *	- 0.62 *	0.16 ns
	15	- 0.61 *	- 0.64 *	- 0.40 ns
	30	0.20 ns	0.12 ns	0.41 ns
	45	0.81 **	0.85 *	0.80 **
	60	0.60 *	0.55 ns	0.69 *
AOA	7	0.39 ns	0.12 ns	- 0.37 ns
	15	0.53 ns	0.76 **	0.38 ns
	30	0.72 **	0.71 **	0.69 *
	45	- 0.17 ns	0.52 ns	- 0.23
	60	0.46 ns	0.77 **	0.45 ns

Significance levels: *** = P<0.001, ** = P<0.01, * = P<0.05*; ns, not significant (P>0.05).

Enzymes: AAP: Alanine aminopeptidase; LAP: Leucine aminopeptidase; TLP: Thermolysin-like proteases.

Gene abundance: AOB: ammonia oxidizing bacteria; AOA: ammonia oxidizing archaea

Factor time: 7, 15, 30, 45 and 60 days

Yellow color: significant positive correlation

Red color: significant negative correlation

S: Tabel 2. Pairwise person correlation matrix enzyme activities and nitrifiers gene abundance across soil pH and time (attach in below link "sheet B")

https://drive.google.com/drive/folders/1psk0ay6KwZgYfyPNPjrdcvKQZOqQP2tL?usp=sharing

Author's declaration

I hereby declare that I have written this thesis independently without using other sources than stated herein. Further, I declare that the research work has not been presented in other university for any degree or academic certificate.

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Hohenhein/ September 2021

Isaac Balume Kayani