

**Impact of environmental and socio-economic factors on soil fertility  
variability and microbial carbon use efficiency in tropical smallholder  
farming systems**



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# **1 General Introduction**

## **1.1 Population dynamics and food security challenges**

### 1.1.1 Population dynamics

The Food and Agriculture Organization of the United Nations (FAO) estimated the global human population will reach 9.27 billion by 2050 and 11.2 by the year 2100 (FAO, 2017). According to this projection, even though the absolute population is increasing, the average world population growth rate is anticipated to decrease. However, the population growth rate will continue until mid to end of the 21<sup>st</sup> century in Asia and Sub-Saharan Africa (SSA), respectively (FAO, 2017). The average annual growth rate for the world population is 1.1 percent; for low and high income countries, it is 2.6 and 0.5, respectively (UN, 2019). Considering this decreasing trend in growth rate, surprisingly there was an absolute annual human population increment of 80 million in the past five years. With a slight decrease per year, this annual population increment will reach 55 million in 2050 as compared to 2015 (FAO, 2017). The population of SSA is expected to reach 2 billion and will grow by 119% in 2050 (Bjørndal et al., 2016). Ethiopia, among the most populous countries in Africa, is expected to reach 205 million people in 2050 with a growth rate of 2.7% per year (UN, 2019). Comparably, the population of the Democratic Republic of Congo (DRC) was 77 million in 2014 and is projected to double in the next 20-25 years (WBG, 2018). The projected annual growth rate for the DRC is 3.2%, becoming 194 million people by 2050 (UN, 2019).

### 1.1.2 Food security challenges

The concept of food security is directly related to hunger, poverty and humanitarian aspects. Maggio et al. (2015) explained that food security is a multi-layered concept that encompasses availability, physical and economic access to food, utilization based on cultural and dietary requirements and the stability of its provision. The aim of the second Sustainable Development Goal (SDG 2) was designed to end hunger, achieve food security, improve nutrition and promote sustainable agriculture simultaneously by 2030 (FAO, 2017). Based on the medium growth rate, it is expected that the world should feed 9.7 billion people in 2050, i.e. 2.0 billion extra people compared to the current population, while sustaining the natural resources for the next generation

(UN, 2019; Maggio et al., 2015). Maggio et al. (2015) further argued that the world will need 50% more food in 2030 and 80-100% in 2050 as of today, to achieve the Sustainable Development Goal 2 (SDG2). Furthermore, FAO (2017) forecasted that food production should be increased by 70% from 2005/07 to 2050 to create a world “free of hunger and malnutrition”. Researchers reported that SSA is the greatest food security risk region, because of its population growth with 2.5 fold, which would approximately demand three fold cereal crops in 2050 (Van Ittersum et al., 2016). In the Sub-Saharan region, even though there is an increased projection of food production in the future, the gap between demand and supply remains a challenge (Onyutha, 2018). Ethiopian food production failed to fulfill the food demand of the country, because population growth outpaces agricultural production (Degife et al., 2019). Degife et al. (2019) further stated that about 96% of the agricultural production in Ethiopia was subsistence small scale crop-livestock mixed farming. The crop production of the country was dominated by cereals, which covered about 81% of the total cultivated area owned by a total of 15.2 million farmers (CSA, 2019). According to the International Food Security Assessment report (Meade & Thome, 2017), Ethiopia needs to fulfill the food gap of 903, 000 tons (t) food by 2027. Comparably, DRC has over 120 million ha of land suitable for crop production even though only 10 percent is currently used (Kane et al., 2004). Due to the low area coverage of the cultivated land and productivity, there existed a huge difference between the food demand and supply in DRC with a projection gap of approximately 2.6 million tons foods in 2027 (Meade & Thome, 2017).

To fill the gaps between food demand and supply, 90 and 80% of the expected crop production in developed and developing countries, respectively, is anticipated from improved productivity per unit area per unit time and increased cropping intensity (FAO, 2009), while the remainder comes from land expansion. Recent studies indicate that this might be achieved if the gaps between the current farm yield and potential yield are closed (van Ittersum et al., 2016). To achieve the yield potential, crop production constraints, such as soil fertility depletion, stress due to water limitations, pests (such as plant hopper), disease and weed prevalence, and lack of proper crop management have to be controlled. However, among the constraints, soil fertility depletion has been a challenge for SSA smallholder farmers due to the inherently low soil fertility on the one hand, and on the other hand continuous mining of plant nutrients due to crop harvest without replenishment.

## **1.2 Soil fertility management for food security**

### 1.2.1 The state of soil fertility management for food security

Soil is a physico-chemical entity and composed of solids, liquids and gases; soluble and insoluble and organic as well as inorganic substances (Osman, 2013b), which makes it an important source of food and energy for plants, and for the growth of soil microorganisms. In soils, there are ions and compounds, salts, acids, bases, minerals and rock fragments. Soils also have very fine soil particles called colloids, which consist of humus, fine silicate clays, and oxides and hydroxides of iron and aluminum (Osman, 2013b). Furthermore, colloids carry both positive and negative electro-chemical charges and are important sites of soil reactions, which determine soil pH and fertility. Decline in soil fertility is, among other factors, a major constraint to the productivity of major crops. Soil fertility used interchangeably here with soil quality, is not only a concept but also a phenomenon (Bünemann et al., 2016). The term originated from the German word “Bodenfruchtbarkeit”. At this point, it was predominantly aligned to yields (Patzel et al., 2000; Bünemann et al., 2016). However, soil fertility is a complex term, which illustrates the physical, chemical, and biological soil parameters and processes, as well as environmental conditions and crop yield (Patzel et al., 2000). Therefore, not only yield, but also other soil functions such as biomass production and human and environmental services are important considerations for soil fertility (Bahr, 2015).

Important soil provisions are food, wood, fiber, raw materials, and physical support for infrastructure. Furthermore, soils provide regulating services, such as flood mitigation, filtering of nutrients and contaminants, carbon storage and greenhouse gas regulation, detoxification and the recycling of wastes, and regulation of pests and disease populations (Dominati et al., 2010; Kopittke et al., 2019). More specifically, according to Mäder et al. (2002), a fertile soil is defined as a soil that provides not only essential nutrients for crop growth, supports a diverse and active biotic community, exhibits a typical soil structure, but also allows for an undisturbed decomposition. On the contrary, degraded soil is primarily characterized by depletion of soil organic matter (SOM) and plant nutrients, reduced water holding capacity, and also reduced activity of soil microbial biomass (Lal, 2001; Scherr, 1999). In the tropics, this phenomenon is associated with the inherent poor fertility of soils (Koning & Smaling, 2005). Furthermore, soils

in the tropics are associated with high rates of erosion, leaching, removal of crop residues and animal manure, continuous cultivation of the land without adequate fertilization or fallowing (Tadesse, 2001; Lal, 2001).

### 1.2.2 Soil fertility in Sub-Saharan Africa (SSA)

The number of smallholder farmers owning less than 1 ha on average in SSA was about 60 million, and are responsible for supplying most foods on the continent (Donovan & Casey, 1998). In SSA, the status of soil fertility is considered to be more depleted over time, as a result of the inherent poor fertility and population pressure (Drechsel et al., 2001; Sanchez et al., 1997). In the Soil Atlas of Africa, soil degradation was reported as a threat to about one-quarter of productive lands of the continent (Stoorvogel & Smaling, 1990; Jones et al., 2013). Soil nutrient mining through crop residue removal for fuel and animal feed and very little replenishment of organic and inorganic resources were the recurrent problems that resulted in soil nutrient depletion in SSA (Stewart et al., 2020). For example, Stoorvogel & Smaling (1990) reported negative balances of 20 N, 10 P<sub>2</sub>O<sub>5</sub> and 20 K<sub>2</sub>O kg ha<sup>-1</sup> up to a maximum of 40 N, 20 P<sub>2</sub>O<sub>5</sub> and 40 K<sub>2</sub>O kg ha<sup>-1</sup> in SSA smallholder cropping systems. Nutrient capital reserves of 40% of SSA soils are low, aluminum toxicity covers 25% of soils, and 18% of soils have a high leaching potential (Tully et al., 2015; Sanchez et al., 2003). The above mentioned situations have resulted in lower productivity of irrigated, rain-fed and pasture lands to below 7, 14 and 45% of their potential productivity respectively (Donovan & Casey, 1998).

### 1.2.3 Soil fertility status in Ethiopia

Ethiopia faces a wide set of soil fertility challenges, because of diverse agro-ecological (elevation, topography, climate, vegetation), socio-cultural (market access, indigenous knowledge) and biophysical variants (slope, aspect, land use, land cover). SOM depletion, severe topsoil loss through erosion, soil nutrient mining, lack of site-specific and locally tailored soil fertility management options are among the most important limitations. For example, reports from Soil Conservation Plots indicated that Ethiopia has experienced alarming rates of soil erosion, averaging (and sometimes exceeding) 137 t ha<sup>-1</sup> per annum (IFPRI, 2010). This means that the country has one of the highest nutrient depletion rates in Africa, i.e. -41, -6, and -26 kg ha<sup>-1</sup> yr<sup>-1</sup> of nitrogen (N), phosphorus (P), and potassium (K), respectively (Stoorvogel & Smaling, 1990). Soil nutrient balance assessments in central Ethiopia showed that nutrient losses

have even worsened and reached an amount of 122 kg N, 13 kg P and 82 kg K ha<sup>-1</sup> year<sup>-1</sup> (Hailelassie et al., 2005). The status of SOM in Ethiopian cultivated lands ranged from 2.34 to 4.44 due to harvesting of crop residues for animal feed and manure for fuel (Agumas et al., 2014; Endalew et al., 2014; Zeleke et al., 2010). Reduced organic matter resulted in poor soil porosity and infiltration, which in turn affects water and nutrient cycling (Tully et al., 2015). In addition to the poor nutrient and organic matter status, aluminum toxicity and phosphorous fixation are other constraints in Ethiopian soils. Aluminum toxicity and phosphorous fixation are apparent in soils with a pH of less than 5.5, which enhance nutrient limitations and toxicity (Agegnehu & Amede, 2017; Agegnehu et al., 2006). As an attempt to reverse this situation and to improve the productivity of major crops in Ethiopia, 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). These pioneering mapping approaches could initialize site-specific integrated soil fertility management (ISFM) adaptations; a combined use of fertilizers and organic inputs as well as improved germplasm with the full knowledge on how to adapt these practices to local conditions (Vanlauwe et al., 2010). However, they did not address essential drivers of soil fertility like agro-ecology (i.e topography, elevation, climatic conditions, vegetation types), farmers' resource endowment and indigenous knowledge. Out of these drivers, agro-ecological factors can be the most dominant influencing soil fertility variability (1) as elevation in Ethiopia ranges from below 500 meters above sea level (m.a.s.l) in the Denakil depression to 4620 m.a.s.l in the mount Ras Deshen (Mengistu, 2003), (2) the climatic conditions range from very cool moist highlands conditions to very hot dry lowlands (Hurni, 1998).

#### 1.2.4 Soil fertility status in Democratic Republic Congo

The rural population increase in DRC is among the highest in SSA with an annual rate of 3.2% (UN, 2019). This demographic pressure caused the soil to become depleted without any renewal measures. In DR-C, the common agricultural practice by smallholder farmers is a slash-and-burn method, in which the immediate uptake of nutrients due to burning of biomass for ash fertilization enhances crop growth (Hauser & Norgrove, 2013). After 3 to 4 years of cultivation, the farmers fallow the land for the next 10-20 years (Wasseige et al., 2012). However, due to rapid population growth, the fallow period is shortened and continuous cropping without renewal measures on the one hand, and increased use of marginal lands for agricultural purpose on the

other hand resulted in serious soil fertility depletion (Thienpondt, 2016; Giller & Palm, 2004; Sanchez and Logan, 1992). In addition, the fertilizer application rate in DRC is the lowest, which was approximately 10 kg ha<sup>-1</sup> or nonexistent compared to the global rate of 110 kg ha<sup>-1</sup> (Henao & Baanante, 2006; Munyahali et al., 2017). Thus, the nutrient balance for DRC is reported as the most negative in nutrient balance in the world (IFDC, 2010). According to the Catalyzed Accelerated Agricultural Intensification for Social and Environmental Stability (CATALIST) project of the International Fertilizer Development Center (IFDC, 2010) survey, agricultural land has lost around 100 kg of soil nutrients ha<sup>-1</sup> year<sup>-1</sup> in this region. Furthermore, the soil in this region has a low cation exchange capacity (CEC) and SOM, high acidity and aluminum toxicity, and low phosphorous availability due to high fixation (Ngongo et al., 2009; Thienpondt, 2016). This is aggravated by an extremely steep relief, making it highly susceptible to soil erosion (IFDC, 2010).

#### 1.2.5 Implications of soil fertility status on food security

According to Kopittke et al. (2019), agricultural land productivity has doubled in the last five decades and about 98.8 % of the daily calories consumed by humans comes from soils and only 1.2% from aquatic sources. However, Tan et al. (2005) stated that soil fertility degradation is a crucial concern directly linked with food insecurity. This is because the worlds' one-third soils as compared to the total cultivated land soil have lost agricultural production capacity since the 1970s owing to unwise soil fertility management, which resulted in severe soil fertility depletion (Rojas et al., 2016). Persistent lack of nutrient renewal and organic matter management of depleted soils, as well as loss of nutrients through wind and water erosion not only aggravated soil degradation, but also hampered agricultural sustainability in these regions (Tan et al., 2005; Sheldrick et al., 2002).

The biggest challenge to soil nutrient and organic matter depletion is soil erosion. Topographical variables such as elevation, slope as well as climatic factors such as temperature and rainfall conditions are biophysical factors responsible for soil erosion; hence soil fertility depletion (Rodrigues et al., 2021). The estimated amount of productivity loss due to soil erosion in Europe was reported to be 0.43% of annual crop productivity, equivalent to a cost of €1.25 billion (Panagos et al., 2018). Similarly, the estimated productivity loss due to soil erosion in Africa ranges from 2-40%, equivalent to 15 million USD (Lal, 2004; ELD & UNEP, 2015). However,

the impact of soil nutrient depletion on food security varied depending on geographical location, due to the inherent poor soil fertility coupled with lack of appropriate soil management (Mugwe et al., 2019; FAO, 2015). Moreover, the average yield gap of cereals was 3.5 and 6.5 Mg ha<sup>-1</sup> in SSA compared to Asia and Latin America, respectively. For the last 40 years, soils in most African countries are depleted and were considered as a crisis to the region and continued until today (AGRA, 2016; Mugwe et al., 2019).

In many parts of Africa, Asia and Latin America, long term declines in crop yields were evident as a result of low input and unbalanced fertilization (Tan et al., 2005). In Ethiopia, the estimated wheat yield loss is 3 t ha<sup>-1</sup>, due to soil nutrient depletion (Abdulkadir et al., 2017; Tadesse, 2001; Zeleke et al., 2010). On the other hand, studies showed that if proper soil fertility management is undertaken there is a huge potential to fill the gaps between food demand and supply in Ethiopia (Abdulkadir et al., 2017). According to Kihara et al. (2017), the country has the potential to achieve food security by using both organic such as farmyard manure, compost and mulch/crop residues and synthetic fertilizers. The positive response of cereals to N, P and S in Ethiopia showed that closing the gap of these nutrients leads a step closer to food security (Kihara et al., 2017). In DRC, poor soil fertility and lack of proper soil management resulted in 41-50 % of the potential yield loss of cassava, which is equivalent to 4.5-6.5 t ha<sup>-1</sup> (Kintché et al., 2017). Cassava is considered as one of the drought-resistant crops and moves from subsistent food to one of the major commercial crops (Kintché et al., 2017). In this context, by overcoming these constraints it is possible to achieve food security in DRC. Therefore, present and future food security challenges depend on how soil fertility management problems concerning crop productivity are addressed in these regions. One highly acknowledged strategy to reverse the food security challenge under smallholder crop livestock farming systems is the integrated soil fertility management (ISFM) approach (Vanlauwe et al., 2010). Nevertheless, its adoption across different regions of SSA is limited (Vanlauwe et al., 2015), as a result of diverse soil fertility variability.

## 1.3 Factors responsible for soil fertility variation

### 1.3.1 Agro-ecological factors

Spatial and temporal variability of soil fertility are common phenomena in agricultural fields. Agricultural fields could be low responsive fertile, highly responsive infertile and poorly responsive degraded fields (Chikowo et al., 2014). Spatial and temporal soil fertility variability can occur due to natural or anthropogenic (human made) factors. Natural soil fertility variability may come as a result of complex interactions between geology, topography, climate, vegetation as well as soil use (Yasrebi et al., 2008; Ayoubi et al., 2007). The geology of the soil determines mainly soil physical properties such as texture, structure, water holding capacity and clay content while the topography of an area affects the storage of SOM and nutrients, due to microclimate, runoff, evaporation and transpiration (Yoo et al., 2006). Furthermore, clay and sand content and pH were highly correlated with topographic position; higher contents of clay and soil pH were found in the terrace than back slope (Karaca et al., 2018). The process of soil formation and development is guided by changes in climatic conditions (e.g. temperature and rainfall), through energy consumption and water balance, which can affect soil fertility status positively or negatively (Pareek, 2017). According to Pareek (2017), topography with climate-induced changes in vegetation types, plant growth rates, rate of soil water extraction by plants and effects on CO<sub>2</sub> consumption level could also control soil fertility status. The effect of climate change on soils are expected mainly through alteration in soil moisture conditions and increase in soil temperature and CO<sub>2</sub> levels (Pareek, 2017). These conditions resulted in alteration of soil functional process such as biomass production and soil microbial activities which has a direct impact on soil fertility. Changes in vegetation types and soil nutrient concentrations have often been found along the altitudinal gradient in crop-livestock mixed agricultural systems (He et al., 2016). Collectively all these factors can be considered as agro-ecological factors responsible for soil fertility variability. Deressa et al. (2018) noted that in Ethiopia soil fertility status varied due to agro-ecological differences (i.e differences in altitude (for example below 500 m.a.s.l in the Danakil Depression to 4200 m.a.s.l in mount Ras Dashen) of the country which determines moisture regime and temperature). As a result, soils can have marked spatial or temporal variability at macro or micro-scale and therefore, understanding soil fertility variability is of paramount importance to devise site-specific soil management strategies. Site-specific soil

fertility management approaches became recognized as the best fit strategy for (1) enhancing input efficiency; (2) increasing the economic returns of crop production; and (3) reducing environmental risks (Yasrebi et al., 2008). However, lack of clear understanding of site specific and agro-ecologically based soil fertility problems that led to niche-based soil fertility management strategies was considered as one of the research limitations that need investigation to improve soil health in general and fertility status in particular in Ethiopia and DR-C. Therefore, to fill this research gap, agro-ecologically based soil fertility assessment was conducted in this study.

### 1.3.2 Socio-Economic factors

Socio-economic factors are responsible for the variability in soil fertility including, but not limited to, farm typology and farmers' indigenous knowledge (Chikowo et al., 2014; Tittonell et al., 2005a; 2010). One source of soil fertility variability is farmers' wealth status. This is because farmers' management decisions are mostly dependent on their economic capacity to invest in soil fertility (Tittonell et al., 2005a; 2005b; Agumas et al., 2021; Balume et al., 2020). Those farmers, who can invest in labor and input, can manage their soil resources better and earn more benefits from their field, than those farmers who do not follow this approach. The variability in management decisions on soil fertility due to resource endowment differences of farmers has created soil fertility variability. Farmers with high number of livestock, livestock density (expressed in TLU ha<sup>-1</sup>) and value (USD), have positive nutrient balances on their fields and positively correlated with nutrient balance (Onduru & Preez, 2007), due to high production of animal manure used for soil fertility improvement. However, farmers' land holding has negative effect on nutrient balance. Onduru & Preez (2007) reported that farmers with large farm sizes negatively affected nutrient balance for they could not provide adequate organic and/or inorganic fertilizer to their farm.

The other socio-economic source of soil fertility variability is farmers' indigenous knowledge. Farmers' indigenous knowledge is a traditional, local and native knowledge adapted to the local environment to create unique indigenous farming practices (David et al., 2014). Farmers are well aware of their soil fertility status (Laekemariam et al., 2017). Recognizing the farmers' knowledge who have been interacting for a long time with their soil and environment, might lead

to a better understanding of the status of soil fertility (Kuldip et al., 2011). Farmers' perception of soil fertility based on farmers' local knowledge guided farmers' soil management decisions. Hence, based on the indigenous knowledge and farmers' resources, farmers can decide on how to manage their soil. In general, a higher priority is given to homestead fields than out-fields to apply both organic and inorganic inputs to soils (Hailelassie et al., 2007; Tittonell et al., 2005b). Even though, farmers' knowledge on soil fertility is acknowledged, synchronizing objectively based scientific knowledge with subjectively based farmers' indigenous knowledge, is lacking in smallholder SSA countries necessitating further investigation.

Furthermore, lack of clear evidence on the interrelated effect of the main drivers of soil fertility variability, such as biophysical (agro-ecology, site) and socio-economic factors (market distance, farmers' resources endowment, indigenous knowledge), on the heterogeneity of soil fertility were critical knowledge gaps that demand further research.

## **1.4 Assessment of soil fertility status**

### 1.4.1 Parameters to be considered for soil fertility assessment

#### *Chemical soil properties*

The concept of soil fertility is ambiguous and cannot be measured directly, as it is manifested by the functions and services it delivers (Guillaume et al., 2016). Hence, it is typically assessed by selecting and interpreting changes of properties or processes recognized as important indicators of soil fertility (Guillaume et al., 2016). Among the indicators of soil fertility status, ion exchange capacity, solubility of chemical substances, availability and uptake of nutrients and tendency of the soil to be reduced or oxidized (Osman, 2013b), are described as chemical properties. More specifically, the most important and frequently measured chemical indicators in relation to soil fertility status are plant nutrients, ion exchange capacity and soil reactions. Plant essential nutrients, which include calcium (Ca), magnesium (Mg), nitrogen (N), potassium (K), phosphorus (P), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), boron (B), and nickel (Ni) are present in the minerals and the soil solution. Soil chemical reactions influence solubility, mobility, and exchange of plant nutrients in the soil ecosystem (Osman, 2013b). These processes affect the plant nutrients availability of the soil, which

determines the extent of soil fertility. The presence of these nutrients in the soil below the critical level and/ thresholds leads to lower soil productivity due to nutrient deficiency. Excess availability of micronutrients, such as Fe, Mn, Zn, Cu, Mo, and B can become toxic to plants (Osman, 2013b). A calculated single value, such as soil fertility index (SFI), can be used to express the fertility status. Based on SFI (depending on the summerized concentration or availability of plant nutrients in the soils), the state of fertility can be either fertile (110-200), medium fertile (80-110) or poor (0-80) (FAO, 1980).

### *Physical soil properties*

Physical soil fertility indicators are soil texture, bulk density, soil structure, porosity, field capacity and soil color. The extent of bulk density, texture, soil structure, porosity and field capacity describes the level of soil fertility. For agricultural soils, each of the above physical properties has critical values (thresholds) to categorize the soil as fertile, medium fertile or poor even though some of these properties depend on local conditions. For example, soils dominated by sandy particles have lower water and nutrient holding and soil buffering capacity compared to clay textured soils. Depending on the local climatic conditions, sand textured soils can be fertile or poor; in moisture deficient areas this type of soil is poor while in moisture sufficient areas it may be fertile.

Depending on the extent and disturbance of soil aggregation, soil structure can affect aeration, water relations, plant root growth, soil temperature, soil compaction and resistance to erosion (Tueche, 2014). The extent of soil compaction can be described by bulk density; it affects soil productivity and key process. Bulk density shapes infiltration, rooting depth/restrictions, available water capacity, soil porosity, plant nutrient availability, and soil microorganism activity (Chaudhari et al., 2013). Bulk density mostly depends on soil texture, structure and SOM; its critical value for plant growth depends on soil texture. For clay and sandy soils, a normal value of bulk density ranges from 1-1.6 g/cm<sup>3</sup> and 1.2-1.8 g/cm<sup>3</sup>, respectively while potential root restriction occurred at  $\geq 1.4$  g/cm<sup>3</sup> for clay and  $\geq 1.6$  g/cm<sup>3</sup> for sand textured soils (Chaudhari et al., 2013).

Similarly, soil color can also be taken as an indicator for soil fertility depending on local conditions (Belachew & Abera, 2010; Corbeels et al., 2000; Yeshaneh, 2015). Farmers use soil

color as easily observable physical horizons to distinguish the state of soil fertility (Kuldip et al., 2011). In this regard, many authors argue that based on farmers classification black soils are considered fertile, while red soils are poor (Yeshaneh, 2015). Furthermore, soil color can often reflect the soils hidden parent material properties, which determines specific soil characteristics (Corbeels et al., 2000). Hence, it was of paramount importance to link soil color with spectral data, such as Diffuse Reflectance Infrared Fourier-Transform Spectroscopy (midDRIFTS). This is because, spectral data has been used to calculate the colour of the soil (Barouchas & Moustakas, 2004) which can be manifested by its soil organic carbon (SOC) and phosphorous content.

### *Biological soil properties*

Since soils are regarded as living entities, the third group of soil fertility indicators are soil biological properties. A large number of organisms including all plants and animals, as well as microorganisms are living in the soil. Therefore, soils do not only provide food for soil flora and fauna, but also provide habitats to a large group of organisms on earth (Osman, 2013a; Bölker & Blume, 2002). Different organisms (e.g higher plants, protists and bacteria) in soil photosynthesize, respire, reproduce, produce organic matter, consume organic matter and decompose it (Osman, 2013a). The most sensitive soil fertility indicators are biological soil properties and labile SOC pools. For example, biological biomass, soil respiration, and extracellular enzymatic activities changed immediately with any land use or soil management changes (Demyan et al., 2012; Guillaume et al., 2016; Heitkamp et al., 2009). The most commonly used parameters for soil microflora studies to assess the status of soil fertility are soil respiration (mineralization of soil organic carbon) and microbial biomass (Valsecchi et al., 1995). Furthermore, the activities of extracellular enzymes are also taken as indicators of soil fertility status, as extracellular enzymes gauge the potential of the soil to support the biochemical processes that are essential for the maintenance of soil fertility (Długosz, 2014). To better understand the environmental drivers of soil microorganisms, scientists derived other parameters to assess the status of soil fertility; amongst others CO<sub>2</sub>-C respiration per biomass C and time (Długosz, 2014). The other derived parameter that can be used as a biological indicator for soil fertility depiction is specific enzymatic activity (the activity of extracellular enzymes per unit biomass production per time) (Lashermes et al., 2016).

#### 1.4.2 Soil organic carbon (SOC) as a soil fertility indicator

Through the positive effect on soil physical, chemical and biological properties of soils, SOC has a beneficial effect on soil fertility (Panakoulia et al., 2017). Degradation of SOC pools affects biomass productivity, microbial activity, soil structure and water holding capacity (Lal, 2004). SOC can be considered as a beneficial pool to enhance nutrient retention capacity and water availability of the soil (Lal, 2006); it also decreases the loss of essential plant nutrients, and increases nutrient use efficiency of soils. In general, the quality of the soil is affected through severe depletion of SOC. Therefore, an increase of SOC contents increases soil fertility in general and crop productivity and production in particular (Bauer & Black, 1994). The net increase of SOC in the soil ecosystem (SOC stabilization) occurred when an input of carbon is more than the output of carbon in the decomposition process of organic residues. However, SOC stabilization of plant and animal-derived organic matter depends on different factors, such as organic matter protection (Kleber et al., 2007; Manzoni et al., 2012; Sollins et al., 1996) and how effectively the decomposer community converts carbon (C) to biomass, relative to how much C is lost in gaseous (CO<sub>2</sub> and CH<sub>4</sub>) or soluble form (Six et al., 2006). The biological process regulating this conversion depends on several factors such as quality and quantity of organic residues, climatic conditions, nutrient status of the soil and abundance, as well as structure of soil microorganisms (Manzoni et al., 2012). A key parameter used to quantify how C is partitioned between microbial growth and respiration is called carbon use efficiency (CUE).

##### *SOC functional group determination*

Different methods such as physical and chemical fractionation, thermal and nuclear magnetic resonance have been employed to determine SOC functional groups (Demyan, 2013; Margenot et al., 2015). Size and density separation has been used as the most suitable physical fractionation method of SOM depending on the size and density of soil particles (Virto et al., 2008; von Lützow et al., 2007). Chemical oxidation methods, such as permanganate oxidation and chloroform fumigation extraction, are amongst the chemical extraction methods of labile SOC functional groups consisting of permanganate-oxidizable C (POXC), microbial biomass C (MBC) and N (MBN). Furthermore, hot and cold water extractions are used to characterize different fractions of SOM (Demyan et al., 2012). All the above methods are quantifications of SOC contents. However, not only SOC quantities (e.g., SOC, microbial biomass C and N, POM)

but also SOC qualities (e.g., aliphatics, aromatics, amides, phenols, carboxylates, polysaccharides) have significant contribution for the status of soil fertility; because SOC qualities depend on the local climatic conditions, the type of vegetation and cropping systems. Both physical and chemical fractionation methods are time-consuming, poorly reproducible in different laboratories and the accuracy depends on the methods used (Demyan et al., 2012). Diffuse reflectance infrared Fourier transform spectroscopy (midDRIFTS) spectral analysis is not only considered as a robust, time and cost-effective method (Shepherd & Walsh, 2002), but also as a useful tool to quantify more sensitive SOC quality indicators subjected to small changes in quality and quantity of organic inputs as well as management effects on SOM (Margenot et al., 2015; Essington, 2004).

MidDRIFTS measures the diffuse reflectance of bending and stretching vibrations of different functional groups in the mid-infrared range from 4000 to 400  $\text{cm}^{-1}$  (Demyan et al., 2012; Margenot et al., 2015). Relative peak area integration among the different approaches was used to assess the potential SOM quality or composition indicators (aliphatics, aromatics, amides, phenols, carboxylates, polysaccharides) in midDRIFTS spectral vibrations (Demyan et al., 2012). Furthermore, the ratio of the functional groups aromatic and C-O poly-alcoholic and ether groups versus aliphatic groups are commonly calculated as an SOC stability index, which is used as soil quality indicator (Demyan et al., 2012; Inbar et al., 1989). Due to a lack of direct translation of size and density, as well as chemical oxidation fractions of SOC to spectral vibrations, relationships were developed by scientists to associate SOC fractions with SOC functional groups (Demyan et al., 2012; Margenot et al., 2015). Hence, positive and highly significant correlations between aliphatic C-H SOC functional groups versus light fraction C content ( $<1.8 \text{ g cm}^{-3}$ ), hot water extractable C, permanganate-oxidizable C (POXC), microbial biomass C (MBC) and N (MBN) indicated that C-H SOC functional groups are interpreted as active and most labile pools (Demyan et al., 2012; Margenot et al., 2015). Negative and significant correlations between these fractions and aromatic C=C SOC functional groups led to interpret this functional groups as stable and recalcitrant SOC pool (Demyan et al., 2012; Margenot et al., 2015).

### 1.4.3 Methodological consideration for soil fertility assessment

Achieving and maintaining a higher level of soil fertility is crucial for crop production. To successfully assess soil fertility status, the determination of quantitative and qualitative soil fertility indicators is necessary. To quantify and assess the extent of these indicators, different methods are employed, including laboratory analysis of soil chemical, physical and biological properties using wet chemistry and soil spectral analysis, crop responses in field conditions and visual assessments using some key observable indicators. Soil analysis using wet chemistry has been used as a key tool to assess the status of plant nutrients, physical soil conditions and microbial activities in the soils for decades (Motsara & Roy, 2008). In addition to soil analysis using wet chemistry, currently spectroscopic analysis using different spectral frequencies have become popular (Gourlay et al., 2017; Shepherd & Walsh, 2002). Even though model calibration is necessary, different spectroscopic (mid, near and visible infrared) analyses are robust and used frequently (Shepherd & Walsh, 2002; Wetterlind et al., 2013). Coupling of spectral analysis with wet chemistry has led to prepare different soil fertility maps, for example in Africa (Soil Atlas of Africa) and Ethiopia (Ethiopian Soil Atlas) (Gelaw et al., 2018; Vågen et al., 2013). The third type of soil fertility assessment is using farmers' indigenous knowledge based on observable and subjective judgments (Belachew and Abera, 2010). However, there is a big challenge to synchronize objective soil fertility assessment methods e.g., laboratory analysis (both wet chemistry and spectroscopic) with farmers' indigenous knowledge. This is because, there are no methodologies developed to link objectively measured soil chemical and physical properties such as SOC, available P and soil texture with observable soil fertility indicators such as soil color and depth.

## **1.5 Carbon use efficiency as determinant of soil fertility status**

Carbon use efficiency (CUE), the ratio of C allocated to biosynthesis and the amount of C accumulated and respired, is a key determinant of SOM dynamics and terrestrial C fluxes, with strong implications for soil C cycles (Geyer et al., 2016). The capacity of soil to store C and enhance long term stabilization depends on CUE, with a strong role for soil C sequestration potential. In most models, CUE was considered as an indicator of the C sequestration potential and related to climate change concepts (Qiao et al., 2019). Accumulation of C in the soil, however, was not only used to sequester C, but also to improve soil fertility. Soils with a high C

content increase cation exchange capacity, improves soil aggregate formation and stabilization, water holding capacity, aeration, and plant nutrient content (Bationo et al., 2007). The positive relationships between soil nutrients and CUE can be used as a determinant of soil fertility status (Manzoni et al., 2012).

The sufficiency or deficiencies of soil nutrients regulate CUE; for example, in the time of greater nutrient availability higher ecosystem CUE was observed, while under nutrient-deficient conditions a lower CUE was observed (Zhang et al., 2019). This is because, in nutrient-deficient conditions, C would be in excess, and there would be less fruitful respiration cycle that lost a proportion of C (Chambers et al., 2004; Zhang et al., 2019). This can lead to lower CUE. In lower CUE, the conversion of plant and animal-derived C to soil microbial growth will be low. This leads to less C storage in the soil ecosystem, which might again affect soil structure, water holding capacity, nutrient provision and soil fertility in general. A lower CUE increases C system losses, and reduces C storage. Thus, CUE is an essential trait influencing community assembly dynamics (i.e species diversity through trait modification and filtering in ecological communities, Hernawati et al., 2015) and nutrient cycling (Kallenbach et al., 2019); hence, CUE can be considered as soil fertility indicator. Higher soil respiration, but lower microbial activity, was observed in poor soils, than fertile soils indicating lower CUE in poor soils than its counterparts (Warembourg & Estelrich, 2001). Therefore, quantification of CUE as a more sensitive indicator for soil fertility status in agricultural fields might be per amount important. However, further evidence showing the linkage of CUE as indicator for soil fertility status in small holder agricultural systems are missing. Hence, to fully understand the relationship between the extent of C dynamics and the status of soil fertility with CUE in soils of SSA, further research is suggested.

### 1.5.1 Factors responsible for CUE variability

#### *Environmental factors*

Temperature and moisture are the two most important environmental factors shaping CUE among others (Manzoni et al., 2012; Herron et al., 2009; Apple et al., 2006). An increase in temperature above a certain level could lead to not only depletion of available substrates and nutrient limitation (Kirschbaum, 2004), but also increase the rate of biomass turnover (Hagerty et

al., 2014), which in turn affects microbial growth and CUE (Zhang et al., 2019). Similarly, alteration of soil moisture condition from normal to either dry or very moist conditions could shift microbial CUE (Manzoni et al., 2012). Change in soil water content could lead to either a shift in the composition of the active soil microbial community, nutrient limitation and/or change in the physiology of the microorganisms (Herron et al., 2009).

The other environmental factor responsible for controlling CUE in soil microbial process is soil pH. At lower soil pH, especially at  $\text{pH} < 4.5$  microbial growth and activity could be affected, which leads to lower CUE. This could be due to two major reasons: (1) shift in microbial structure and trait modification because of aluminum toxicity; and (2) nutrient limitations as a result of lower pH (Rousk et al., 2009; Jones et al., 2019). The third environmental factor affecting microbial CUE is the source of substrates for microorganisms, where the quality of the substrates affects CUE in two key different ways (Manzoni et al., 2012): (1) lower quality substrates require a large number of enzymatic steps to decompose the substrates and convert it into new biomass (Agren & Bosatta, 1987), (2) different qualities of substrates require different metabolic pathways to be completely assimilated, therefore requiring a high respiration rate per unit of C biomass (van Hees et al., 2005). Both of these mechanisms undertaken by microorganisms to degrade substrates could lead to a higher energy investment, which in turn lowers CUE. Lower quality substrates also provide a small amount of nutrients, which could be responsible for nutrient limitation and lowering CUE. However, there is no clear evidence showing the effect of the interacted effect of different environmental factors in general, and the effect of residue quality and soil pH in particular on CUE in soils of the tropics. Therefore, detailed understanding of the interacting effects of more complex residue quality with soil pH provides a clearer insight on microbial CUE in soils of the tropics.

#### *Methodological variability for CUE*

Quantification of CUE for soil microorganisms is difficult, therefore, currently different methodological approaches are used (Agumas et al., 2021; Geyer et al., 2019). Both direct and indirect approaches are used to estimate microbial CUE, including the C balance approach that considers increments in microbial biomass and in respired carbon dioxide ( $\text{CO}_2$ ) (Blagodatskaya et al., 2014; Herron et al., 2009). The C balance approach uses the microbial yield coefficient as equivalent to CUE during the growth period (Blagodatsky et al., 2002). If microorganisms are in

a state of maintenance and no distinct biomass increase is recorded, the metabolic quotient ( $qCO_2$ , the rate of  $CO_2$ -C evolution per microbial biomass C) is used to evaluate microbial metabolic efficiency (Blagodatskaya et al., 2014; Puttaso et al., 2011). The basic assumption of this method is that C gain in the microbial biomass originates solely from the substrate. This assumption excludes the possible reuse of microbial biomass C without explicit microbial growth (Hagerty et al., 2014). Uncertainty about this approach exists when biochemically complex organic residues are considered as substrates during decomposition, as the turnover of microbial biomass may distort C balance calculations.

Stoichiometric modeling of decomposition has been introduced as an alternative method (Sinsabaugh et al., 2016). In this case, CUE of the soil microbial community is considered as a function of the difference between nutrient requirements for growth and nutrient composition of the substrate, whereby extracellular enzyme activities (EEA), C:N (C:P) ratio of microbial biomass, and bioavailable organic matter are considered to calculate CUE. The main principle of using extracellular enzyme activities (EEA) to calculate CUE is that extracellular enzyme activities connect the stoichiometric theory of ecology. It reflects the equilibrium between the elemental composition of microbial biomass and detrital organic matter on the one hand and the efficiencies of nutrient assimilation and growth on the other hand (Sinsabaugh & Follstad Shah, 2011). The principal advantage of this approach is that the required parameters for CUE calculation can be obtained easily, and that it can be applied at various spatio-temporal resolutions (Sinsabaugh et al., 2016). However, it was not clear so far whether single enzymatic or multi-enzymatic stoichiometry (modified from single enzymatic) is an appropriate approach to quantify CUE with regard to different stages of plant residue decomposition. Furthermore, comparisons of different CUE estimation methods in acidic soils amended with organic residues remained elusive, leaving a critical knowledge gap necessitating further investigation. This included a modification of the single stoichiometric modeling approach.

## **1.6 Problem statement and justification of the study**

Adoption of integrated soil fertility management approaches (ISFM) across different regions of SSA including Central and Eastern Africa remains a major challenge (Vanlauwe et al., 2015). Heterogeneity of soil fertility does not allow uniform soil management strategies in larger areas; hence there is a need to unravel the complex dynamics of soil fertility gradients to develop ISFM

strategies adjusted to local contexts. To tailor demand-oriented ISFM interventions for smallholder conditions under different local contexts, it is critical to understand the main drivers of soil fertility variability and to use this knowledge to develop explicit ISFM strategies. Socio-economic factors such as farmers' resource endowment as well as their indigenous knowledge of soil fertility status could also be speculated amongst the main drivers of soil fertility variability (Tittonell et al., 2005b; Vanlauwe et al., 2015). Not only socio-economic factors but also biophysical variations such as agro-ecology and site (geographical location) are key drivers of soil fertility variability. Furthermore, the interrelated effect of biophysical with socio-economic factors such as market access, farm typology and farmers' indigenous knowledge to soil fertility status is missing. This is because not only the individual drivers but also the synchronization of both biophysical and socio-economic factors may contribute for soil fertility variability beyond the individual factors. Nevertheless, 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). To tackle these gaps and understand the extent of soil fertility status across different geographical locations, socio-economic factors and design tailor made soil fertility management strategies, as well as develop appropriate natural resources management policies, cost and time efficient soil analysis methods are of paramount importance. Hence, midDRIFTS analysis, among others, has been approved as a suitable tool to assess and map soil fertility variability in and among African agricultural farming systems (Cobo et al., 2010; Shepherd & Walsh, 2007; Vågen et al., 2006). Furthermore, midDRIFTS does not only allow the quantitative prediction of soil chemical properties (e.g., total soil N and C content as conventional soil fertility indicators) across large spatial scales. It also enables the spectroscopic assessment of SOC quality indicators (e.g., functional groups of SOC (i.e., aliphatic (labile) and aromatic (recalcitrant) compounds) as a function of soil fertility (Baes & Bloom, 1989; Demyan et al., 2012). Hence, the use of midDRIFTS spectral frequencies of SOC functional groups as soil fertility indicators during soil fertility assessment might provide further insights. This is due to (1) different SOC functional pools (aliphatic versus aromatic) provide the extent of decomposition and mineralization of SOC. Moreover, these functional groups influence the capacity of soils to provide nutrients to plants and energy to microorganisms (Demyan et al., 2012; Margenot et al., 2015; Haynes, 2005). The ratio of aromatic to aliphatic SOC functional

pools (SOC stability index) has been regarded as indicators for the extent of decomposition and mineralization process; hence, they display important features of the soil fertility status (Demyan et al., 2012; Margenot et al., 2015; Hsu and Lo, 1999). On top of the midDRIFTS, integrating subjectively based farmers' indigenous knowledge with objectively based detailed laboratory analysis and characterization of soil fertility status using farmers' indigenous knowledge is also important. Likewise, not only the analytical approaches, but also the use of more sensitive parameters such as CUE is missing and has to be considered for soil fertility assessment. CUE is a critical parameter to give insights about whether the soil C stabilized or mineralized and was lost as CO<sub>2</sub> to the atmosphere. Even though, CUE is a very useful parameter, however, a wide range of CUE estimates found in literature necessitates an investigation into the sources of this variability, which is critical for quantification of C dynamics in agricultural ecosystems (Manzoni et al., 2012; Geyer et al., 2016; 2019). The source of CUE variability can be categorized as genetic, environmental and methodological; genetically diverse microorganisms have different CUE (Pfeiffer et al., 2001; Molenaar et al., 2009). Genetically diverse soil microorganisms are also influenced by environmental factors, such as substrate quality and soil pH, with respective effects on microbial CUE (Manzoni et al., 2008; Jones et al., 2019). However, the interrelated effect of environmental factors, such as complex plant residues with soil pH on microbial CUE is less understood and/or nonexistent in agricultural soils of the tropics. Therefore, understanding the interrelated effect of biochemical quality of complex plant residues with soil pH on CUE provides a good insight into C dynamics in agricultural soils. Quantification of CUE for soil microorganisms is difficult. Therefore, different methodological approaches as a third source of variability were discussed recently (Geyer et al., 2019). The effect of methodological difference in CUE becomes even more challenging when an interrelated effect of complex plant residues with soil pH is considered. To better explore the complex interaction effects of different biochemical qualities of plant residues and soil pH, we used both the direct C-balance method, the indirect single enzymatic stoichiometric and multi-enzymatic stoichiometric methods to quantify CUE. Therefore, in this study we provided suitable methods for CUE when complex plant residue interacted with soil pH.

## 1.7 Objectives

The goal of this study was to explore the interrelated effect of biophysical and socio-economic factors on soil fertility variability and understand the effect of environmental and methodological variations on CUE in crop-livestock mixed agricultural systems of smallholder farmers in Ethiopia and DRC. Therefore, the main objectives of this study were to:

- i. explore the interrelated effect of biophysical and socio-economic factors on soil fertility variability in crop-livestock mixed agricultural systems in Ethiopia and to verify that farmers' indigenous knowledge would be also driven by inter-related effects of these factors, considering the continuous knowledge transfer among farmers within and across agro-ecological zones.
- ii. understand the interrelated effect of market distance and farm typology on soil fertility variability in DRC and to verify whether farmers' indigenous knowledge is a valuable proxy to assess soil fertility status.
- iii. compare the different CUE estimating methods and evaluate the influence of environmental factors on CUE as well as to test whether CUE can be used as soil fertility indicator.

## 1.8 Hypothesis

The following hypotheses were addressed in this study:

- i. For the assessment of soil fertility status across a regional scale, not only individual factors, but also interrelated effects of agro-ecology and farmers' resource endowments on soil fertility variability have to be considered, while farmers' indigenous knowledge on soil fertility status would be guided by the inter-related effects of these factors. This assumption was based on the continuous knowledge transfer among farmers within and across agro-ecologies (Leta et al, 2018).
- ii. Specific market access would be suggested as a determinant of agricultural development; it was hypothesized that with increasing market distance, the soil fertility status of smallholder farming systems would decrease since farms in remote areas, irrespective of

their wealth status, do not have the opportunity to invest in soil fertility management strategies.

- iii. By using multi-C cycling enzymes stoichiometry modeling (MCE-STM) in the estimation of CUE, a more accurate insight would be provided on microbial CUE in soils amended with complex plant residues as compared to the use of single C-cycling enzymes (BGL) and the conventional C balance approach. Furthermore, higher microbial CUE would occur in less acidic soil amended with high-quality plant residues than in more acidic soils amended with lower quality plant residues.

## **1.9 Outline of the thesis**

This doctoral thesis comprises six chapters. Chapter 1 presents the general introduction, followed by three chapters detailing the research outputs of this PhD study. Chapter 2 of this thesis has been published in the journal “Soil Use and Management” entitled ‘Agro-ecology, resource endowment and indigenous knowledge interactions modulate soil fertility in mixed farming systems in Central and Western Ethiopia. The main focus of this chapter was to assess the interrelated effect of agro-ecology and farm typology on soil fertility status in smallholder farmers in Central and Western Ethiopia. Besides, it also verifies that farmers indigenous knowledge on soil fertility status was not varied due to both agro-ecology and farm typology. The consecutive chapter 3 “Market access and resource endowment define the soil fertility status of smallholder farming systems of South-Kivu, DR Congo” has been published in the journal “Soil Use and Management”. The aim of this chapter was to explore the interrelated effect of market distance and farm typology on soil fertility status. It also verified under contrasting socio-economic and agro-ecological conditions the concept of chapter 2 that farmers’ indigenous knowledge is a valuable proxy to assess the soil fertility status. Hence, farmers’ indigenous knowledge can be implemented into a generic sampling strategy to further validate the soil fertility variability across study sites assessed by a science-based approach. Chapter 4 of this thesis entitled “Microbial carbon use efficiency during plant residue decomposition: comparison of multi-enzyme stoichiometry and C balance approach” has been published in the journal of “Applied Soil Ecology”. This chapter emphasized the development of a multi-enzyme stoichiometry model to estimate and verify soil microbial CUE influenced by environmental factors as additional proxies to understand soil fertility variability. In a final step, the three

research chapters are discussed (Chapter 5) and concluding remarks along with suggestions for prospective research are provided in Chapter 6.

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## **Chapter 2**

### **Agro-ecology, resource endowment and indigenous knowledge interactions modulate soil fertility in mixed farming systems in Central and Western Ethiopia**

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## **2 Agro-ecology, resource endowment and indigenous knowledge interactions modulate soil fertility in mixed farming systems in Central and Western Ethiopia**

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## 2.1 Abstract

Site-specific soil fertility management requires a fundamental understanding of factors that modulate soil fertility variability in the local context. To verify this assumption, this study hypothesized that soil fertility variability across two regions in Central and Western Ethiopia is determined by inter-related effects of agro-ecological zones and farmers' resource endowment ("wealthy" versus "poor" farmers). Mid-infrared spectroscopy coupled to partial least squares regression (midDRIFTS-PLSR) and wet-lab analyses were used to assess the soil fertility (i.e., soil pH, total soil carbon (TC), total soil nitrogen (TN), plant-available phosphorous ( $P_{av}$ ) and potassium ( $K_{av}$ )) across four agro-ecological zones: "High-Dega" (HD), "Dega" (D), "Weina-Dega" (WD) and "Kola" (K). MidDRIFTS peak area analysis of spectral frequencies (2930 (aliphatic C-H), 1620 (aromatic C=C), 1159 (C-O poly-alcoholic and ether groups)  $cm^{-1}$ ) was applied to characterize soil organic carbon (SOC) quality and to calculate the SOC stability index (1620:2930). Higher TC in HD, as well as higher TN and  $K_{av}$  contents in K were found in fields of wealthy compared with poor farmers. Resource endowment dependent soil fertility management options revealed SOC of higher quality in wealthy compared with poor farms in D. Agro-ecological zones distinctions contributed to these soil fertility differences. Farmers distinguished visually fertile and less fertile fields based on 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. To conclude, tailor-made soil fertility management in the local context must consider agro-ecological zones and resource endowment interactions along with farmers' indigenous knowledge.

**Keywords:** midDRIFTS; soil fertility variability; resource endowment; farmers' indigenous knowledge; SOC stability index; agro-ecological zones

## 2.2 Introduction

Integrated soil fertility management (ISFM) is an intervention strategy to counteract the problem of soil fertility depletion of smallholder farming systems in sub-Saharan Africa (SSA) (Vanlauwe et al., 2010). Its adoption across different regions of SSA remains, however, challenging (Vanlauwe et al., 2015). This is mainly due to resource shortcomings (e.g., land size, capital) that force resource-constrained farmers to expand into marginal lands, while wealthy farmers continue investing in fertile lands. This situation is aggravated by insecure tenure systems, prohibiting farmers from investing in their land, along with limited access to fertilizer inputs (Stevenson et al., 2019). These features have led to highly variable soil fertility levels across and within regions, magnified by inherent heterogeneity of agro-ecological zones and a wide range in socio-economic status among smallholder farmers (Tittonell et al., 2005a). Heterogeneity of soil fertility does not allow uniform soil management strategies in larger areas, making ISFM adjusted to local contexts more essential. To tailor demand-oriented ISFM interventions to smallholder conditions under different local contexts, factors modulating soil fertility variability must be understood, considering farmers' resource endowment (i.e. their wealth) and indigenous knowledge (Vanlauwe et al., 2015; Tittonell et al., 2005b).

Previous soil fertility assessments in Eastern (e.g., Kenya) and Southern (e.g., Zimbabwe) Africa revealed the influence 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). These studies were, however, not based on generic and harmonized soil surveying procedures, making direct comparisons across different agro-ecological zones and smallholder farming systems difficult. Africa Soil Information Service (AfSIS) (Vågen et al., 2010) and Ethiopian Soil Information System (EthioSIS) (Amare et al., 2018) have made important progress in consolidating existing soil fertility survey protocols for several African countries, including Ethiopia. Nevertheless, (1) the inter-related effects of agro-ecological zones and farmers' resource endowments, along with (2) farmers' indigenous knowledge as additional proxies for soil fertility assessment have so far been neglected and thus need further investigation. This is justified as it could be suggested that continuous knowledge transfer among farmers within and across agro-ecological zones (Leta et al., 2018), as well as contrasting agro-ecological and geological contexts (Mengistu, 2003) modulate soil fertility variability. Hence, it was our first objective to perform a local soil fertility

survey to test the hypothesis that not only individual but also inter-related effects of agro-ecological zones and farmers' resource endowments affect soil fertility variability in a local context. Our second objective was to verify 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 transfer of knowledge among farmers within and across agro-ecological zones (Leta et al., 2018).

## 2.3 Material and methods

### 2.3.1 Site selection and farm typology characterization

The soil fertility survey was conducted in four contrasting agro-ecological zones of Central and Western Ethiopia, which were defined according to Mengistu (2003) and Hurni (1998): (i) “Kola” (K) (<1500 m a.s.l., average temperatures of 15 to 27°C, average rainfall of 2037 mm), and (ii) “Weina-Dega” (WD) (1500-2500 m a.s.l., 15 to 27°C, 1376 mm), (iii) “Dega” (D) (2500-3200 m a.s.l.,  $\leq 9^\circ\text{C}$ , 938 mm) and (iv) “High-Dega” (HD) (3200-3500 m a.s.l.,  $\leq 9^\circ\text{C}$ , 938 mm). Agro-ecological zones of K (Lelisadimtu (36°24'E; 9°02'N)) and WD (Fromsa (36°45'E; 9°03'N)), are subsistence maize dominated crop-livestock farming systems and Nitisols with clay texture (FAO, 2015), while D (Kolugelan (38°9'E; 9°22'N)) and HD (Chilanko (38°11'E; 9°20'N)) are dominated by market-oriented potato/barley systems as well as Luvisols and Alisols with clay texture (FAO, 2015). Lelisadimtu and Fromsa were located in Diga District (Western Ethiopia), while Kolugelan and Chilanko were located in Jeldu district (Central Ethiopia) (Table 1; Fig. S1).

Farm typologies (resource endowment) at the target sites (villages) were defined during village meetings and focus group discussions. Two to three focus group discussions with a total of 16-18 household heads with an equal share of females and males as well as young and old farmers were held in each agro-ecological zone. The main farm typology indicators were farm size (landholdings (LH)), livestock ownership and level of agricultural inputs (i.e., chemical fertilizer) (Hailelassie et al., 2006; Kebede et al., 2019). Thresholds set by farmers in all villages were <2 ha farm size, <6 tropical livestock units (TLU), and relatively low chemical fertilizer rates to categorize farmers as “Eyeessaa (poor)”, while a LH of  $\geq 4$  ha,  $\geq 8$  TLU and use of full

fertilizer rates (100 kg urea and 100 kg DAP) were defined as “Ditta (wealthy)”. This is because wealthy farmers frequently intend to maximize crop productivity by applying fertilizer, whereas poor farmers cannot follow a similar strategy due to a lack of cash to purchase fertilizer. To confirm the agreed farm typology thresholds, detailed data on farm typology indicators were collected on 90 predefined wealthy and poor households (10% of the total population) (Table 1).

**Table 1.** Average values of socio-economic indicators (farm size, number of livestock and amount of fertilizer used) for the different farm typologies in the selected study regions (Lelisa Dimtu (Kola (K)), Fromsa (Weina-Dega (WD)), Kolu-Gelan (Dega (D)) and Chilanko (High-Dega (HD)); Number of households = 90.

Agro-ecology	Typology	Farm size [ha]	Livestock holding [TLU <sup>1</sup> ]	Fertilizer rate [kg ha <sup>-1</sup> ]
Kola (K)	Wealthy (Ditta)	5.7 (1.0) <sup>ab</sup>	11.7 (1.8) <sup>a</sup>	117 (25) <sup>bc</sup>
	Poor (Eyeessaa)	0.8 (1.0) <sup>d</sup>	3.2 (1.8) <sup>d</sup>	64 (35) <sup>c</sup>
Weina-Dega (WD)	Wealthy (Ditta)	4.4 (0.9) <sup>abc</sup>	8.6 (1.59) <sup>abc</sup>	121 (35) <sup>abc</sup>
	Poor (Eyeessaa)	1.1 (1.0) <sup>d</sup>	4.5 (1.8) <sup>cd</sup>	72 (35) <sup>c</sup>
Dega (D)	Wealthy (Ditta)	4.9 (0.9) <sup>ab</sup>	9.02 (1.5) <sup>abc</sup>	192 (46) <sup>ab</sup>
	Poor (Eyeessaa)	1.8 (1.1) <sup>cd</sup>	5.9 (2.0) <sup>bcd</sup>	180 (30) <sup>ab</sup>
High-Dega (HD)	Wealthy (Ditta)	7.0 (1.0) <sup>a</sup>	9.5 (1.7) <sup>ab</sup>	198 (27) <sup>a</sup>
	Poor (Eyeessaa)	1.8 (1.0) <sup>cd</sup>	5.4 (1.70) <sup>bcd</sup>	135 (20) <sup>abc</sup>
P-level (agro-ecology)		Ns	Ns	***
P-level (typology)		***	**	ns
P-level (agro-ecology × typology)		Ns	Ns	ns

Significance levels: ns, not significant at  $P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

<sup>1</sup>TLU = Tropical livestock unit.

### 2.3.2 Soil sampling

In each agro-ecological zone ( $n = 4$ ), 14 individual households (7 wealthy, 7 poor) per farm typology were 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 selected for soil sample collection (Vågen et al., 2012). According to Yeshaneh (2015), farmers use soil color as the most important indicator of soil fertility, where black and brown soils were considered as fertile and red soils as less fertile.

During soil sampling, the household head indicated the color of the specified soil of the field plot. According to the sampling procedure, a total number of 224 geo-referenced soil samples were collected (4 agro-ecological zones (K, WD, D, HD)  $\times$  2 farm typologies (wealthy, poor)  $\times$  7 farms per typology  $\times$  2 fields per farm (fertile and less fertile)  $\times$  2 soil depths (0-20 cm, 21-50 cm)). Soil samples were air-dried and passed through 2 mm sieve prior shipping to the University of Hohenheim (Stuttgart, Germany) for further analysis.

### 2.3.3 Soil analysis

Soil pH ( $\text{CaCl}_2$ ) was measured according to Houba et al. (2000). Total carbon (TC) and nitrogen (TN) were analyzed by dry combustion. Available phosphorus ( $P_{av}$ ) was measured colorimetrically at 720 nm using Bray1 method (Bray & Kurtz (1945). Available potassium ( $K_{av}$ ) was analyzed using ICP-OES (Agilent 5100) (Schüller, 1969). Calcium-acetate-lactate was used as an extractant for both phosphorous and potassium.

MidDRIFTS-based analyses were performed according to Mirzaeitalarposhti et al. (2015), Rasche et al. (2013), and Demyan et al. (2012). 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 v7.5 (Bruker Optik GmbH) (Rasche et al., 2013). Similarly, peak area integration by midDRIFTS using OPUS 7.5 software (Bruker Optik GmbH) (Demyan et al., 2012) was conducted to provide an additional measure of the soil fertility status. Three prominent peaks (i.e., 2930, 1620 and 1159  $\text{cm}^{-1}$ ) with their respective integration limits (3000-2800, 1770-1496, 1180-1126  $\text{cm}^{-1}$ ) 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  $\text{cm}^{-1}$  represents less stable aliphatic C-H groups, components of the active SOC pool (Demyan et al., 2012; Laub et al., 2019). Peak 1620  $\text{cm}^{-1}$  represents more stable aromatic C=C bonds as part of the recalcitrant SOC pool (Demyan et al., 2012; Laub et al., 2019). The third peak at 1159  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  are commonly calculated as the SOC stability index, which is used as a soil quality indicator. Further methodological details are given in the supplementary materials of this paper.

#### 2.3.4 Statistical analysis

Univariate analysis using Kolmogorov-Smirnov tests was conducted to determine if the data met the assumptions of normality. Except for  $P_{av}$  and  $K_{av}$ , all soil chemical properties met the assumption. For  $P_{av}$  and  $K_{av}$ , logarithmic and square root transformations were performed respectively. Factorial analysis of variance (ANOVA) was conducted to assess the effect of agro-ecology, farm typology (resource endowment class), farmers' indigenous knowledge and their interaction on soil fertility status, using a mixed model with restricted maximum likelihood (REML) (Piepho et al., 2003) (SAS statistical software, version 9.4, SAS Institute, North Carolina, USA). Agro-ecology, farm typology and soil fertility status as defined by farmers 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).

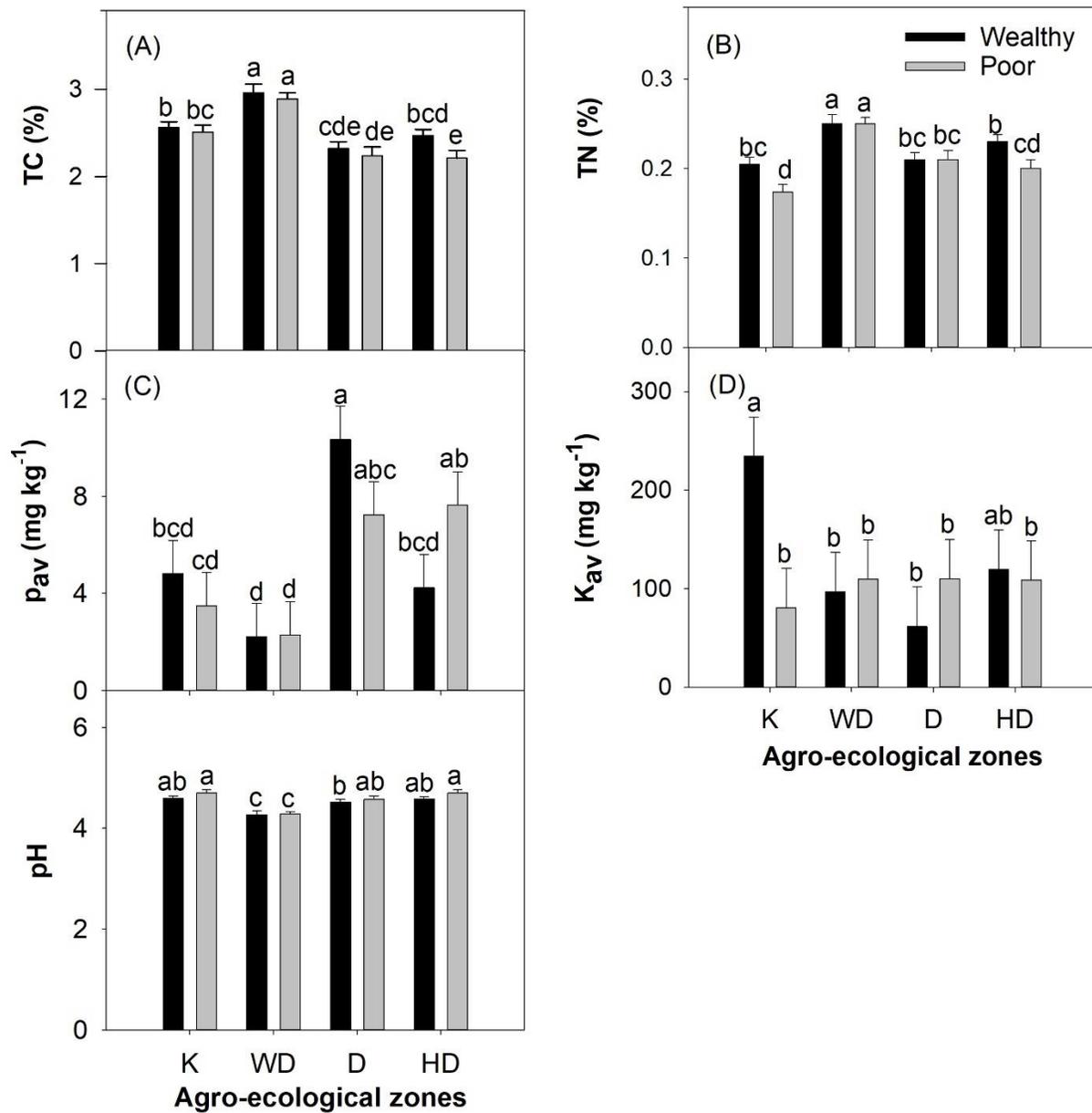
## 2. 4 Results

### 2.4.1 Interrelated effect of agro-ecological zones and farmers' resource endowment on soil fertility

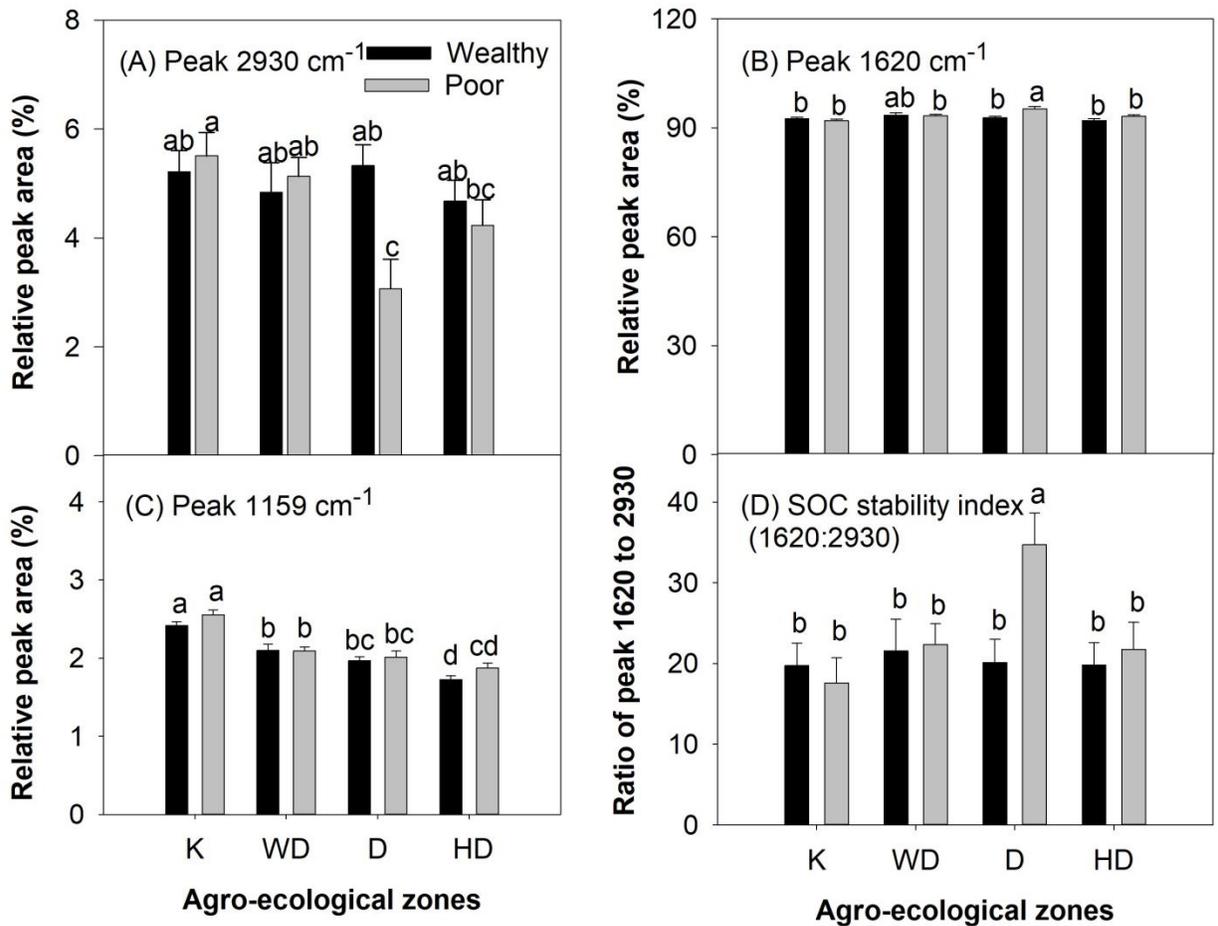
Analysis of variance showed that not only agro-ecological zone but also farmers' resource endowment had a significant effect on soil fertility indicators (i.e., TC, TN,  $K_{av}$ ;  $P < 0.01$ ) (Fig. 1). However, pH and  $P_{av}$  were only influenced by agro-ecological zone ( $P < 0.01$ ). An interaction effect between agro-ecological zone and resource endowment was observed for  $K_{av}$  ( $P < 0.01$ ) (Fig. 1D). The higher  $K_{av}$  values (234  $\text{mg kg}^{-1}$ ) were noted for fields of wealthy farmers in "Kola" (K), while the lowest  $K_{av}$  values (62  $\text{mg kg}^{-1}$ ) were recorded on wealthy farms in "Dega"

(D) ( $P < 0.01$ ) (Fig. 1D). The highest values of TC and TN were observed in “Weina-Dega” (WD) in both farm typologies, while the lowest TC was found in fields of D ( $P < 0.01$ ) (Fig. 1A). In “High-Dega” (HD), higher TC and higher TN contents were found in fields of wealthy compared with less wealthy farmers ( $P < 0.01$ ) (Fig. 1A & 1B). Agro-ecological zone influenced soil pH and  $P_{av}$  ( $P < 0.001$ ) (Fig. 1C & 1E), where lowest values were observed in WD. No effect of farm typology was found for pH and  $P_{av}$  ( $P > 0.05$ ) (Fig. 1C & 1E).

Three dominant relative peak areas representing SOC functional groups were identified and used as proxies for SOC quality: (i)  $2930\text{ cm}^{-1}$  (C-H- aliphatic groups), (ii)  $1620\text{ cm}^{-1}$  (C=C- aromatic groups), (iii)  $1159\text{ cm}^{-1}$  (C-O poly-alcoholic and ether group) (Fig. 2A to 2C). The relative peak areas of these SOC functional groups and the SOC stability index, calculated as the ratio of aromatic to aliphatic area (peak  $1620\text{ cm}^{-1}$  to  $2930\text{ cm}^{-1}$ ), varied across agro-ecological zones and farmers resource endowment with respective interaction effects ( $P < 0.05$ ) (Fig. 2A to 2D). The highest (5.5%) and lowest (3.1%) peaks at  $2930\text{ cm}^{-1}$  were noted on fields of poor farmers in K and D, respectively. Similarly, fields of wealthy farmers revealed a larger peak area at  $2930\text{ cm}^{-1}$  than those of poor farmers in D ( $P < 0.05$ ) (Fig. 2A). In contrast, the highest (95.2%) and lowest (91.9%) values of relative peak area at  $1620\text{ cm}^{-1}$  peak were found in fields of poor farmers in D and K, respectively ( $P < 0.05$ ) (Fig. 2B). The highest relative peak area of  $1159\text{ cm}^{-1}$  was observed in K fields of both farm typologies, while the lowest were found in HD ( $P < 0.01$ ) (Fig. 2C). The highest and lowest SOC stability indice were calculated for fields of poor farmers in D and K, respectively ( $P < 0.001$ ) (Fig. 2D). In D, a larger index was noted in fields of poor than wealthy farmers ( $P < 0.05$ ). Furthermore, significant positive correlations of pH and TOC with C-H aliphatic SOC (pH:  $r^2 = 0.39$ ; TOC:  $r^2 = 0.51$ ) were found, while negative relationships were calculated for C=C aromatic SOC (pH:  $r^2 = -0.39$ ; TOC:  $r^2 = -0.47$ ) ( $P < 0.001$ ) (data not shown). Correlation between the stability index and TOC ( $r^2 = -0.45$ ) and TN ( $r^2 = -0.24$ ) ( $P < 0.001$ ) were negative, while no correlation was found for soil pH.



**Figure 1.** Soil chemical properties (A = total carbon (TC) (%); B = total nitrogen (TN) (%); C = available phosphorus (P<sub>av</sub>) (mg kg<sup>-1</sup>), D = available potassium (K<sub>av</sub>) (mg kg<sup>-1</sup>); E = soil pH) obtained from soils of fields of wealthy and poor farmers' fields across the four agro-ecological zones (K (Kola), WD (Weina-Dega), D (Dega), HD (High-Dega)). N = 215 for TC, TN and pH while 96 for (P<sub>av</sub>) and (K<sub>av</sub>). Bars with different letters on top of standard error indicate significant differences at P < 0.05.



**Figure 2** MidDRIFTS relative peak areas ((A) 2930 cm<sup>-1</sup>, (B) 1620 cm<sup>-1</sup>, (C) 1159 cm<sup>-1</sup>) and ratio of 1620:2930 (D) obtained from soils of fields of wealthy and poor farmers' fields across the four agro-ecological zones (K (Kola), WD (Weina-Dega), D (Dega), HD (High-Dega)). N = 107; Bars with different letters on top of standard error indicate significant differences at P < 0.05.

#### 2.4.2 Farmers' indigenous knowledge

Farmers' indigenous knowledge on soil fertility agreed with 75% (8 out of 12 soil fertility indicators) of scientifically generated soil fertility indicators across agro-ecological zones (Tables 2 & 3). Soil color as a soil fertility indicator for farmers suggested that black and brown soils were considered as fertile, while red soils were assessed to less fertile soils. This was confirmed by laboratory analysis, i.e. black and brown soils had generally higher TC, TN, P<sub>av</sub>

and pH than the red soils, except soil pH at HD (Table 2). The capability of farmers' indigenous knowledge to identify fertile and less fertile soils was further verified by a higher relative peak area of 1159 cm<sup>-1</sup> in less fertile fields; a similar trend was noted for the SOC stability index ( $P < 0.01$ ) (Table 3).

**Table 2.** Selected soil fertility indicators (TC, total carbon; TN, total nitrogen; P<sub>av</sub>, available phosphorus, pH, soil pH) in relation to different soil colors (red, less fertile; black and brown; fertile) across agro-ecological zones. Stand errors are given in brackets. N = 24.

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

Significance levels: ns, not significant at  $P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

**Table 3.** Relative peak areas and stability index as indicators of soil organic carbon (SOC) quality with regard to farmers' perception of fertile and less fertile fields. Standard errors are given in brackets, N = 107.

SOC quality indicators	Fertile	Less fertile	P level
Peak 2930 cm <sup>-1</sup>	4.95 (0.22)	4.55 (0.22)	ns
Peak 1620 cm <sup>-1</sup>	92.88 (0.26)	93.18 (0.26)	ns
Peak 1159 cm <sup>-1</sup>	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  $\text{cm}^{-1}$ , aliphatic C-H; Peak 1620  $\text{cm}^{-1}$ , aromatic C=C; Peak 1159  $\text{cm}^{-1}$ , C-O poly-alcoholic and ether groups of SOC functional groups.

Significance levels: ns, not significant at  $P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

## 2.5 Discussion

### 2.5.1 Inter-related effect of agro-ecological zones and farmers' resource endowment on soil fertility

It was a key finding that the soil fertility status in the study region was determined by an inter-related effect of farmers' resource endowment (farm typology) and agro-ecological zone. This effect was most pronounced between the wealthy and poor farms located in the lowland (K) and highland (HD) agro-ecological zones, 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-ecological zone. This finding is in line with Nyamangara et al. (2011) and Masvaya et al. (2010) those who observed higher TN, SOC,  $P_{av}$  and cation exchange capacity (CEC) in wealthy than poor farmers' fields in two different agro-ecological zones in Zimbabwe.

The effect of resource endowment in the lowlands was explained by the better soil nutrient status (e.g., TN,  $K_{av}$ ) in the fields of wealthy compared with 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 (Corbeels et al., 2000; Tian et al., 2005; Haileslassie et al., 2006). These interventions provide sufficient resources to replenish the soil nutrient pool (Haileslassie et al., 2007; Cobo et al, 2010). With this, 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). Even though poor farmers have a higher livestock density and may potentially provide more manure per area of land; these farmers commonly use livestock manure for cooking fuel rather than applying it to fields for fertilization purpose. The use of manure as fuel is essential for poor farmers as they do not have extra land to cultivate biomass for firewood production, unlike for wealthy farmers.

Apart from the obvious differences in the soil nutrient status in the lowlands, no clear effect of resource endowment on TC content and SOC quality was observed. 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 (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 polyphenol content) of respective crop residues enhanced the SOC pool (Wang et al., 2015b). Irrespective of the typology classes in the low and medium altitude agro-ecological zones, 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 farmers than those wealthy farmers due to higher fertilization (Wang et al., 2015b). This high potential of C stabilization was corroborated by the presence of recalcitrant SOC pools (i.e., C-O poly-alcoholic and ether groups). In the highlands, in contrast to low- and midlands, there was a distinct difference of TC content, which was higher in the fields of wealthier farmers. This was substantiated by the option of wealthy farmers to combine organic and inorganic fertilizer inputs, leading to an increase of 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 farmers.

The application of inorganic fertilizer resulted most likely in greater plant biomass production, providing additional inputs to accelerate the 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-ecological zone, indicating fewer 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 farmyard manure. The higher labile SOC pool with a lower SOC stability index may be an indicator for high soil fertility as compared to higher C=C aromatic and high stability index. In contrast, C=C aromatic pools were shown to increase soil C stabilization (Haynes,

2005). It is acknowledged that the labile SOC pool can benefit important soil functions; including soil aggregate formation and nutrient supply as well as serve as essential microbial energy source (Kunlanit et al., 2020; Maia et al., 2007; Haynes, 2005; Ghani et al., 2003).

### 2.5.2 Farmers' perception of soil fertility across agro-ecological zones and farm typologies

This study confirmed the capability of farmers' indigenous knowledge to define the soil fertility status, a capacity influenced by neither agro-ecological zone nor farm typology. The identification of soil fertility status based on farmers' indigenous knowledge is often in close agreement with soil chemical properties analyzed in the laboratory (Huynh et al., 2020). Irrespective of their wealth status and geographic location, farmers confirmed their capacity to assess soil fertility variability using indigenous knowledge accumulated through generations 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-ecological zones may have been responsible for the common farmer perception that red soils are less fertile than black and brown soils. Farmers describe and classify their soils using a holistic approach and use relatively homogeneous soil classification indicators across agro-ecologies (Laekemariam et al., 2017). Accordingly, farmers have been using soil color, soil texture, soil depth, topography and drainage, as well as crop performance as criteria to categorize their land into fertile and less fertile fields (Belachew & Abera, 2010; Corbeels et al., 2000; Yeshaneh, 2015; Karlton et al., 2013). 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 in red soils in the midlands and lowlands, respectively. Higher  $P_{av}$  values in black than in 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). This might indicate that organic matter and soil mineralogy are the most important soil properties that govern soil color (Poppiel et al., 2020).

No difference between farm typologies was observed with respect to the identification of fertile and less fertile fields based on indigenous knowledge (Table S2), a likely result of the informal communication channels among social institutions: e.g. ‘iddir’ (indigenous and local self-help association), ‘debo’ (collective labor support group), and ‘dado’ (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 in soil fertility exist, both wealthy and poor farmers have comparable indigenous knowledge to identify fertile and less fertile fields.

Indigenous knowledge is generally used by farmers to design management strategies for site-specific soil fertility problems. Farmers in the lowlands, for example, fallow, burn organic residues and apply higher farmyard manure 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).

## **2.6 Conclusions**

This study verified that inter-related rather than individual effects of agro-ecological zones and farmers’ resource endowment (farm typology) must be considered to explain soil fertility variability of smallholder farms across regions and wealth classes. Accordingly, it was inferred that prospective ISFM strategies must be niche-based, considering such contrasting but inter-related agro-ecological zones and farm typologies to reduce the inherent depletion of soil fertility across smallholder farms in the study region of Ethiopia. Moreover, across agro-ecological zones, farmers identified fertile and less fertile fields based on their indigenous knowledge, which was corroborated by the laboratory-based soil fertility survey. Hence, farmers’ indigenous knowledge was verified as a valuable proxy for this local soil fertility survey.

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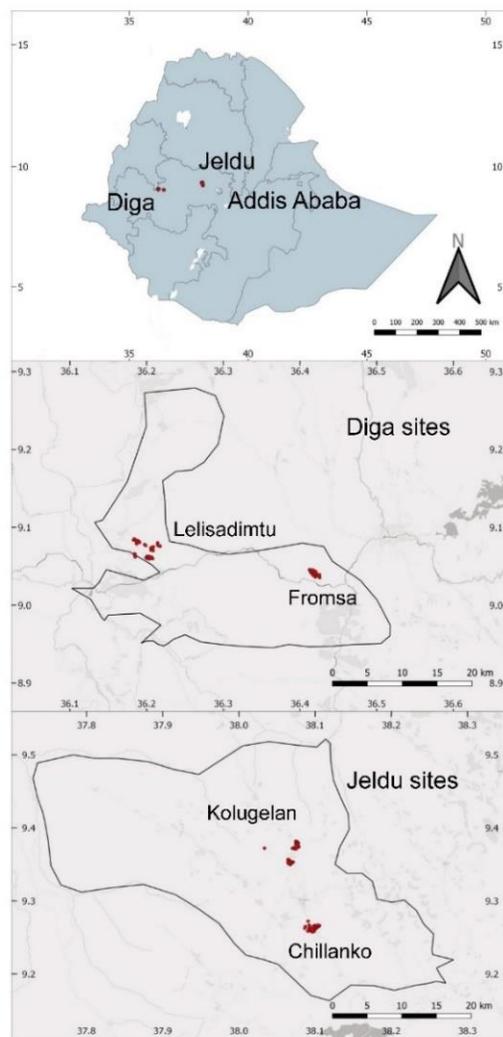
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## 2.9 Supplementary materials

### 2.9.1 Site location and soil sampling

#### Site location



**Figure S1.** Study regions with respective sampling points located in Central (Dega (D), High-Dega (HD)) and Western (Kola (K), Weina-Dega (WD)) Ethiopia.

## Soil sampling

The soil fertility survey was conducted in four contrasting agro-ecologies of Central and Western Ethiopia, which were defined according to Mengistu (2003) and 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). In both sites of K (Lelisadimtu (36°24'E; 9°02'N)) and WD (Fromsa (36°45'E; 9°03'N)), subsistence maize dominated crop livestock farming systems and clayey Nitisols (WRB classification; FAO, 2014) are predominant. Agro-ecology (iii) “Dega” (D) (cold) and (iv) “High-Dega” (HD) (moist cold) (2500-3500 (m a.s.l.)) show average temperatures of  $\leq 9^{\circ}\text{C}$  and average annual rainfall of 938 mm, whereby representative sites of D (Kolugelan (38°9'E; 9°22'N)) and HD (Chilanko (38°11'E; 9°20'N)) soils are dominated by clayey Luvisols and Alisols (WRB classification; FAO, 2014). The dominant cropping systems in D and HD are market-oriented potato/barley systems. Lelisadimtu and Fromsa were located in Diga District (Western Ethiopia), while Kolugelan and Chilanko were located in Jeldu district (Central Ethiopia) (Fig. S1).

From an official farmer list provided by the local agricultural development agent, farmers were categorized into three wealth groups. These were based on the agreed thresholds set by the focus group discussions. Farm typologies (resource endowment) at the target sites (villages) were defined during village meetings and focus group discussions, with an equal share of female and male as well as young and old farmers. Main farm typology indicators were farm size (land holdings (LH)), livestock ownership and level of agricultural inputs (i.e., chemical fertilizer). Thresholds set by farmers in all villages were <2 ha farm size, <6 tropical livestock units (TLU), and relatively low chemical fertilizer rates to categorize farmers as “Eyeessaa (poor)”, while a LH of  $\geq 4$  ha,  $\geq 8$  TLU and use of full fertilizer rates were defined as “Ditta (wealthy)”. To confirm the agreed farm typology thresholds during focus group discussions, detailed data on farm typology indicators were collected using a quick baseline survey on predefined wealthy and most poor 90 households (10% of the total population). Due to the fact that the study area is characterized by subsistence smallholder farmers and that the entire population is generally poor, we referred purposely only to the two contrasting typology classes better off and the most poor. On each farm, the head of the household was requested to indicate the most and least fertile field plots based on their indigenous knowledge about soil fertility status. 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.

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: (i) 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 (ii) a parallel study in the Democratic Republic of Congo (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., 2021 published in journal of soil use and management). We have considered 2 countries to increase the overall sample number, a prerequisite to increase the accuracy of the prediction model development explained below (i.e. MidDRIFTS analysis and PLSR-based prediction of soil chemical properties). 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.

### 2.9.2 Soil sample analysis

#### **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 ( $\text{CaCl}_2$ ) extracting solution with a soil-to-solution ratio of 1:2.5 (Houba et al., 2000). Soil pH results showed values of  $<7.4$ , so that total carbon estimation was regarded as equivalent to total SOC (Wang et al., 2015a; Schumacher, 2002). Total carbon (TC) and nitrogen (TN) were analyzed by dry combustion (vario MAX CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). Plant available phosphorus ( $P_{\text{av}}$ ) was analyzed using the Bray1 method (Bray & Kurtz, 1945), and plant available potassium ( $K_{\text{av}}$ ) was analyzed using the method of Schüller (1969).

### 2.9.3 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  $\text{cm}^{-1}$ . MidDRIFTS-PLSR-based prediction models for each soil chemical property (i.e., TC, TN, pH,  $P_{\text{av}}$ ,  $K_{\text{av}}$ ) were constructed with OPUS-QUANT2 package of OPUS v7.5 (Bruker Optik GmbH) (Rasche et al., 2013). For this, the spectral range was set to exclude the background carbon dioxide region (2300-2400  $\text{cm}^{-1}$ ) and edges of detection limits of the spectrometer ( $<700$  and  $>3900$   $\text{cm}^{-1}$ ) 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 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 S1) 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.

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 S1). 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. For  $P_{av}$  and  $K_{av}$  wet chemistry data were

used for further data analysis. Figure S2 shows the relations between measured and predicted values based on the 'ComCount' prediction models described in Table S1. 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. S2). 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 S1).



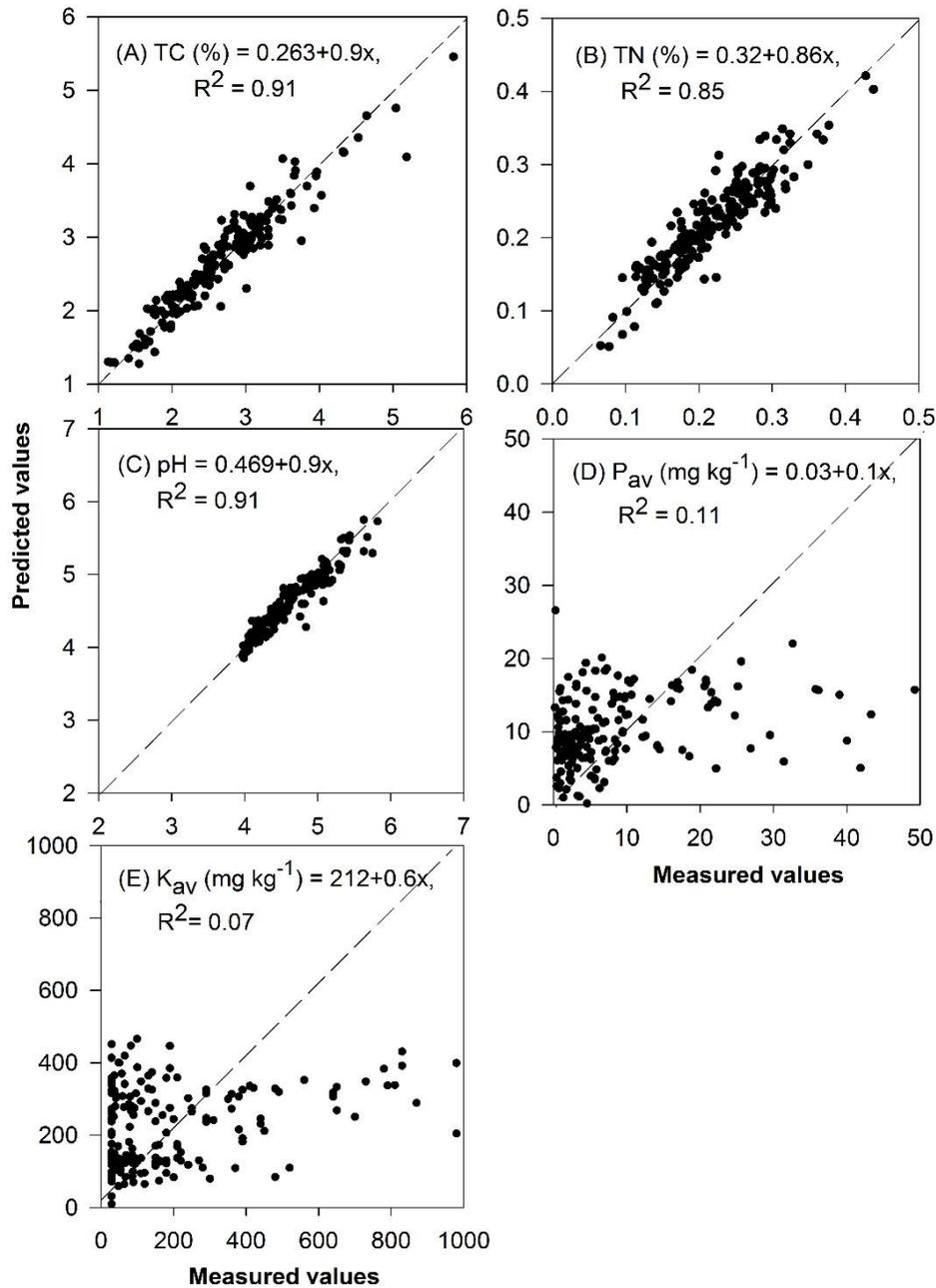
**Table S1.** Calibration results of midDRIFTS spectra of bulk soils across both countries (Ethiopia, DR Congo), based on independent test validation (n = 183).

Soil chemical properties <sup>1</sup>	Model name	Number of calibrated/ validated samples	Pre-processing method <sup>2</sup>	Spectral frequencies	Model accuracy parameters <sup>3</sup>		
					<i>R</i> <sup>2</sup>	RPD	RMSEP
pH	pH ComCount	123/61	1 <sup>st</sup> D +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	1 <sup>st</sup> D	2980-2399,1959-1279, 941-698	0.86	2.71	0.03
P <sub>av</sub> [mg kg <sup>-1</sup> ]	p <sub>av</sub> ComCount	123/61	COE	3658-3317,2980-2399,2301-1957	0.14	1.08	11.5
K <sub>av</sub> [mg kg <sup>-1</sup> ]	K <sub>av</sub> ComCount	123/61	VN	1620-939	0.05	1.03	710

<sup>1</sup>Soil chemical properties: pH, soil pH; TC, total carbon; TN, total nitrogen, P<sub>av</sub>, plant-available phosphorous; K<sub>av</sub>, plant-available potassium.

<sup>2</sup>Pre-processing methods (optimization): 1<sup>st</sup>D, first derivative; VN, vector normalization; SLS, straight line subtraction; COE, constant offset elimination.

<sup>3</sup>Model accuracy parameters: *R*<sup>2</sup>, coefficient of determination; RPD, residual prediction deviation; RMSEP, root mean square error of prediction.



**Figure S2.** 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 ( $P_{av}$ ) ( $\text{mg kg}^{-1}$ ), E = available potassium ( $K_{av}$ ) ( $\text{mg kg}^{-1}$ )), using respective ‘ComCount’ prediction model described in Table S1.

## 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 of Ethiopia. Three prominent peaks (i.e., 2930, 1620 and 1159  $\text{cm}^{-1}$ ) with their respective integration limits (3000-2800, 1770-1496, 1180-1126  $\text{cm}^{-1}$ ) 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  $\text{cm}^{-1}$  represents less stable aliphatic C-H groups, components of the active SOC pool (Demyan et al., 2012; Laub et al., 2019). Peak 1620  $\text{cm}^{-1}$  represents more stable aromatic C=C bonds as part of the recalcitrant SOC pool (Demyan et al., 2012; Laub et al., 2019). The third peak at 1159  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  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; Laub et al., 2019).

**Table S2** Selected soil fertility indicators (TC, total carbon; TN, total nitrogen;  $P_{av}$ , available phosphorus, pH, soil pH) in different wealth groups and farmer defined soil fertility groups Stand errors are given in brackets.

Wealth status (WS)	Fertility class (FC)	TC (%)	TN (%)	$P_{av}$ ( $\text{kg}^{-1}$ )	pH
Wealthy	Less fertile	2.56 <sup>a</sup> (0.06)	0.23 <sup>a</sup> (0.01)	2.99 <sup>b</sup> (1.2)	4.5 <sup>ab</sup> (0.07)
	Fertile	2.60 <sup>a</sup> (0.06)	0.26 <sup>a</sup> (0.01)	7.89 <sup>a</sup> (1.2)	4.64 <sup>a</sup> (0.07)
Poor	Less fertile	2.38 <sup>b</sup> (0.06)	0.26 <sup>a</sup> (0.01)	4.23 <sup>a</sup> (1.2)	4.44 <sup>b</sup> (0.07)
	Fertile	2.55 <sup>ab</sup> (0.06)	0.26 <sup>a</sup> (0.01)	6.08 <sup>a</sup> (1.2)	4.59 <sup>a</sup> (0.07)
P level (WS × FC)		Ns	Ns	*	Ns
P value (FC)		Ns	Ns	**	**
P value (WS)		Ns	Ns	ns	Ns

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## Chapter 3

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

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

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### 3.1 Abstract

This study verified the inter-related effect of “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 soil fertility variability in smallholder farming systems in two distinct regions (Bushumba, Mushinga) of South-Kivu, DR Congo. A total of 384 soil samples were selected from representative farmers’ fields and analyzed for soil pH, soil organic carbon (SOC) content and quality, as well as nutrient contents, using midDRIFTS (mid-infrared diffuse reflectance Fourier transform spectroscopy) and wet chemistry analyses. MidDRIFTS was also used to calculate SOC stability indexes as SOC quality proxies. “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. “Farm typology” was related to exchangeable calcium and magnesium, while “site” resulted in a difference of plant available phosphorus. SOC quality indexes were related to “site”, interacting with “market distance”. A “market distance” effect became obvious in the medium wealthy and poor farms of Mushinga, where a lower SOC quality in remote fields plots was noted with increasing market distance. In agreement with farmers’ indigenous knowledge, soil fertility levels were higher in deep than shallow soils, which were reflected in higher nutrient stocks in deep soils receiving organic amendments. Our results inferred that soil fertility variability across smallholder farms must consider various inter-related determinants as basis for site-specific fertility management interventions.

**Keywords:** market distance, soil fertility variability, midDRIFTS, farm typology, farmers’ indigenous knowledge.

## 3.2 Introduction

In the South-Kivu region of the Democratic Republic of Congo (DRC), the rural population currently has approximately 3.8 million people (250 inhabitants per km<sup>2</sup>) (World Bank, 2018; Mbadu Muanda et al., 2018). More than 80% of this population are smallholders relying on subsistence agriculture as their main activity for income generation (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 (Vanlauwe et al., 2017; Pypers et al., 2011). A similar trend has been noted in many other regions of sub-Saharan Africa (SSA) (Tadele, 2017; Tully et al., 2015). As a consequence, food insecurity has become a major societal challenge putting people in South-Kivu at severe risk (FAO et al., 2018; Murphy et al., 2015). 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, including both organic and mineral fertilizers, remains challenging (Vanlauwe et al., 2010; Sanginga & Woomer, 2009).

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 & 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; Crawford et al., 2003; Minten & Kyle, 1999), 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 (Tittonell et al., 2010; Ojiem et al., 2006). 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 barrier 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 (Vanlauwe et al., 2016; Tittonell & Giller, 2013; Crawford et al., 2003). 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 as to 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 would be useful 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 present realistically local conditions with their complex socio-economic characteristics. Indigenous knowledge of smallholder farmers could be a critical aid to guiding agricultural interventions to sustain farm productivity and 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 evaluated as a suitable tool to assess soil fertility variability in and among African agricultural farming systems (Cobo et al., 2010; Shepherd & Walsh, 2007; Vågen et al., 2006). Basically, midDRIFTS employs a non-destructive

estimation of physico-chemical soil properties allowing the analysis of spatial variability of soil properties across agro-ecologies (Shepherd & Walsh, 2014; McCarty et al., 2002). Coupled with partial least squares regression (PLSR)-based prediction, midDRIFTS is suited to process large batches of soil samples (Rasche et al., 2013; Cobo et al., 2010). 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 (Mirzaeittalarposhti et al., 2015; Demyan et al., 2012).

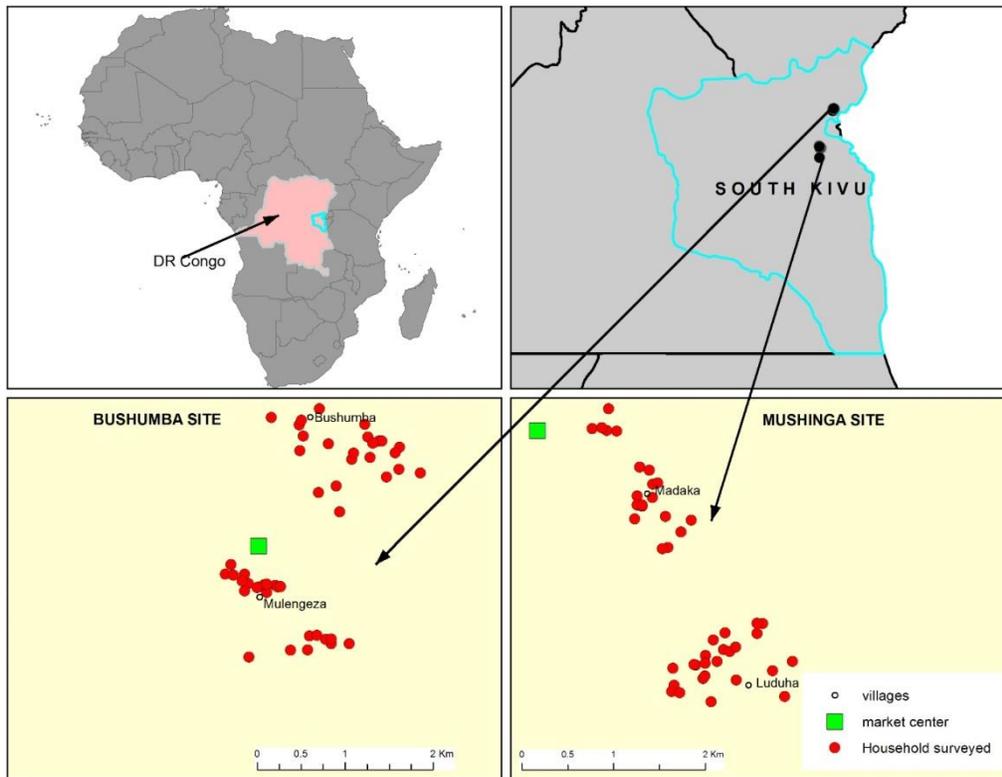
The first objective of this study was to assess the inter-related influence of market distance and resource endowment classes on soil fertility status of smallholder farming systems of South-Kivu in DRC, as a case study for Central Africa. The second objective was to verify, under contrasting socio-economic 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 statuses 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.

### **3.3 Material and methods**

#### **3.3.1 Study site description**

The soil fertility survey was conducted in the “Territoire de Kabare”, “groupement” of Bushumba (Site #1, 2° 21'S and 28° 49'E, 1740 m above sea level (m a.s.l.)), and “Territoire de Walungu”, “groupement” of Mushinga (Site #2, 2° 46'S and 28° 41'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%) and 25-27% sand. Total carbon ranged from 1.6 to 5.2%, pH (CaCl<sub>2</sub>) was 5.1, and total nitrogen was approximately 0.45% (Muliele et al., 2015, Lunze et al., 2012). Soils in Mushinga (Ferrasols; (IUSS Working Group WRB, 2014) are characterized by a wide variation in textural fractions of clay (17-70%), a sand content of 20-29%, pH (CaCl<sub>2</sub>) of 4.8, low base saturation (6.6 cmol<sub>(+)</sub> kg<sup>-1</sup>) 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 (Baert et al., 2012; Moeyersons et al., 2004). 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 1.** Maps of the two study sites Bushumba (bottom left) and Mushinga (bottom right) in South-Kivu (DR Congo). The 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).

### 3.3.2 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<sup>-2</sup> were considered. Walking distance from the field plots to the closest regional 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 (Chikowo et al., 2014; Rusinamhodzi et al., 2012; Tittonell et al., 2005). 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 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. 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).

### 3.3.3 Soil sampling and soil analysis

Soil samples were obtained using the Y-shaped scheme technique (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  $\times$  2 villages per site  $\times$  3 farm typologies per village  $\times$  8 farms per typology  $\times$  2 plots per farm  $\times$  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).

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 (i.e., SOC, TN, soil pH,  $P_{av}$ ,  $K_{av}$ ) was done according to Mirzaeitalarposhti et al. (2015). As prerequisite for property prediction, a defined proportion of the entire sample set was subjected to wet chemistry (see supplementary materials of this manuscript). Briefly, soil organic carbon (SOC) and total soil nitrogen (TN) contents 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 & Kurtz, 1945), and plant available potassium ( $K_{av}$ ) according to Schüller (1969). Since predictions of exchangeable calcium ( $Ca_{ex}$ ) and magnesium ( $Mg_{ex}$ ) were not successful, all soil samples were processed by wet chemistry according to Mehlich (1984).

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 midDRIFTS 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).

**Table 1** MidDRIFTS peaks representing organic functional groups considered for SOC quality analysis

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

<sup>a</sup>Baes and Bloom, 1989; <sup>b</sup>Demyan et al., 2012.

### 3.3.4 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). 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 SOC and midDRIFTS peak data (i.e., relative peak area, SOC stability indexes). The chi-squared test for independence was applied to determine significant differences within local soil fertility indicators used by smallholder farmers.

### 3.4 Results

#### 3.4.1 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. 2). The inter-related effect of market distance and sites showed a significant effect for TN ( $P < 0.001$ ) (Table 2, Fig. 2). As a single factor, however, market distance revealed a significant effect for SOC ( $P < 0.01$ ), TN ( $P < 0.001$ ), and  $Mg_{ex}$  ( $P < 0.05$ ) (Table 2, Fig. 2). This was corroborated by linear regression analyses showing negative relations between market distance and SOC (“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. 2 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** Effects of market distance, farm typology and sites with their interactions on soil fertility properties (for data values see Fig. 3 and 4)

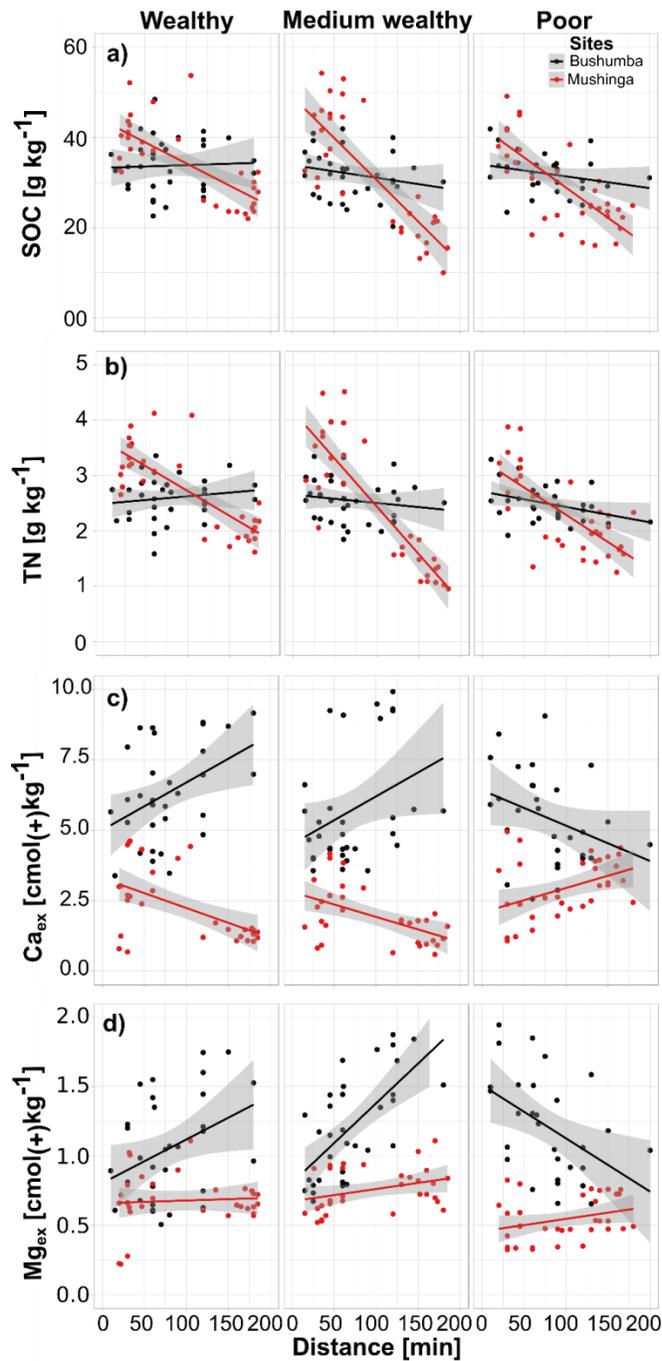
Properties	Factors and interactions				
	Market distance	Farm typology	Site	Market distance × Farm typology	Market distance × Site
SOC [g kg <sup>-1</sup> ]	**	ns	ns	Ns	*
TN [g kg <sup>-1</sup> ]	***	ns	***	Ns	***
Soil pH [CaCl <sub>2</sub> ]	ns	ns	ns	Ns	*
$P_{av}$ [mg kg <sup>-1</sup> ]	ns	ns	*	Ns	Ns
$K_{av}$ [mg kg <sup>-1</sup> ]	ns	ns	ns	Ns	Ns
$Ca_{ex}$ [cmol <sub>(+)</sub> kg <sup>-1</sup> ]	ns	**	***	*	Ns
$Mg_{ex}$ [cmol <sub>(+)</sub> kg <sup>-1</sup> ]	*	***	*	***	Ns
Peak 2930 [cm <sup>-1</sup> ]	ns	ns	***	Ns	**
Peak 1620 [cm <sup>-1</sup> ]	***	ns	**	**	Ns
Peak 1530 [cm <sup>-1</sup> ]	***	ns	ns	Ns	***
Peak 1159 [cm <sup>-1</sup> ]	**	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:  $P < 0.001$  ‘\*\*\*’,  $P < 0.01$  ‘\*\*’,  $P < 0.05$  ‘\*’,  $P > 0.05$  ‘not significant (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.



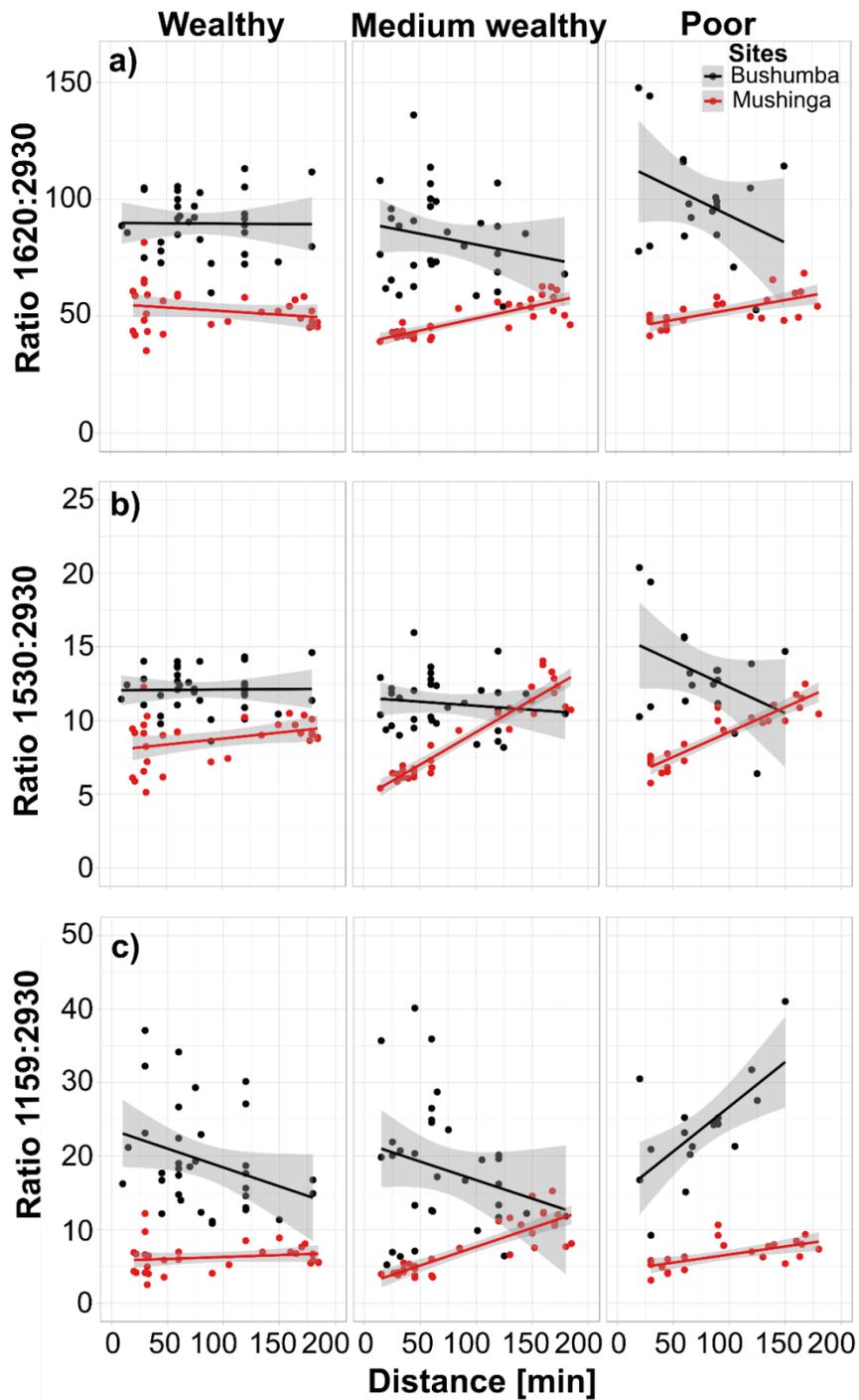
**Figure 2** Contents of total carbon (SOC,  $P < 0.05$ ; a) and total nitrogen (TN,  $P < 0.05$ ; b), as well as exchangeable calcium ( $\text{Ca}_{\text{ex}}$ ,  $P > 0.01$ ; c) and magnesium ( $\text{Mg}_{\text{ex}}$ ,  $P < 0.01$ ; d) in soils of surveyed smallholder households in the two sites Bushumba and Mushinga considering the two factors “farm typology” and “market distance”. Gray color in scatter plots representing the confidence interval.

The relative peak areas of 4 representative peaks at 2930 (aliphatic C-H stretching), 1620 (aromatic C=C and COO<sup>-</sup> stretching), 1530 (aromatic C=C stretching), 1159 (C-O bonds of poly-alcoholic and ether groups) cm<sup>-1</sup> and respective SOC 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<sup>-1</sup> (P < 0.01) (Table 2, Fig. 3). 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<sup>-1</sup> (P < 0.01) (Table 2, Fig. 3). Peaks 2930 and 1530 cm<sup>-1</sup> revealed a significant interaction between market distance and site (P < 0.01); as market distance increases, peaks 2930 and 1530 cm<sup>-1</sup> in Bushumba increased, while they were reduced in Mushinga for the medium wealthy class (Table 2, Fig. 3). Similar results were noticed for 1530 cm<sup>-1</sup> 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. 3). Except for the ratio of 1620:2930, all midDRIFTS-derived SOC quality indicators revealed a significant positive correlation with SOC content (Table 3).

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

<b>Variables</b>	<b>R</b>	<b>F test</b>
Peak 2930 [cm <sup>-1</sup> ]	0.24	**
Peak 1620 [cm <sup>-1</sup> ]	0.48	***
Peak 1530 [cm <sup>-1</sup> ]	-0.27	***
Peak 1159 [cm <sup>-1</sup> ]	-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’.



**Figure 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.

### 3.4.2 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 4** Proportional contribution (%) of farmers to the ranking (Chi<sup>2</sup>) of selected soil fertility indicators across sites

<b>Indicators for soil fertility</b>	<b>Chi<sup>2</sup></b>	<b>Proportion (%)</b>
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: not significant’.

Complementary, laboratory analysis revealed higher concentrations of SOC and P<sub>av</sub> in “deep” than “shallow” soils (P < 0.05) (Fig. 4 a-b), with similar trends for TN, K<sub>av</sub>, Ca<sub>ex</sub>, and Mg<sub>ex</sub> (Table 5). In agreement with farmers' indigenous knowledge, wet chemistry analyses revealed higher concentrations of P<sub>av</sub> in “dark” than “red” soils (P < 0.05) (Table 5, Fig. 4 d). SOC, on the other hand, disagreed with farmers' indigenous knowledge, revealing higher values in the “red” than “dark” soils (P < 0.05) (Table 5, Fig. 4 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 4).

**Table 5** Averages of selected local soil fertility indicators in soil chemical properties measured across the two sites from top- and subsoil (SOC, TN, soil pH, Ca<sub>ex</sub>, Mg<sub>ex</sub>, n = 360), and (P<sub>av</sub>, K<sub>av</sub>, n = 96)

Selected indicator	Site	Soil chemical properties														
		SOC		TN		Soil		P <sub>av</sub>		K <sub>av</sub>		Ca <sub>ex</sub>		Mg <sub>ex</sub>		
		[g kg <sup>-1</sup> ]	( )	[g kg <sup>-1</sup> ]	( )	pH	( )	[mg kg <sup>-1</sup> ]	( )	[mg kg <sup>-1</sup> ]	( )	[cmol(+)kg <sup>-1</sup> ]	( )	[cmol(+)kg <sup>-1</sup> ]	( )	
Soil depth [0-50 cm]	Deep	B	3.05	(1.20) <sup>ab</sup>	0.24	(0.11) <sup>ab</sup>	4.87	(0.52) <sup>b</sup>	12.54	(8.53) <sup>c</sup>	222.07	(208.40) <sup>ab</sup>	5.20	(2.40) <sup>b</sup>	1.04	(0.43) <sup>b</sup>
	Shallow	B	2.80	(1.12) <sup>a</sup>	0.22	(0.10) <sup>a</sup>	4.53	(0.49) <sup>a</sup>	9.16	(7.99) <sup>b</sup>	186.77	(169.85) <sup>a</sup>	4.38	(2.11) <sup>b</sup>	0.81	(0.36) <sup>a</sup>
	Deep	M	3.45	(1.22) <sup>b</sup>	0.27	(0.11) <sup>b</sup>	4.70	(0.54) <sup>ab</sup>	8.75	(6.20) <sup>ab</sup>	273.9	(191.07) <sup>b</sup>	2.63	(2.36) <sup>a</sup>	0.77	(0.40) <sup>a</sup>
	Shallow	M	2.98	(1.22) <sup>a</sup>	0.24	(0.11) <sup>ab</sup>	4.60	(0.45) <sup>a</sup>	5.67	(8.63) <sup>a</sup>	223.64	(200.03) <sup>ab</sup>	2.32	(2.36) <sup>a</sup>	0.71	(0.42) <sup>a</sup>
			**		*		***		***		*		*		*	
Soil color	Black	B	2.90	(1.03) <sup>a</sup>	0.23	(0.09) <sup>a</sup>	4.75	(0.45) <sup>a</sup>	11.26	(7.33) <sup>b</sup>	194.56	(174.83) <sup>a</sup>	4.98	(2.03) <sup>b</sup>	0.94	(0.37) <sup>c</sup>
	Red	B	2.95	(1.01) <sup>a</sup>	0.23	(0.09) <sup>a</sup>	4.65	(0.44) <sup>a</sup>	10.44	(7.21) <sup>b</sup>	214.28	(156.89) <sup>a</sup>	4.60	(1.93) <sup>b</sup>	0.91	(0.34) <sup>bc</sup>
	Black	M	2.60	(1.05) <sup>a</sup>	0.20	(0.10) <sup>a</sup>	4.63	(0.47) <sup>a</sup>	9.32	(7.54) <sup>b</sup>	242.78	(170.12) <sup>a</sup>	2.68	(2.05) <sup>a</sup>	0.77	(0.37) <sup>ab</sup>
	Red	M	3.84	(1.05) <sup>b</sup>	0.31	(0.10) <sup>b</sup>	4.67	(0.48) <sup>a</sup>	5.10	(7.76) <sup>a</sup>	254.76	(175.59) <sup>a</sup>	2.27	(2.09) <sup>a</sup>	0.71	(0.34) <sup>a</sup>
			**		***		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 ‘\*\*’

## 3.5 Discussion

### 3.5.1 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 SOC 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 & 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 with the opportunity to sell surplus yields of crops. This generates extra income to support increased access to 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 (Yamano & Kijima, 2010; Tittonell et al., 2005).

The survey of the SOC 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 had 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 SOC 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  $\text{cm}^{-1}$ . This corroborated the former argument that primarily wealthy farmers were able to purchase farm yard manure as the only locally available fertilizer (Soule & 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  $\text{cm}^{-1}$ , representing the labile SOC pool (Baes & 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 SOC 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 wealthy and medium wealthy farmers. It was assumed that these farmers with market proximity had favorable economic opportunities, exerting considerable production pressure on their land to maximize yield and income (Kansiime et al., 2018; Bationo et al., 2006). Due to such continuously high cultivation pressure, the poor farmers in Mushinga depleted their soils in Ca and Mg. Meanwhile in Bushumba, wood ash derived from kitchen waste (Bekunda & Woome, 1996) is broadcasted on farm plots close to the market increasing soil nutrient contents. The positive effect of this fertilization strategy is more pronounced on farms with less land (<1 ha) than on wealthy and medium wealthy farms that have more land (>2 ha), as observed by Place et al. (2003). In contrast to farm plots near to markets, remote field plots are less depleted of nutrients because of lower cultivation pressure. Consequently, adequate levels of Ca and Mg stocks are maintained in the soil.

### 3.5.2 Indigenous knowledge to validate soil fertility status across market gradients

Existing farmers' knowledge to assess soil fertility has been based mainly on local indicators, including soil color and soil depth (Dawoe et al., 2012; Desbiez et al., 2004). This study has evaluated correspondence and discrepancies between farmers' indigenous and scientific knowledge about the soil fertility status of contrasting farm typologies, by testing whether soils considered fertile or less fertile by farmers show a similar fertility status according to science-based measurements using the midDRIFTS approach. 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 are 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 were 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, SOC and TN contents were higher in red than black soils. We assumed that soil color was more related to soil physical properties such as soil texture. Dawoe et al. (2012) as well as Gray & Morant (2003), also found a red soil color to indicate a sandy soil texture, while a grey color was 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).

## 3.6 Conclusions

This study has found that the inter-related effect of market distance and farm typology are a main driver of soil fertility variability across the study sites. Soil fertility, as displayed by SOC and TN concentrations, decreased with increasing market distance, with exception of the wealthy farm 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. Such advanced knowledge will contribute essentially to the development of niche-based ISFM intervention strategies in soil fertility constrained smallholder farming systems across sub-Saharan Africa.

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## Chapter 4

### **Microbial carbon use efficiency during plant residue decomposition: integrating multi-enzyme stoichiometry and C balance approach**

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## **4 Microbial carbon use efficiency during plant residue decomposition: integrating multi-enzyme stoichiometry and C balance approach**

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## 4.1 Abstract

Accurate estimation of microbial carbon use efficiency (CUE) in soil is challenged by a high degree of genetic and environmental variability. Different methods vary in their estimates of soil microbial CUE giving the room to select the optimal method for a specific research task, while integrating different methods could improve our understanding of processes controlling CUE variability. Aiming to estimate CUE during plant residue decomposition in different soils, we applied the conventional C-balance method, single C-cycling enzymatic stoichiometry (SCE-STM) and newly proposed “multi”-C-cycling enzymatic stoichiometry (MCE-STM) methods. The STM approach derives CUE from elemental ratios of microbial biomass, substrate, and activities of C and nutrient (e.g. N) acquiring exoenzymes. The extended MCE-STM is a modification of the SCE-STM method, where we used the sum of three C-cycling enzymatic activities ( $\beta$ -glucosidase (BGL),  $\beta$ -D-cellobiohydrolase (BCL),  $\beta$ -xylosidase (BXL)) as proxy for CUE calculation, instead of using a single C-acquiring enzyme (BGL). We hypothesized that MCE-STM provides a more reliable estimation of microbial CUE in soils amended with complex plant residues than the SCE-STM or the C balance approach. The comparison of methods was done in a laboratory incubation experiment, using two soils differing mainly in acidity level mixed with two specimen of plant residues differing in lignin (L) and polyphenol (PP) content. We anticipated a higher microbial CUE in less acidic (pH 5.1) soil amended with higher quality (lower (L+PP)/N ratio)) plant residues than in more acidic (pH 4.3) soils amended with medium quality (higher (L+PP)/N ratio) plant residues, due to less energy investment in microbial metabolism the former case. Microbial CUE estimations were completed at 7, 15, 30, 45 and 60 days. Lower CUE values (0.09-0.18) were recorded by MCE-STM as compare to those (0.24-0.47) obtained by C-balance and SCE-STM methods. Irrespective of applied CUE estimation methods, higher CUE was recorded in less acidic (pH 5.1) soil amended with residues of higher quality than the other three combinations. Microorganisms invested more energy to support growth in low pH soil in order to tolerate soil acidity, which, in turn, suppressed N-acquiring enzymatic activity and further decreased CUE. The modification of the MCE-STM method for CUE determination proposed in this work was capable to quantify the combined effect of soil pH and plant residue quality on efficiency of microbial metabolism. It,

therefore, improved the original stoichiometric modelling approach (SCE-STM), which relies only on the nutrient availability concept.

**Keywords** extracellular enzymes, residue quality, soil acidity,  $q\text{CO}_2$ , microbial resource acquisition.

### **Highlights**

- Carbon use efficiency (CUE) was estimated by 3 methods during plant residues decomposition
- New modification of multi-enzymatic stoichiometry modelling (MCE-STM) was applied
- MCE-STM was comparable with single-C enzyme CUE estimates
- Both soil pH and residue quality shaped microbial CUE estimated by MCE-STM

## 4.2 Introduction

Microorganisms control carbon (C) decomposition and sequestration in soils, with strong effects on terrestrial C cycle (Singh et al., 2010). The ratio between C sequestration and decomposition in soil is determined inter alia by carbon use efficiency (CUE) of soil microorganisms. CUE is equal to the microbial biomass C increment during growth per amount of organic C used, or more generally, as the ratio of C allocated to biosynthesis and the amount of accumulated C. CUE is thus an important ecological characteristic of microbial metabolism and soil C cycling (Manzoni et al., 2018); Spohn et al., 2016). CUE is commonly calculated in the range of 0.2 – 0.8 (Manzoni et al., 2012), making quantification of soil C budgets uncertain. The source of this variability can be categorized into three groups: genetic, environmental and methodological. Genetic variability of soil microbial communities on CUE has been documented by pure culture studies of individual bacterial taxa ( Keiblinger et al., 2010; Molenaar et al., 2009; Pfeiffer et al., 2001). A genetically diverse soil microbiome with its CUE potential is influenced by environmental factors, including plant residue quality and soil pH ( Jones et al., 2019; Puttaso et al., 2011). Despite the fact that the individual effect of these factors on microbial metabolism and CUE has been documented (Malik et al., 2019; Rousk et al., 2009, their interrelated effects on CUE during the course of decomposition of complex plant residues in agricultural soils remain elusive.

Generally, quantification of soil microbial CUE is difficult, whereby different methods as another source of variability have been acknowledged (Geyer et al., 2019). Both direct and indirect approaches were used to estimate microbial CUE. The C balance approach considers increments in microbial biomass and respired carbon dioxide (CO<sub>2</sub>) (Blagodatskaya et al., 2014; Herron et al., 2009), and uses the microbial yield coefficient as equivalent to CUE during growth (Blagodatsky et al., 2002). If microorganisms are in state of maintenance and no distinct biomass increase is recorded, the metabolic quotient (qCO<sub>2</sub>, the rate of CO<sub>2</sub>-C evolution per microbial biomass C) is used to evaluate microbial metabolic efficiency (Blagodatskaya et al., 2014; Puttaso et al., 2011). The basic assumption of this method is that C gain in microbial biomass originates solely from the substrate. This assumption excludes the possible recycling of microbial biomass C without explicit microbial growth (Hagerty et al., 2014). Uncertainty exists

when biochemically complex organic residues are considered as substrates during gradual decomposition, because the turnover of microbial biomass may distort C balance calculations.

Alternatively, stoichiometric modeling of decomposition can be used for CUE estimation (Sinsabaugh et al., 2016). In this case, CUE of the soil microbial community is considered as a function of the difference between nutrient requirements for growth and nutrient composition of the substrate, whereby extracellular enzyme activities (EEA), C/N (C/P) ratio of microbial biomass, and available organic matter are considered to calculate CUE. The main principle of using EEA reflects the equilibrium between the elemental composition of microbial biomass and detrital organic matter in the one hand and the efficiencies of nutrient assimilation and growth in the other (Sinsabaugh & Follstad Shah, 2012). The advantage of this approach is that it utilizes common soil analysis for CUE calculation, and can be applied at various spatio-temporal scales (Sinsabaugh et al., 2016).

The indicator enzymes most commonly used to quantify CUE by stoichiometric modeling are  $\beta$ -1,4-glucosidase (BGL), leucine aminopeptidase (LAP),  $\beta$ -1,4-N-acetylglucosaminidase (NAG), and acid (alkaline) phosphatase (APH) (Sinsabaugh & Follstad Shah, 2012; Sinsabaugh et al., 2013; 2016). However, this enzyme combination may not be applicable for accurate characterization of microbial CUE in complex soil ecosystems (Hu et al., 2011; Voříšková et al., 2011). This is justified since plant residue degrading, C-cycling enzymes other than BGL may also control the rate of decomposition, hence microbial CUE. Thus, it may be a limitation to use BGL as a sole proxy to estimate CUE (Sinsabaugh & Follstad Shah, 2012; Sinsabaugh et al., 2013; 2016). Moreover, NAG, as a chitin degrading enzyme (Allison & Vitousek, 2004; Baldrian, 2009), was used as input parameter for nitrogen (N) acquisition (Sinsabaugh & Follstad Shah, 2012; Sinsabaugh et al., 2013; 2016). The ecological function of NAG remains uncertain, as it is also considered as a C-acquiring enzyme (Gooday, 1990; Kramer et al., 2013; Wieczorek et al., 2014). This duality of the NAG effect complicates its applicability to calculate microbial CUE. According to Jan et al. (2009), a main limiting step in the breakdown of organic N in the plant residues is protein degradation. This step includes, among others, the enzymes Leucine-aminopeptidase (LAP), Succinyl-Alanyl-Alanyl-phenyl (SAA) and Alanyl-Alanyl-phenyl aminopeptidase (AAP) (Enowashu et al., 2009; Obayashi et al., 2017). It could be

deduced that consideration of such N-acquiring enzymes as alternatives to NAG may provide a clearer picture of nutrient assimilation efficiency, which finally drives microbial CUE.

Decomposition of plant residues requires a suite of enzymatic steps. Substrates differing in the availability of C and N require different metabolic pathways to be completely decomposed and assimilated. This leads to a wide range of respired C-CO<sub>2</sub> per unit C assimilated, namely microbial CUE (Manzoni et al., 2012; van Hees et al., 2005). The main determinant of biochemical quality reflecting C and N availability and hence CUE during decomposition is the ratio of lignin (L) and polyphenol (PP) to N ((L+PP)/N). It could be suggested that stoichiometric modeling for CUE estimation using multiple extracellular C and N cycling enzymes provide a more coherent insight into the ecology and efficiency of microbial decomposition of biochemically contrasting plant residues in soils. In this respect, we propose that not only BGL, but also other plant residue degrading and rate limiting (Hu et al., 2011; Voříšková et al., 2011) C-cycling enzymes (e.g.,  $\beta$ -D-cellobiohydrolase (BCL),  $\beta$ -xylosidase (BXL)) should be implemented in CUE estimation models. We therefore hypothesized that the integration of multi-C cycling enzymes stoichiometry modeling (MCE-STM) with single C-cycling enzyme (BGL) stoichiometry modeling (SCE-STM) and the conventional C balance approach reveals a more accurate estimation of microbial CUE in soils amended with complex plant residues. Besides the differences in substrate (plant residues) quality, the soil environment in general, including specifically soil pH, would affect the CUE (e.g. Jones et al., 2019; Rousk et al., 2009). Both factors determine in an interrelated manner the energy investment by soil microorganisms during growth. We anticipated a higher microbial CUE in less acidic soil amended with high quality (low (L+PP)/N)) plant residues than in more acidic soils amended with lower quality (high (L+PP)/N)) plant residues, due to less energy investment in the former case.

## 4.3 Materials and methods

### 4.3.1 Soil and plant analysis

Soil samples of S4.3 (pH of 4.3) and S5.1 (pH of 5.1) were collected from farmers' fields at a depth of 0-20 cm in Lelissa Dimtu Kebele, an administrative unit of Diga district, Ethiopia (36°24'E; 9°02'N). The dominant soil type was a Nitisol according to WRB classification (Berhanu et al., 2013; Deressa et al., 2013; FAO, 2014). Soils were air dried, sieved through <2 mm sieve, and transported to the University of Hohenheim, Stuttgart, Germany. Soil pH was measured in 0.01 M CaCl<sub>2</sub> extracts with a soil-to-solution ratio of 1:2.5 (Houba et al., 2000). Total carbon (TC) and nitrogen (TN) were analyzed by dry combustion (vario MAX CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) (Nelson & Sommers, 1996). Soil pH results showed values of <7.4 so that carbonate content was considered as negligible and total carbon was regarded as equivalent to total soil organic C (SOC) (Schumacher, 2002; Wang et al., 2015). S4.3 had 23.2 g kg<sup>-1</sup> SOC, 1.5 g kg<sup>-1</sup> TN, a C/N ratio of 15.75, and a pH 4.3. S5.1 had 26.0 g kg<sup>-1</sup> SOC, 1.8g kg<sup>-1</sup> TN, a C/N ratio of 14.73, and a pH 5.1. S4.3 and S5.1 described further as a “very strongly” and “moderately” acidic soils, respectively (Ahem et al., 1995; Hazelton & Murphy, 2007).

Above-ground residues (leaves, twigs) of the tropical shrub *Calliandra calothyrsus* were collected in Kenya (medium quality residue (MQR)) and Democratic Republic of Congo (high quality residue (HQR)) and analyzed individually for dry matter content, TC, TN, total extractable polyphenol (PP), neutral detergent fiber, acid detergent fiber, and acid detergent lignin (VDLUFA, 2012). Hemicelluloses and cellulose were calculated by subtracting acid from neutral detergent fiber and acid detergent lignin from acid detergent fiber, respectively. TC and TN were measured using a Euro EA 3000 elemental analyzer (Hekatech, Wegberg, Germany). Dry matter content was determined according to AOAC (1990). Polyphenol was determined according to Makkar et al. (1993). Biochemical quality, thus 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. Detailed biochemical data of plant residues are displayed in Table 1.

**Table 1** Biochemical composition of Calliandra plant (*Anneslia calothyrsus* (Meissn)) residues collected from Kenya (medium quality (MQR)) and Democratic Republic of Congo (high quality (HQR))

RT	C	N	C/N	PP	Hem	Cell	L	PP/	L/	(L+
	g kg <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	N	N	PP)/
		1								N
MQR	429.7	19.9	21.6	63.4±5.4	101±10.3	208±3.9	102±8.7	3.18	4.9	8.1
HQR	408.8	22.5	18.2	46.2±1.5	143±11.7	197±10.2	68±0.8	2.05	3.0	5.1

RT= residue type, C=Carbon, N=Nitrogen, C/N=carbon to nitrogen ratio, PP=polyphenol, Hem=Hemicelluloses, Cell=Cellulose, L=lignin, PP/N=ratio of polyphenol to nitrogen, L/N= ratio of lignin to nitrogen, (L+PP)/N=ratio of the sum of polyphenol and lignin to nitrogen.

#### 4.3.2 Microcosm experimental set up

Leaves and twigs of air-dried residues of *C. calothyrsus* were chopped to 5 to 8 mm length. 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 pre-incubation, 33 g sub-samples of the soils were mixed each with 0.33 g of each MQR and HQR. Mixtures were transferred into cylindrical jars. 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 without plant residues) × 5 sampling dates (7, 15, 30, 45, 60 days of incubation) × 3 replications. The soil samples were stored at -28°C until further analysis.

#### 4.3.3 Microbial activities

##### 4.3.3.1 Respiration

To trap the evolving carbon dioxide (CO<sub>2</sub>), a small plastic vessel containing 1 M NaOH solution was placed inside jars. The jar was sealed tightly, while the small cylinder was left open. NaOH solution was changed regularly after every sampling of alkali for titration. CO<sub>2</sub>-C production was measured every 1 to 2 days during the first 2 weeks, followed by every 6 to 7 days for the remaining incubation period (15 to 60 days). The amount of non-used NaOH was determined titrimetrically in an aliquot of 0.5 ml with 0.1 M HCl (Anderson, 1982).

#### 4.3.3.2 Potential enzymatic activities

The potential activities of C-cycling enzymes, including  $\beta$ -D-glucosidase (BGL),  $\beta$ -D-cellobiohydrolase (BCL) and  $\beta$ -xylosidase (BXL) were determined using hydrolysable substrates containing the fluorescent 4-methylumbelliferone (MUF) (i.e., MUF- $\beta$ -D-Glucoside (BGL); MUF- $\beta$ -D-Cellobioside (BCL), MUF- $\beta$ -D-Xylopyranoside (BXL) (Sigma-Aldrich, St. Louis, MO, USA) (Marx et al., 2001). Potential activities of leucine-aminopeptidases (LAP), Alanyl-Alanyl-phenyl aminopeptidase (AAP) and thermolysin-like neutral metalloproteases (SAA) were determined as the rates of fluorescence of an enzymatically hydrolyzed substrate containing the highly fluorescent compound 7-amino-4-methyl coumarin (AMC) (i.e., L-Leucine-AMC hydrochloride (LAP) and Ala-Ala-Phe-AMC hydrochloride (AAP) (Sigma-Aldrich), Suc-Ala-Ala-Phe-AMC hydrochloride for SAA (Bachem AG, Bubendorf, Switzerland)), with slight modifications according to Marx et al. (2001) and Rasche et al. (2017). One g of each soil was weighed into a 100 ml beaker and suspended with 50 ml of deionized H<sub>2</sub>O. Each sample (including negative control) was dispersed by an ultrasound bar for 2 min at 35 J s<sup>-1</sup>.

After sonication, an aliquot of each sample (50  $\mu$ l) along with 50  $\mu$ l (MES) and TRIZMA buffer (Sigma-Aldrich) and 100  $\mu$ l substrate working solution were pipetted into each well of microplate. 4-Morpholineethanesulfonic acid sodium salt (MES) and TRIZMA-Base (Tris (hydroxymethyl) aminomethane) reagent as well as TRIZMA-HCl (Tris (hydroxymethyl) aminomethan-hydrochlorid (TRIZMA) buffers were used for C and N- cycling enzymes, respectively. Substrates, standard stock and working solutions were prepared according to Rasche et al. (2017). For each analysis, a negative control (without soil, only H<sub>2</sub>O) was used. A standard plate of 4-MUF and 7-AMC for C-cycling and N-cycling, respectively, was prepared in the concentration range of 0 to 120 mM as detailed in Rasche et al., (2017). Plates were incubated at 30°C over a period of 3 hours and substrate hydrolysis were measured after 30, 60, 120 and 180 min. However, for SAA and AAP, the incubation period was extended up to 5 hours and measurements were taken after 90, 120, 180, 240, 300 min. Then, fluorescence recording on a microplate reader (FLX 800, Microplate Fluorescence Reader, Bio-Tek Instruments, Inc., Winooski, VT, USA) was done at 360/460 nm wavelength. Finally, calculation of enzymatic activity kinetics was performed according to Marx et al. (2001).

#### 4.3.4 Ammonium and nitrate content in soil

Ammonium ( $\text{NH}_4^+$ -N) and nitrate ( $\text{NO}_3^-$ -N) were extracted with 0.05 M  $\text{K}_2\text{SO}_4$  (soil to extractant ratio (w/v) of 1:4), shaken on a horizontal shaker for 30 min at 250 rpm, centrifuged for 30 min at 4400 g and filtered (Rotilabo-Rundfilter AP55.1, Carl Roth GmbH). Concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were measured colorimetrically on an auto-analyzer (Bran & Luebbe, Autoanalyzer 3, SEAL Analytical, Hamburg, Germany) (Bamminger et al., 2014).

#### 4.3.5 Microbial biomass and dissolved organic C and N

Determination of microbial biomass C (MBC) and N (MBN) was done by chloroform-fumigation-extraction (Vance et al., 1987), using conversion factors of  $K_{\text{EC}}$  0.45 (Joergensen, 1996) and  $K_{\text{EN}}$  0.54 (Joergensen and Mueller, 1996), respectively. Ten grams of non-fumigated and fumigated soil subsamples were mixed with 40 ml of a 0.5 M  $\text{K}_2\text{SO}_4$  –solution (1:2, w/v, soil/extractant ratio) followed by shaking for 30 min at 250  $\text{rev min}^{-1}$  on a horizontal shaker and centrifuged for 30 min at 4400 g. Dissolved organic C (DOC) and total N (TN) were measured in the supernatants of both fumigated and non-fumigated samples, using a DOC/TN-analyzer (multi N/C 2100S from Analytik Jena, Jena, Germany). DOC was calculated from total C concentrations in the supernatants of non-fumigated samples ((Müller et al., 2016), while DON was calculated by subtracting mineral N (sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) from total N concentration obtained from DOC/TN-analyzer.

#### 4.3.6 Microbial carbon use efficiency (CUE) and metabolic quotient ( $q\text{CO}_2$ )

Microbial CUE was determined either by the C-balance based method (direct method) or by the enzymatic stoichiometry modeling (STM) approach. For the latter, we proposed and validated the extended “multi-C” enzymes stoichiometry modeling approach (MCE-STM), which represented a modification of the single C-enzyme (SCE-STM) method applied earlier (Geyer et al., 2019; Sinsabaugh & Follstad Shah, 2012; Sinsabaugh et al., 2013; 2016). Because the C-balance approach could not be used during the whole period of our incubation study due to

microbial recycling of C and N, we used the data of the first 7 days of incubation to compare the conventional method (C-balance based approach) with STM approach. CUE values in C-balance based method were calculated according to Blagodatsky et al (2002), using MBC increment per amount of consumed C-substrate, which was assumed in turn to be equal to biomass C increment plus CO<sub>2</sub> evolved (Manzoni et al., 2012).

$$\text{CUE} = \Delta\text{MBC}/(\Delta\text{MBC}+\Delta\text{CumCO}_2\text{-C}) \quad (\text{Eq. 1}),$$

where  $\Delta\text{MBC}$  is the net increase in MBC calculated according to Eq.2 and  $\Delta\text{CumCO}_2\text{-C}$  is the net increase in cumulative respiration for day 7 after subtraction of respiration of unamended soil ( $\mu\text{g CO}_2\text{-C g}^{-1}$  of amended soil –  $\mu\text{g CO}_2\text{-C g}^{-1}$  control soil).

$$\Delta\text{MBC} = \text{MBC}_{\text{amended}} - \text{MBC}_{\text{control}} \quad (\text{Eq. 2}),$$

where  $\text{MBC}_{\text{amended}}$  and  $\text{MBC}_{\text{control}}$  are values of microbial biomass C in soil amended with residues and in control (unamended) soil, respectively, measured at the same date, e.g. day 7. We considered only period with distinct biomass increase and assumed in our calculations that priming effect was negligible.

Since CUE was estimated by C-balance method only for the first 7 days,  $q\text{CO}_2$  was used to quantify the microbial metabolic efficiency (Puttaso et al., 2011) for the rest of the incubation period.

$$q\text{CO}_2 = \text{daily CO}_2\text{-C}/\text{MBC} \quad (\text{Eq. 3})$$

Stoichiometry modeling CUE calculations were based on an assumption that the imbalance of microbial C/N and labile pool substrate C/N ratio, as well as ratio between C and N (P) acquiring enzymes are a direct control of microbial CUE. For MCE-STM and SCE-STM (BGL-STM, BCL-STM and BXL-STM) stoichiometry, CUE was calculated based on the scalar ratio ( $\text{Sc:x}$ ) fitted to the Michealis-Menten model (Geyer et al., 2019; Sinsabaugh et al., 2016).

$$\text{CUE}_{\text{c:x}} = \text{CUE}_{\text{max}} * (\text{Sc:x}/(\text{Sc:x}+\text{Kx})) \quad (\text{Eq. 4})$$

$$\text{Sc:x} = (\text{Bc:x}/\text{Lc:x}) * (1/\text{EEAc:x}) \quad (\text{Eq. 5})$$

where  $CUE_{max}$  is set at 0.6 based on thermodynamic constraints and following the original publication of (Sinsabaugh et al., 2016);  $K_x$  is the half saturation constant set at 0.5 (Roels, 1980).  $Bc:x$  is the elemental c/x ratio of microbial biomass; in our study it was the ratio of MBC/MBN ( $x = N$ ).  $Lc:x$  is the elemental c/x ratio of substrate; in our case it was C/N of plant residues for the first 7 days (Table 1). However, after 7 days, we recalculated the remaining C/N ratio of the applied substrates using the following formulas:

$$TC_t = TC_0 - \Delta MBC_t - \Delta CumCO_2-C \quad (\text{Eq. 6})$$

$$TN_t = TN_0 - \Delta MBN_t - \Delta DON - \Delta NO_3^-N - \Delta NH_4^+ N \quad (\text{Eq. 7})$$

where  $TC_t$  and  $TN_t$  are the remaining TC and TN in substrate after decomposition at specific time  $t$  (days 7, 15, 30, 45 and 60).  $TC_0$  and  $TN_0$  is the amount of initial TC and TN applied to the soil, respectively.  $\Delta MBC_t$  and  $\Delta MBN_t$  are the changes in microbial biomass C (N) during the specific incubation period ( $t = 7, 15, 30$  and 45 days) calculated similarly (as exemplified for C in Eq. 8).

$$\Delta MBC_t = MBC_{\text{amended}} - MBC_0 \quad (\text{Eq.8}),$$

where  $MBC_{\text{amended}}$  is microbial biomass in amended soil at specific date and  $MBC_0$  is microbial biomass in soil before experiment.  $\Delta DON$ ,  $\Delta NO_3^-N$ ,  $\Delta NH_4^+N$  are the net increase in DON,  $NO_3^-N$ , and  $NH_4^+N$ , respectively, and was calculated after subtraction of respective N concentration in unamended soil (e.g.  $\mu\text{g MBN g}^{-1}$  of amended soil –  $\mu\text{g MBN g}^{-1}$  of control soil).

Extracellular enzymatic activity ( $EEAc:x$ ) is the ratio of extracellular enzymatic activities directed toward acquiring C and x ( $x = N$  in our study) from the environment. For the multi-enzymes approach (MCE-STM), we used the ratio of the summarized activities of 3 C-cycling enzymes to the summarized activities of 3 N-cycling enzymes, i.e.  $(BGL+BCL+BXL)/(LAP+SAA+AAP)$ , while for the single C-enzyme approach (SCE-STM),  $EEAc:x$  are  $BGL/(LAP +SAA+ AAP)$ ,  $BCL/(LAP +SAA+ AAP)$  and  $BXL/(LAP+SAA + AAP)$  for BGL, BCL and BXL, respectively. The approach applied in this study differs from the original method described earlier by the selected set of N-acquiring enzymes: we used combination of three enzymes (LAP+SAA + AAP) instead of two (LAP+NAG), this modification will be justified in discussion section.

Data used for CUE calculation in both direct and indirect methods are provided in supplementary tables S1 and S2.

#### 4.3.7 Statistical analysis

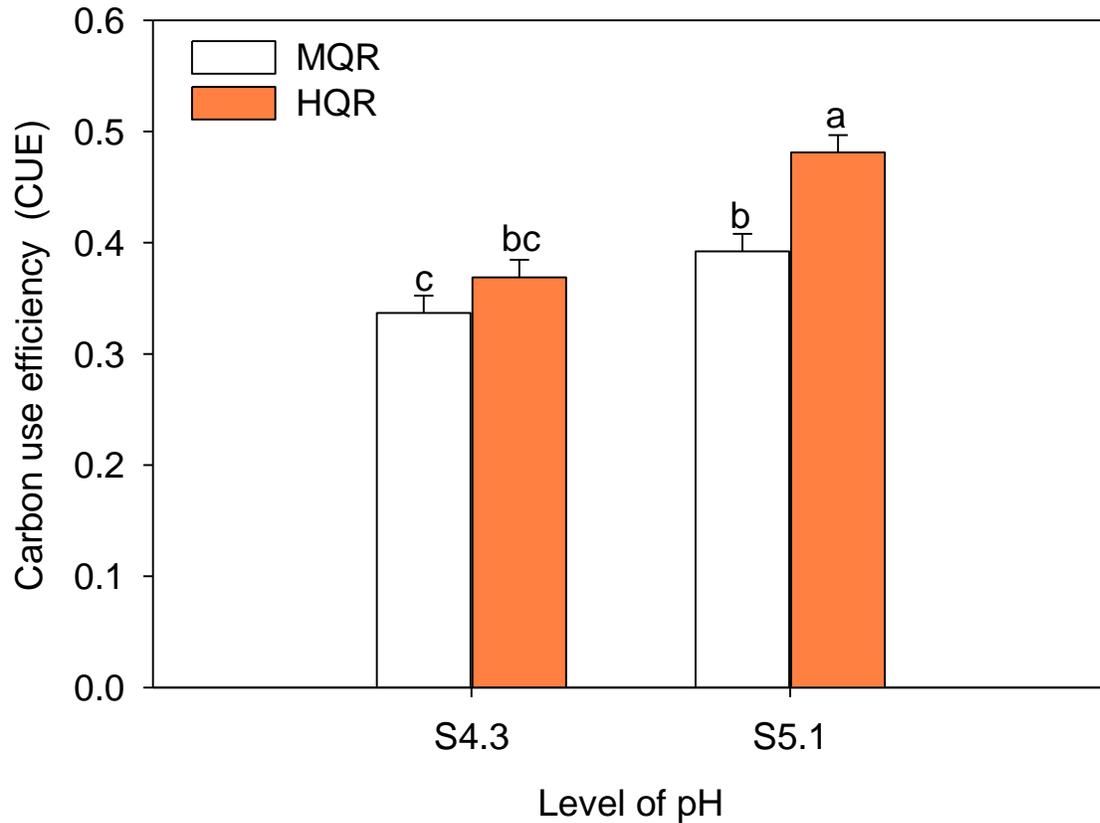
Factorial analysis of variance (ANOVA) was performed as per the requirement of the randomized complete block designed (RCBD) ( $n = 90$ ), using the PROC mixed model with restricted maximum likelihood (REML) (Piepho et al., 2003). Factors “plant residue quality” and “soil pH”, as well as their interaction were considered as fixed, while replications as random effects (Piepho et al., 2003). ANOVA for all parameters considered in this study was conducted using SAS statistical software (version 9.4, SAS Institute, Cary, North Carolina, USA). Means separation ( $P < 0.05$ ) and calculation of standard error (SE) were done using pdiff command in SAS macro %MULT (Piepho, 2012). In addition, Pearson linear correlations were conducted using SAS to assess the relationship between CUE and  $q\text{CO}_2$  values of tested methods. Furthermore, multiple linear regressions were undertaken to examine the relationship between MCE-CUE and the ratio of the sum of potential activities of C and N cycling enzymatic activities in R package, using MCE-CUE as dependent and soil pH, residue quality and the sum of potential activities of C and N cycling enzymatic activities as independent variables.

## 4.4. Results

### 4.4.1 CUE and $q\text{CO}_2$ based on C-balance method

The highest CUE was calculated for S5.1-HQR (0.48), followed by S5.1-MQR (0.39) and S4.3 amended with both residues of S4.3-HQR - 0.37 and S4.3-MQR - 0.34 (Fig. 1). CUE values for S5.1-HQR were significantly ( $P < 0.01$ ) different from the values for other three treatments (S5.1-MQR, S4.3-HQR and S4.3-MQR). Factors “soil pH” and “plant residue quality” also had a distinct effect on  $q\text{CO}_2$  ( $P < 0.01$ ) with the lowest  $q\text{CO}_2$  value of 0.02 in S5.1-HQR from days 30 to 60, and the highest  $q\text{CO}_2$  value of 0.06 for S4.3-MQR on day 30, followed by  $q\text{CO}_2$  value of 0.03 for S5.1-MQR on day 60 (Fig. 2). Intermediate  $q\text{CO}_2$  was recorded in S5.1-MQR (0.02-0.04) and S4.3-HQR (0.02-0.03) from days 15 to 45. In S4.3-MQR and S4.3-HQR, a steady decline of  $q\text{CO}_2$  was observed between days 15 and 45, while inconsistent  $q\text{CO}_2$  values were recorded for S4.3-MQR ( $P < 0.05$ ) (Fig. 2). After 45 days,  $q\text{CO}_2$  measurements in both soils

amended with HQR became constant (0.02) up to 60 days of incubation. Analysis of variance showed that an interrelated significant ( $P < 0.01$ ) effect of soil pH and residue quality on CUE and  $qCO_2$  (Table 2).

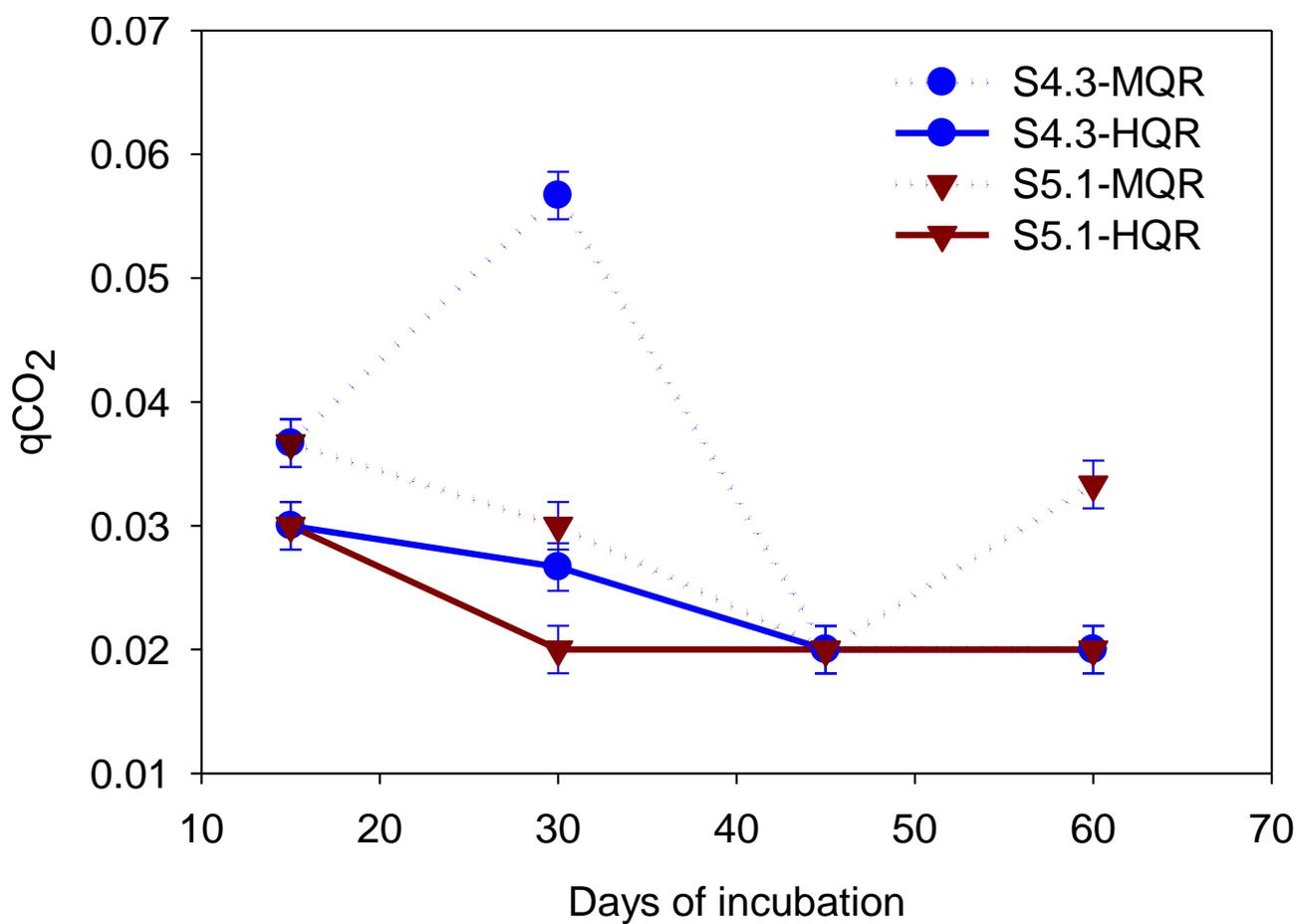


**Figure 1** Estimated CUE values with standard error (SE) based on direct (C-balance) method for both soils with different pH levels (S4.3, S5.1) amended by medium quality (MQR) and high quality (HQR) residues for the first seven days of incubation experiment (N=9);  $P < 0.001$ . Different letters show significant differences between soil pH levels and residue qualities (MQR, HQR) at  $p < 0.01$

**Table 2** Significance of single and interacted factors influencing CUE as evaluated by different methods (3-way ANOVA). Multi-enzymatic approach (MCE-STM), BGL, BCL and BXL enzymatic activities and direct C-balance methods were compared.

Effect	MCE-STM	BGL-STM	BCL-STM	BXL-STM	C-balance
Soil pH	***	***	***	***	***
Residue quality	***	***	***	***	***
Sampling date	***	***	***	***	-
Soil pH*residue quality	**	**	NS	NS	*
Soil pH*residue quality*sampling date	***	***	***	*	-

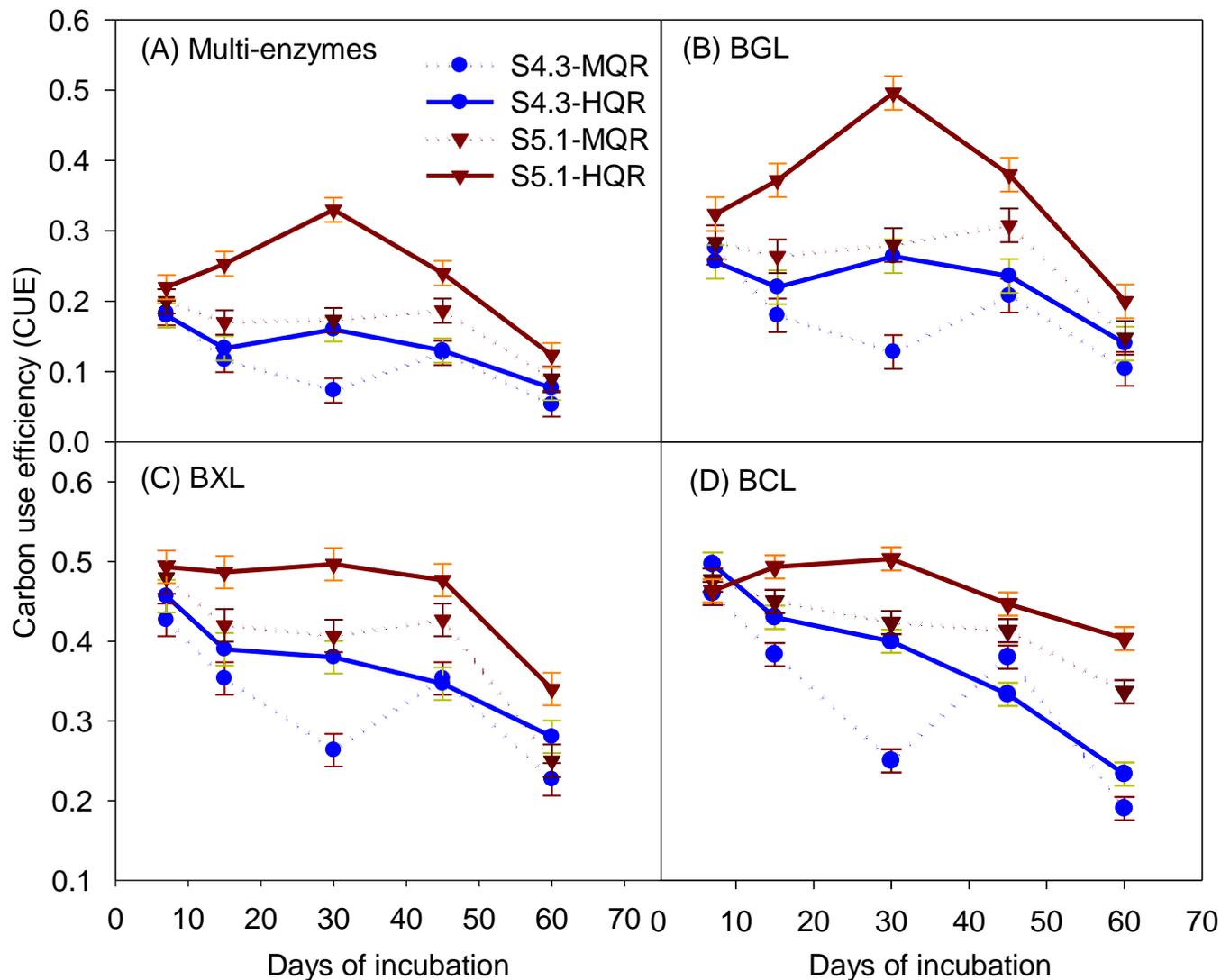
Significant at \*\*\* $p < 0.001$ , \*\* $p < 0.01$  and \* $p < 0.05$ ; NS: Not significant; CUE using C-balance method was calculated only for the first date (7 days after incubation)



**Figure 2** Estimated  $q\text{CO}_2$  values during 15-60 days of incubation for S4.3 (pH 4.3) and S5.1 (pH 5.1) amended by medium quality (MQR) and high quality (HQR) residues. Standard error (SE) bars represent  $\pm 1\text{SE}$ .

#### 4.4.2 Single C-cycling enzymatic stoichiometric model (SCE-STM)

For SCE-STM, activities of individual C-cycling enzymes (BGL, BCL, BXL) were used as proxies for CUE calculation. For all 3 models, the interrelated effect of soil pH and residue quality had a significant ( $P < 0.01$ ) effect on CUE across the incubation, except the first 7 days (Fig. 3 B-D). In all SCE-STM, the highest CUE was recorded for S5.1-HQR, followed by S5.1-MQR, except BXL-STM at day 60. CUE for S4.3-HQR had intermediate values across the incubation, except at day 45 (Fig. 3 B-D). From all models, the lowest CUE was noted for S4.3-MQR.



**Figure 3** Estimated carbon use efficiency (CUE) values across the incubation period based on stoichiometry modeling (A) using Multi C-cycling enzymes (Multi-enzymes); (B)  $\beta$ -D-glycosidase; (C)  $\beta$ -D xylanase and (D)  $\beta$ -D-cellobiohydrolase as an indicator for C-cycling enzymatic activities) for S4.3 (pH 4.3) and S5.1 (pH 5.1) amended with medium quality (MQR) and high quality (HQR) residues. Standard error (SE) bars represent  $\pm 1SE$ .

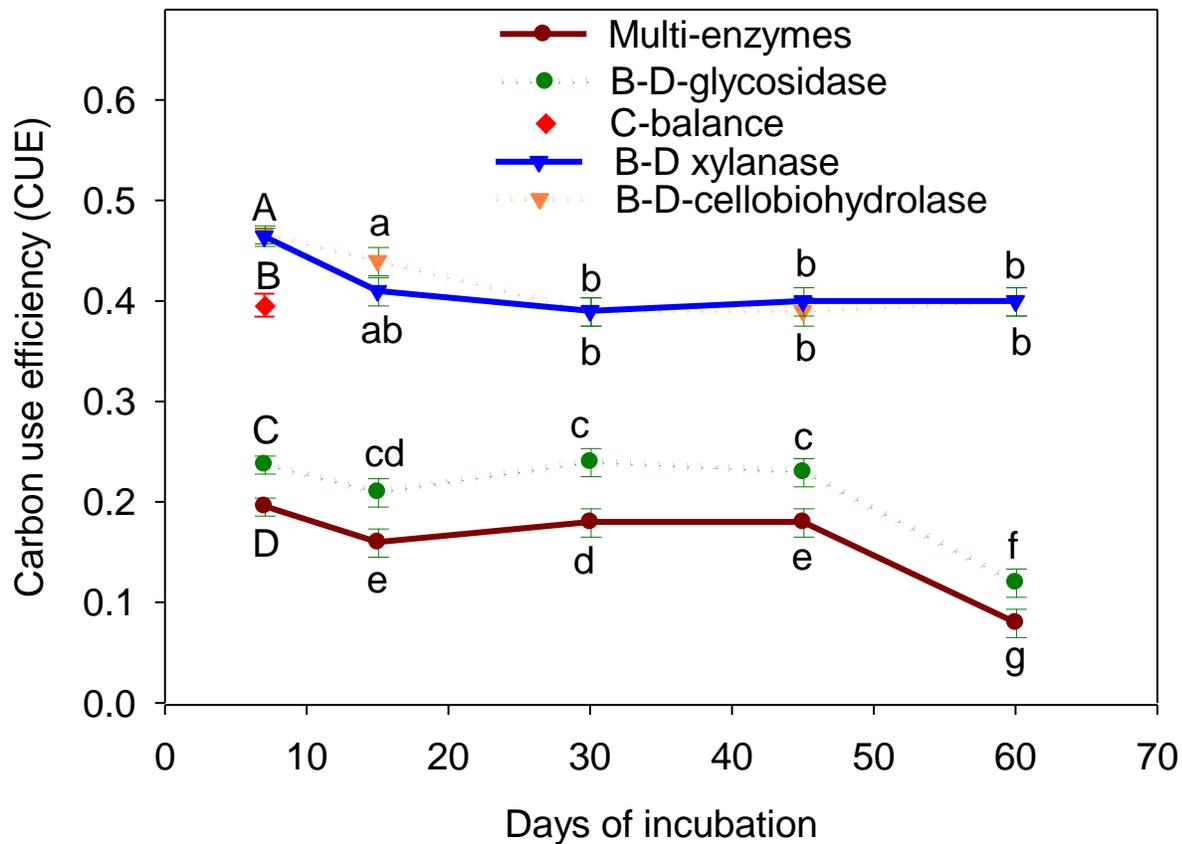
#### 4.4.3 Multi enzymatic stoichiometric model (MCE-STM)

For MCE-STM, the sum of the activities of 3 C-cycling enzymes (BGL, BCL, BXL) was used to calculate CUE. Similar to the direct method (C-balance approach) and SCE-STM, an interrelated significant ( $P < 0.01$ ) effect of soil pH by residue quality interaction was noticed for MCE-STM

(Table 3). Accordingly, the highest CUE of 0.12-0.33 was calculated for S5.1-HQR, followed by 0.09-0.20 for S5.1-MQR across the incubation period (Fig. 3A). In S5.1, the CUE values increased steadily from day 7 to 30 and then declined slowly when amended by HQR. The lowest CUE of 0.05-0.18 was found for S4.3-MQR across the incubation period. Intermediate CUE value of 0.08-0.18 was recorded in S4.3-HQR. The CUE decreased in the course of incubation in S5.1-MQR and S4.3 amended with both residue types.

#### 4.4.4 Correlation and differences between approaches for CUE estimation

Generally, MCE-STM revealed significantly ( $P < 0.0001$ ) lower CUE than the direct C-balance method and SCE-STM across the incubation period (Fig. 4). For the latter (SCE-STM), CUE in both soils amended with both residue types as calculated by BXL-STM (0.46) and BCL-STM (0.46) were higher than BGL-STM (0.21) and the C-balance method (0.40) (Fig. 4). Strong and positive significant ( $P < 0.001$ ) correlations were found between the different indirect methods of CUE estimation (Table 3): MCE-STM versus BGL-STM ( $r^2 = 0.99$ ), BXL-STM ( $r^2 = 0.90$ ), and BCL-STM ( $r^2 = 0.84$ ). A strong positive and significant ( $P < 0.001$ ) relationship was noted between the BGL-STM and the other two SCE-STM (BCL-STM, BXL-STM) (Table 3). The correlation between the C-balance and the indirect methods, which was only calculable at day 7 of incubation, was not significant ( $P > 0.05$ ) (data not shown). Nevertheless, soil pH and residue quality revealed similar effects on CUE, according to both direct and indirect methods (Fig. 1 and 3). There was no statistically significant ( $P > 0.05$ ) correlation between  $q\text{CO}_2$  and all indirect CUE estimation methods (Table 3).



**Figure 4** Estimated Carbon use efficiency (CUE) values by different methods for all treatments. Capital letters indicate mean comparison for all methods at day 7; C-balance method, Multi-enzyme (using sum of three C-cycling enzymes),  $\beta$ -D-glycosidase,  $\beta$ -D-cellobiohydrolase and  $\beta$ -D xylanase as C-cycling indicator enzymes in stoichiometry modeling. Small letters indicate stoichiometry modelling methods comparison in dynamics from day 15 to day 60. Different letters show significant differences between methods at  $P < 0.001$ . Standard error (SE) bars represent  $\pm 1SE$ .

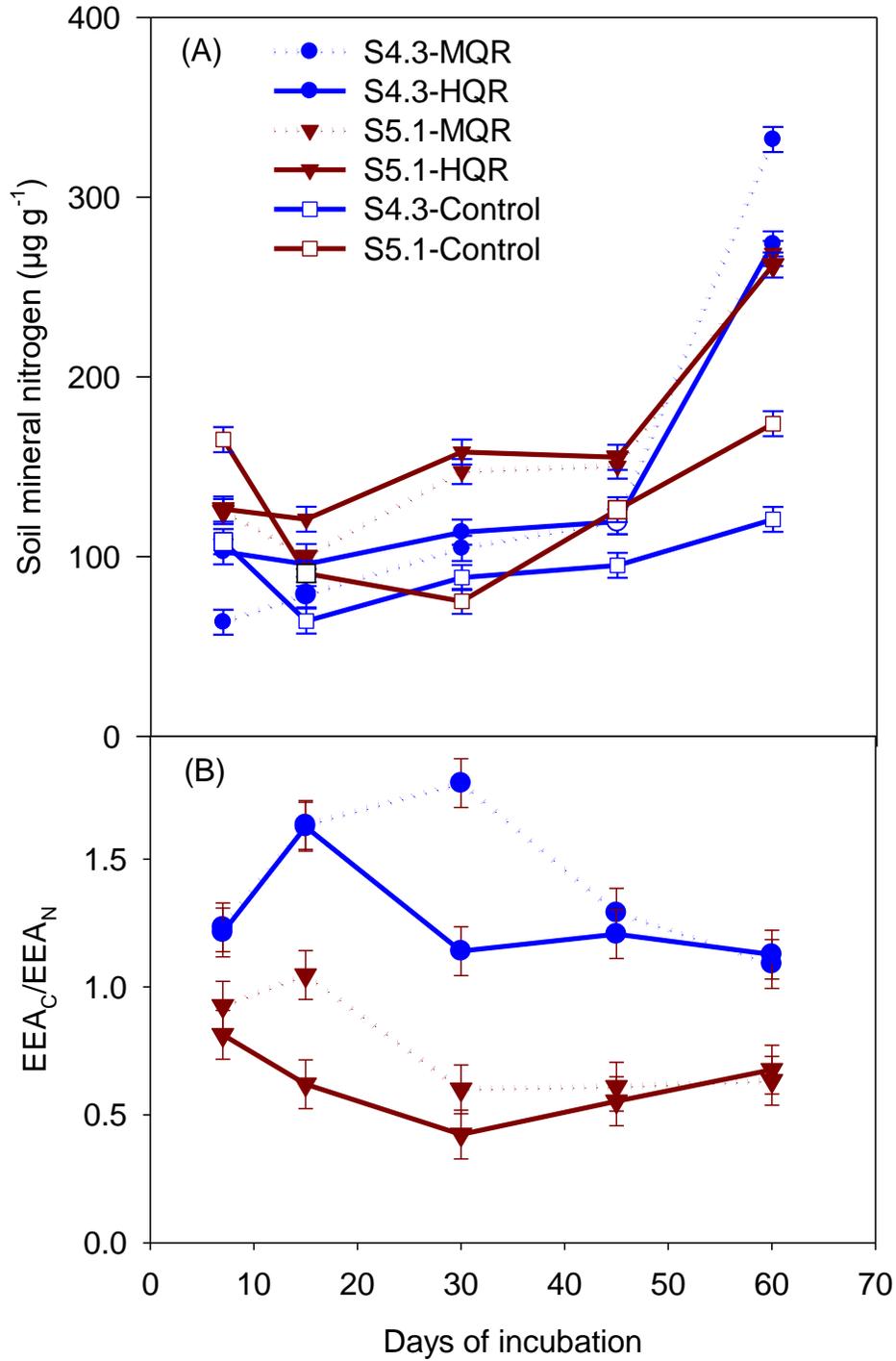
**Table 3** Pearson correlation of CUE estimation approaches and  $qCO_2$  (N=60) across the incubation period following addition of high (L+PP)/N; 5.1) and medium quality (L+PP)/N; 8.1) residues in moderately (S5.1) and very strongly (S4.3) acidic soils; Multi-enzyme = multi-C cycling enzymes Stiochoimetry, using  $\beta$ -D-glycosidase,  $\beta$ -D xylanase and  $\beta$ -D-cellobiohydrolase activity as a proxy for CUE calculation,  $qCO_2$  (Metabolic quotient).

CUE quantification methods	Multi-enzymes	$\beta$ -D-glycosidase	$\beta$ -D-xylanase	$\beta$ -D-cellobiohydrolase	qCO <sub>2</sub>
Multi-enzymes	1.00				
$\beta$ -D-glycosidase	0.99***	1.00			
$\beta$ -D xylanase	0.90***	0.86***	1.00		
$\beta$ -D-cellobiohydrolase	0.84***	0.79***	0.91	1.00	
qCO <sub>2</sub>	0.01NS	-0.08NS	0.13NS	0.18	1.00

\*\*\*<0.001; NS: Not significant

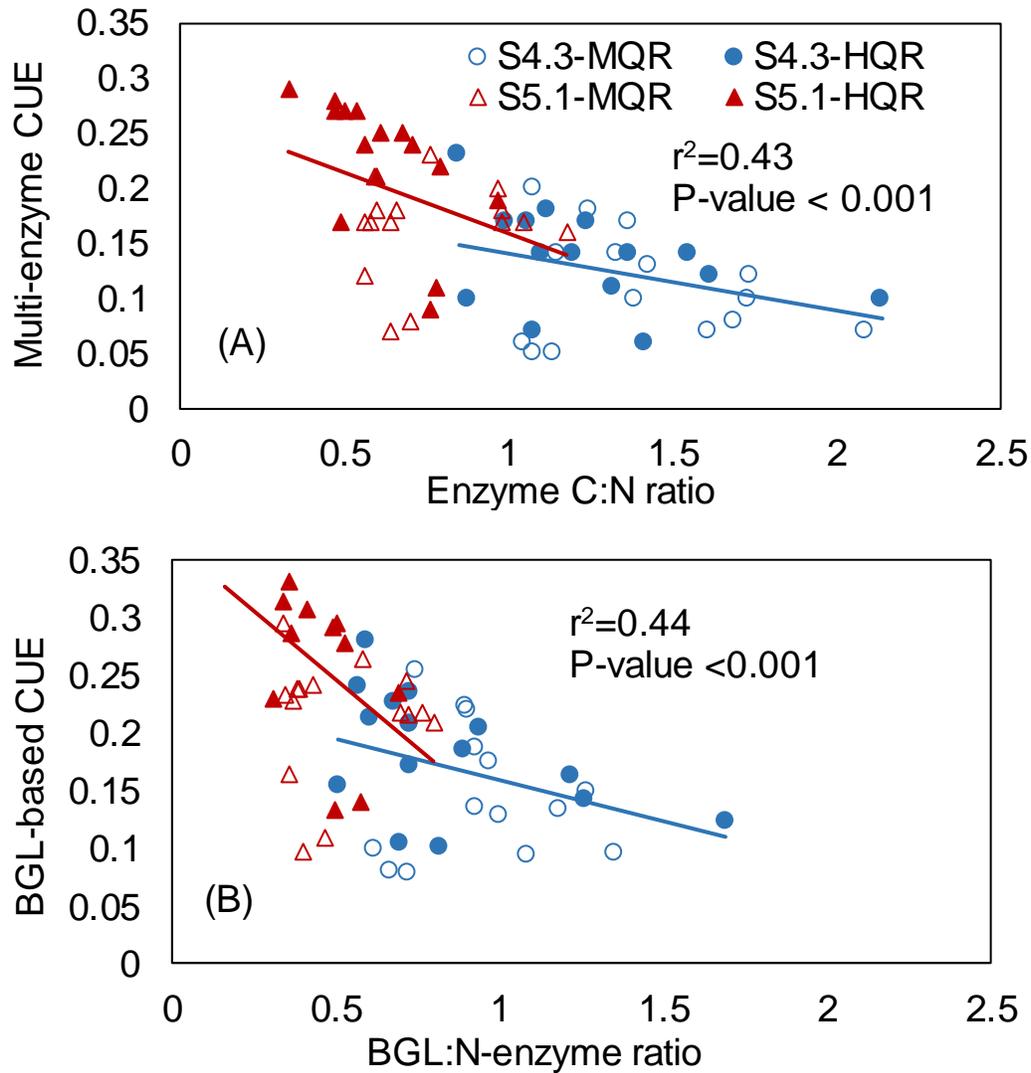
#### 4.4.5 Interactive effect of soil pH and residue quality on microbial CUE

CUE values estimated by indirect MCE-STM and SCE-STM as well as direct C-balance method were significantly affected by the soil pH, residue quality, and incubation duration, including the interaction of the factors (Table 2). For the C-balance method, the effect of residue quality on CUE was more pronounced in the less acidic than more acidic soil (Fig. 1). Despite the recorded differences in N content between medium and high quality residues (MQR versus HQR, Table S1), the amount of mineralized N during incubation depended more on soil pH level rather than on residue quality (Fig. 5A). During the 45 days of incubation, soil mineral N content was lower in S4.3 than in S5.1, while the respective differences between plant residues were moderate (Fig. 5A). Relative prevalence of C- versus N-acquiring enzymatic activities in S4.3 were noted in the mid phase of incubation (Fig. 5B). The interrelated effect of soil pH level and plant residue quality on CUE was substantiated by the clustering of the two soils with different pH, masking the plant residue quality effect (Fig. 6 A & B). The relatively higher contribution of N-acquiring enzymes (lower values on X axes) promoted a balanced microbial growth and higher CUE in the less acidic soils. There was also statistical equivalence of MCE-STM and BGL-STM methods of calculation (Fig. 6A versus 6B), though MCE-STM revealed a broader values distribution due to greater values of C-acquiring enzymatic activities, and, thus, a more distinct vision on the presented relationship.



**Figure 5** Estimated mean values for mineral nitrogen content in soil ( $\mu\text{g g}^{-1}$ ) (A); CN ratio ( $\text{EEA}_C/\text{EEA}_N$ ) of enzymatic activity (unit less) (B) for S4.3 (pH 4.3) and S5.1 (pH 5.1) amended

by medium quality (MQR), high quality (HQR) and without (S4.3-control and S5.1-control) residues across the incubation periods. Standard error (SE) bars represent  $\pm 1SE$ .



**Figure 6** Relationships between carbon use efficiency (CUE) estimated by two methods and ratio between C and N-acquiring enzymatic activity. (A) - CUE is evaluated using multi-enzymatic approach and (B) CUE is evaluated using BGL as indicator enzymes. Soils with pH 4.3 (S4.3) are marked with red triangles and soils with pH 5.1 (S5.1) with blue circles; samples amended by medium quality (MQR) plant residues are presented as unfilled symbols and those amended with high quality (HQR) plant residues as filled symbols.

## 4.5 Discussion

### 4.5.1 Microbial CUE influenced by the choice of calculation method

In the presented study, indirect, stoichiometric modelling (STM) approaches, in addition to the direct C-balance method, were used to evaluate soil microbial carbon use efficiency (CUE) during microbial growth on decomposing plant residues. The usefulness of STM was specifically reflected by the MCE-STM approach, followed by BGL-STM, showing both the more realistic lowest CUE values in all treatments. The C-balance method could overestimate the CUE, because MBC determination by fumigation extraction method does not account for any C lost through microbial enzyme and metabolite excretion (Manzoni et al., 2012; Sinsabaugh et al., 2013; Hagerty et al., 2018).

The principal difference between direct calculation of CUE by C-balance method and STM approaches is that the latter considers the effect of nutrient limitation on CUE. As detailed in the section 4.3.6, enzymatic STM considers the C to nutrient (N, P) ratios of microbial biomass and detrital organic matter and the ratio between C- and N acquiring enzymatic activities (Sinsabaugh & Shah, 2012; Cleveland & Liptzin, 2007). Although BGL was proportionally about 50-75% (Table S2) a major contributor of MCE-STM, the reliability of the STM-based approach benefitted greatly from the addition of complementary BCL and BXL. BCL and BXL activities are rate limiting in plant residue decomposition (Voříšková et al., 2011). These enzymes cleave the reducing or non-reducing ends of cellulose polysaccharide chains and complex structures of hemicelluloses (xylan) liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) (Lynd et al., 2002; Yun et al., 2015). In this respect, it must be emphasized that both BCL and BXL cannot be regarded in isolation (Fig. S1), because cellulose and xylan hydrolysis during litter decomposition occurs simultaneously (Hu et al., 2011; Voříšková et al., 2011); Fig. S1). It could be thus deduced that the lower CUE values calculated by MCE-STM, including three C-cycling enzymes, provided a more realistic picture of plant residue decomposition, reflecting the synergism of microbial extracellular enzymes involved in the hydrolysis of complex (BXL, BCL) and simple (BGL) substrates (Amin et al., 2014; Eriksson et al., 1990; Lashermes et al., 2016).

#### 4.5.2 Use of versatile proteolytic enzymes in MCE-STM modelling

The STM approach is based on the relative activity of both C- and nutrient (N or P)-acquiring enzymes. Thus, the first step was to suggest and verify the extended MCE-STM approach, where the sum of three C-acquiring enzymatic activities (BGL + BCL + BXL) were considered instead of BGL as single enzyme. The next logical step was to increase the accuracy of the CUE calculation by implementing, in addition to LAP, a suite of N-acquiring enzymes covering a range of enzymatic reactions into the model. Therefore, SAA and AAP rather than NAG were used as essential N-acquiring enzymes. This choice was worthwhile because these enzymes are rate limiting in the protein degradation of plant residues (Enowashu et al., 2009; Jan et al., 2009; Obayashi et al., 2017). Fujita et al. (2018) concluded that the synthesis of NAG is not controlled by N availability and that the NAG/BGL ratio did not show a significant negative relationship with available N concentration in arable soils. These authors suggested the use of other enzymes (urease and L-asparaginase) to estimate N-acquiring enzymatic activity in resource allocation models. This finding corroborated the presented approach of including SAA and AAP in MCE-STM, but not NAG. The potential activity of the above-mentioned proteolytic enzymes (specifically the proportion of SAA and AAP activities in total N-acquiring EEA) increased during incubation, a trend in line with the increase of C-acquiring enzymatic activities and changes in the C/N ratio of microbial biomass and substrate (Table S2). During residue decomposition, organic resources released nitrogenous nutrients (Fig. 5A). Therefore, the inclusion of rate limiting protein-degrading enzymes was crucial for the calculation of CUE.

#### 4.5.3 Interactive effect of soil pH and organic residue quality

In our study, we were able to evaluate an integrative effect of environmental factors (soil pH and residues quality) shaping microbial CUE in soil. The lowest CUE was calculated for the soil with a pH of 4.3 amended with medium quality residue (MQR, (PP+L)/N = 8.1). This indicated a higher energy investment to tolerate both soil acidity (Rousk et al., 2009) and accessibility of less decomposable residues (Johnson et al., 2007). Likewise, the highest CUE was recorded in the soil with a pH of 5.1 amended with less lignified residues (HQR, (PP+L)/N = 5.1), a finding in line with earlier studies (Jones et al., 2019; Lashermes et al., 2016; Puttaso et al., 2011). In this particular treatment (S5.1-HQR), soil microorganisms were not N-limited (Table S1), prompting

the utilization of available C for growth rather than increasing respiration as stress response in the contrasting treatment (S4.3-MQR). Schimel et al. (2007) observed similar effects, revealing low soil microbial CUE under drought stress. The experimental design of this study and application of the MCE-STM approach with consideration of activities of several protein-degrading enzymes (LAP, AAP, SAA) provided a precise insight into the mechanism of CUE change under the combined influence of soil acidity and biochemical quality of plant residues. Lower soil pH level (4.3 versus 5.1) suppressed N-acquiring enzymatic activities more than C-acquiring EEA (Table S2, Fig 5B). This suppression of proteolytic EEA was followed by a less intensive N mineralization in the soil with lower pH (Fig. 5A) that in turn may have induced N limitation for decomposing soil microorganisms. This led to the lower CUE efficiency, which was depended on the ratio between  $EEA_C$  and  $EEA_N$  (Fig. 6).

The observed interrelated effects of soil pH and organic residue quality suggested a treatment explicit modification of the microbial decomposer community. This is in line with recent theories by Kallenbach et al. (2019) proposing soil microbial CUE as the result of either “moderating” (prompting all community members) or “filtering” (selecting community members) microbial traits to enable the functional accommodation to a given environmental context. Accordingly, under S4.3-MQR with lowest microbial CUE, the soil microbial decomposer community might have faced a trait modification by selecting specifically resource use efficient community members to maintain decomposition and growth. The negative synergetic effect of both environmental parameters (pH and residue quality) induced a less efficient utilization of complex organic residues to acquire a unit of biomass C, as further verified by a higher metabolic quotient ( $qCO_2$ ) and stimulated activities of individual enzymes in S4.3-MQR. This finding was in line with Puttaso et al. (2011) who reported a higher microbial  $qCO_2$  in more lignified (high L+PP)/N; dipterocarp) than less lignified (low L+PP)/N; tamarind) residues in a long-term field experiment.

The intermediate values of CUE in the strongly acidic soil amended with high quality residues and in less acidic soils amended with medium quality residues were not significantly different from each other. Hence, caution must be taken in interpretation of the results as different environmental factors can have opposite effects on resource acquisition. Under natural conditions, where a combination of complex environmental factors control microbial

metabolism, it is necessary to consider different factors (e.g., residue quality, soil pH) in CUE estimation models (Manzoni et al., 2012), rather than taking only individual factors (Amin et al., 2014; Lashermes et al., 2016; Puttaso et al., 2011).

## **4.6 Conclusions**

The extent of the estimation of microbial CUE depended on the selected method (MCE-STM, SCE-STM, C-balance method). We could deduce that the multi-C cycling enzymatic stoichiometric modeling (MCE-STM) approach, rather than the C-balance, was more appropriate for investigating the combined effects of soil pH and residue quality in the initial stage of decomposition. On the other hand, MCE-STM did not increase the accuracy of CUE estimation in comparison to SCE-STM. Expanding the number of various C- and N-acquiring enzymes for CUE evaluation (MCE-STM), provided, however, CUE estimates with a higher reliability, because cellulose and hemicellulose degrading enzymes in conjunction with protein degrading enzymes are all rate limiting in litter decomposition. This conclusion was especially relevant for the given environmental set-up that deduced explicitly the interrelated effects of soil pH and organic residue quality on soil microbial CUE.

## **4.7 Acknowledgments**

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## 4.9 Supplementary materials

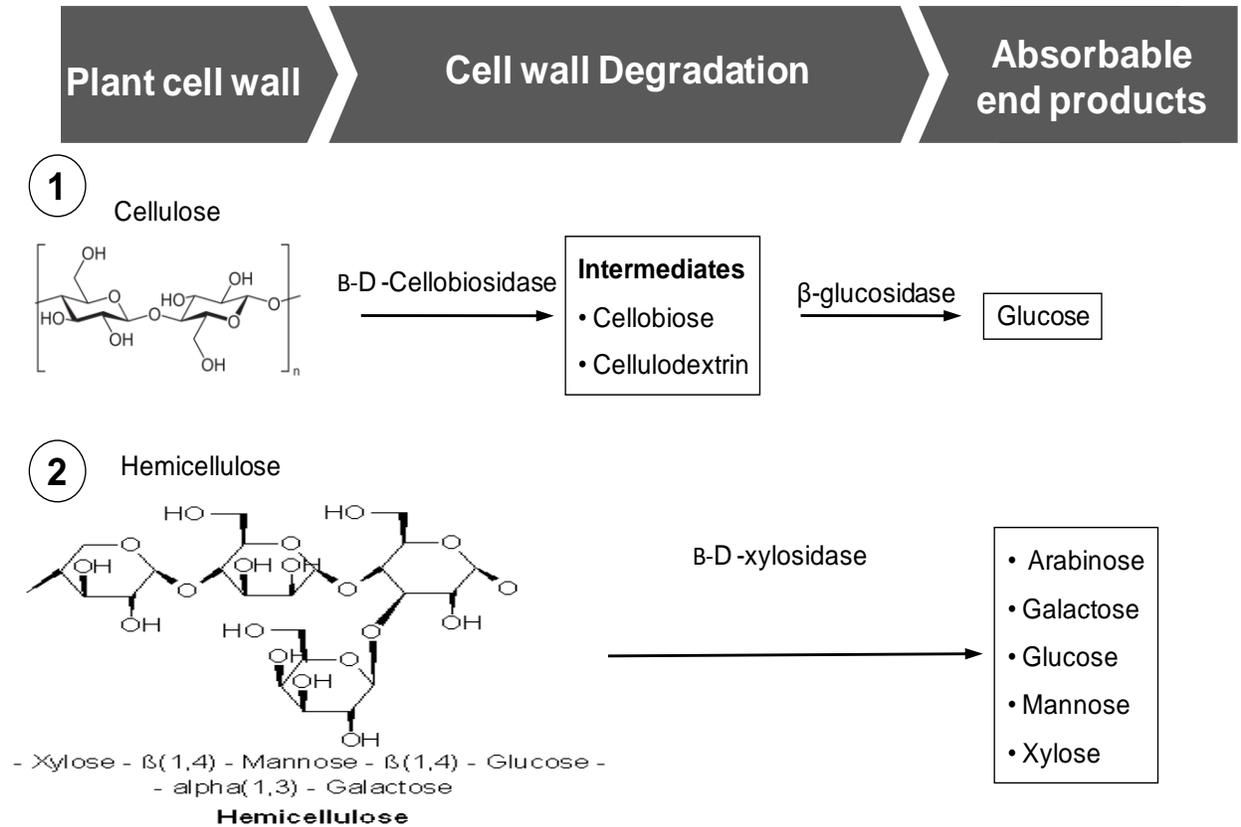


Figure S1 Schematic illustration of plant residues (cell wall) degradation by enzymatic hydrolysis; 1, cellulose degradation and 2, hemicelluloses degradation

Table S1 Mean soil chemical values (N=18 per sampling date) in S\_4.3 (pH; 4.3) and S\_5.1 (pH; 5.1) soils amended with medium quality (MQR) and high quality (HQR) residues in each sampling date (days 7, 15, 30, 45 &60): MBC; microbial biomass carbon, dCO<sub>2</sub>; daily CO<sub>2</sub>-C, CumCO<sub>2</sub>; cumulative CO<sub>2</sub>-C, DOC; dissolved organic carbon, MBN; microbial biomass nitrogen, DON; dissolved organic nitrogen, Min N; mineral nitrogen (sum of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), trt; treatment, LSD; least significant difference, SD sampling date.

Treatments	MBC μg g <sup>-1</sup>	dCO <sub>2</sub> μg g <sup>-1</sup>	CumCO <sub>2</sub> μg g <sup>-1</sup>	DOC μg g <sup>-1</sup>	MBN μg g <sup>-1</sup>	DON μg g <sup>-1</sup>	Min N μg g <sup>-1</sup>	MBC/MBN	DOC/DON
Day 7									
S4.3-MQR	942.2b	56.0b	934.1b	482.5b	226.9ab	285.0ab	63.5d	4.2a	1.7a
S4.3-HQR	1071.0b	45.7c	926.9b	416.1c	240.7ab	302.2ab	102.7c	4.4a	1.4b
S5.1-MQR	1013.9b	62.6a	1049.0a	592.1a	284.7a	357.6a	125.0b	3.6ab	1.7a
S5.1-HQR	1274.7a	56.6b	1073.8a	481.2b	300.2a	377.0a	126.4b	4.2a	1.3b
S4.3	557.0c	9.04d	150.5c	324.5d	193.9b	243.5b	108.3bc	2.9bc	1.3b
S5.1	633.7c	11.1d	189.45c	300.4d	264.2ab	331.7ab	165.1a	2.4c	0.9c
P-level (trt)	***	***	***	***	***	***	***	NS	***
P-level (SD)	***	***	***	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	**	***
LSD value	124.25	3.15	108.8	57.4	80.0	100.3	19.72	1.1	0.25
Day 15									
S4.3-MQR	898.7b	34.2b	1189.4b	401.7a	255.0bc	320.2bc	78.7cd	3.5a	1.3a
S4.3-HQR	928.6b	28.6c	1163.9b	364.9ab	277.0abc	347.6ab	95.7bc	3.4a	1.0ab
S5.1-MQR	1177.9a	41.1a	1362.5a	362.2ab	308.0ab	386.5ab	100.1b	3.8a	0.9bc

S5.1-HQR	1214.9a	35.2b	1407.8a	316.6bc	349.1a	438.4a	120.8a	3.5a	0.7c
S4.3	675.0c	8.3d	193.7c	268.7c	218.4c	274.2c	64.1d	3.2a	1.0ab
S5.1	683.8c	11.2d	250.1c	282.4c	253.0bc	317.7bc	90.6bc	2.7a	0.9bc
P-level (trt)	***	***	***	***	***	***	***	NS	***
P-level (SD)	***	***	***	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	**	***
LSD value	124.25	3.15	108.8	57.4	80.0	100.3	19.72	1.1	0.25
Day 30									
S4.3-MQR	824.4bc	45.6a	2028.0b	347.1a	354.0b	444.5b	104.6bc	2.3c	0.8ab
S4.3-HQR	924.9b	23.7c	1540.8d	315.0ab	293.8b	369.0b	113.6b	3.1bc	0.9ab
S5.1-MQR	1186.6a	34.4b	2222.8a	275.8bc	498.9a	626.5a	147.3a	2.4bc	0.4c
S5.1-HQR	1173.4a	24.6c	1865.1c	226.4cd	322.4b	404.9b	158.2a	4.7a	0.6bc
S4.3	635.6d	8.6d	358.9e	207.4d	184.4c	231.5c	88.3cd	3.5b	0.9a
S5.1	720.5cd	10.3d	462.9e	170.5d	291.8b	366.4b	75.1d	2.5bc	0.5c
P-level (trt)	***	***	***	***	***	***	***	**	***
P-level (SD)	***	***	***	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	**	***
LSD value	124.25	3.15	108.8	57.4	80.0	100.3	19.72	1.1	0.25
Day 45									
S4.3-MQR	923.5b	19.5b	2259.2a	266.04ab	307.0b	385.5b	119.3b	3.1a	0.7ab
S4.3-HQR	848.4bc	15.8c	1755.8c	290a	310.5b	389.9b	119.4b	2.7a	0.7a
S5.1-MQR	1079.5a	23.0a	2313.0a	223.2bcd	401.0a	503.5a	150.3a	2.7a	0.4c

S5.1-HQR	1113.2a	18.4bc	1948.9b	230.4bc	372.3ab	467.6ab	155.2a	3.0a	0.5bc
S4.3	634.7d	10.8d	460.3d	193.3cd	212.9c	267.4c	95.1c	3.0a	0.7ab
S5.1	767.5c	9.0d	547.6d	168.7d	305.1b	383.1b	126.1d	2.5a	0.4c
P-level (trt)	***	***	***	***	***	***	***	NS	***
P-level (SD)	***	***	***	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	***	***
LSD value	124.25	3.15	108.8	57.4	80.0	100.3	19.72	1.1	0.25
Day 60									
S4.3-MQR	939.3a	16.2b	2578.7b	230.2ab	784.5a	985.2a	332.1a	1.2b	0.2b
S4.3-HQR	860.8ab	16.0b	2225.9d	206.7bc	624.5b	784.2b	273.9b	1.4b	0.3b
S5.1-MQR	815.0bc	24.1a	2794.2a	264.3a	758.7a	952.8a	268.7b	1.1b	0.3b
S5.1-HQR	934.9ab	18.9b	2461.8c	199.1bc	666.8b	837.4b	262.2b	1.4b	0.2b
S4.3	586.5d	8.2d	561.7f	193.1bc	212.3c	266.6d	120.7d	3.1a	0.8a
S5.1	713.8c	9.4d	730.4e	162.6c	393.9d	494.7c	173.9c	1.8b	0.4b
P-level (trt)	***	***	***	***	***	***	***	**	***
P-level (SD)	***	***	***	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	***	***
LSD value	124.25	3.15	108.8	57.4	80.0	100.3	19.72	1.1	0.25

P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; Different letters show significant differences between means at P<.0001

Table S2 Mean potential enzymatic activities (nmol g DM-1 h-1) (N=18 per sampling date) in S4.3 (pH; 4.3) and S5.1 (pH; 5.1) soils amended with medium quality (MQR) and high quality (HQR) residues in each sampling date (days 7, 15, 30, 45 &60) and respective ratios: BGL;  $\beta$ -glucosidase, BCL;  $\beta$ -D-cellobiohydrolase, BXL;  $\beta$ -xylosidase, LAP; Leucine-aminopeptidase, SAA; Succinyl-Alanyl-Alanyl-phenyl, AAP; Alanyl-Alanyl-phenyl aminopeptidase, EEAC; extracellular enzyme activities of C-cycling enzymes, (BGL+BCL+BXL) and EEAN; extracellular enzyme activities of N-cycling enzymes (LAP+AAP+SAA), trt; treatment, LSD; least significant difference, soil SD sampling date.

Treatments	BGL	BCL	BXL	LAP	AAP	SAA	EEAC/ EEAN	LAP/EE AN	AAP/ EEAN	SAA/ EEAN	BGL/ EEAC	BCL/ EEAC	BXL/ EEAC
Day 7													
S4.3-MQR	322.91d	60.24b	83.04c	213.98b	129.7b	36.24bc	1.23a	0.57bc	0.34cd	0.1	0.69b	0.13b	0.18b
S4.3-HQR	402.1c	62.78b	68.40cd	229.35b	179.37b	44.44bc	1.21a	0.52cd	0.38bc	0.1	0.75a	0.12b	0.13c
S5.1-MQR	812.41a	132.66a	129.96a	493.36a	585.68a	93.54a	0.93b	0.42e	0.50a	0.08	0.76a	0.12b	0.13c
S5.1-HQR	618.4b	145.73a	106.53b	519.24a	476.15a	102.42a	0.81b	0.49de	0.42ab	0.09	0.71ab	0.17a	0.12c
S4.3	134.59e	32.55c	52.48d	198.93c	100.94b	26.00c	0.68bc	0.61ab	0.31cd	0.08	0.61c	0.15ab	0.24a
S5.1	259.72d	71.85b	77.85c	525.64a	207.82b	57.90b	0.52c	0.66a	0.26d	0.07	0.63c	0.178a	0.19b
P-level (trt)	***	***	***	***	***	***	***	***	***	NS	***	***	***
P-level (SD)	***	***	*	***	**	**	*	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	**	***	***	***	***	***	***	***
LSD value	78.28	17.08	17.52	44.31	117.47	22.81	0.27	0.08	0.08	0.03	0.06	0.03	0.04
Day 15													
S4.3-MQR	356.72cd	67.25c	85.15c	158.96bc	100.38d	52.54c	1.63a	0.51a	0.32d	0.17a	0.7ab	0.13bc	0.17bc

S4.3-HQR	403.62 <sup>bc</sup>	57.33 <sup>c</sup>	78.92 <sup>c</sup>	135.95 <sup>bc</sup>	161.15 <sup>d</sup>	49.78 <sup>c</sup>	1.63 <sup>a</sup>	0.40 <sup>bc</sup>	0.46 <sup>c</sup>	0.14 <sup>ab</sup>	0.74 <sup>a</sup>	0.11 <sup>c</sup>	0.15 <sup>c</sup>
S5.1-MQR	638.29 <sup>a</sup>	119.25 <sup>a</sup>	151.56 <sup>a</sup>	364.30 <sup>a</sup>	422.16 <sup>b</sup>	93.20 <sup>b</sup>	1.05 <sup>b</sup>	0.42 <sup>b</sup>	0.47 <sup>c</sup>	0.10 <sup>c</sup>	0.7 <sup>ab</sup>	0.13 <sup>bc</sup>	0.17 <sup>bc</sup>
S5.1-HQR	475.49 <sup>b</sup>	109.30 <sup>a</sup>	117.03 <sup>b</sup>	363.45 <sup>a</sup>	638.42 <sup>a</sup>	138.04 <sup>a</sup>	0.62 <sup>cd</sup>	0.32 <sup>cd</sup>	0.56 <sup>b</sup>	0.12 <sup>bc</sup>	0.68 <sup>bc</sup>	0.16 <sup>ab</sup>	0.17 <sup>bc</sup>
S4.3	122.01 <sup>e</sup>	28.06 <sup>d</sup>	40.38 <sup>d</sup>	131.39 <sup>c</sup>	285.87 <sup>c</sup>	22.44 <sup>d</sup>	0.44 <sup>d</sup>	0.29 <sup>d</sup>	0.66 <sup>a</sup>	0.05 <sup>d</sup>	0.64 <sup>c</sup>	0.15 <sup>ab</sup>	0.21 <sup>a</sup>
S5.1	313.74 <sup>d</sup>	86.33 <sup>b</sup>	96.23 <sup>c</sup>	178.85 <sup>b</sup>	392.75 <sup>b</sup>	106.55 <sup>b</sup>	0.74 <sup>c</sup>	0.26 <sup>d</sup>	0.58 <sup>ab</sup>	0.16 <sup>a</sup>	0.63 <sup>c</sup>	0.17 <sup>a</sup>	0.19 <sup>ab</sup>
c													
P-level (trt)	***	***	***	***	***	***	***	***	***	***	***	***	***
P-level (SD)	***	***	***	***	***	***	*	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	***	***	***	***	***	***
LSD value	78.28	17.08	17.52	44.31	117.47	22.81	0.27	0.08	0.08	0.03	0.06	0.03	0.04

Table S2 (continued)

Treatments	BGL	BCL	BXL	LAP	AAP	SAA	EEAC/E EAN	LAP/ EEAN	AAP/ EEAN	SAA/ EEAN	BGL/ EEAC	BCL/E EAC	BXL/ EEAC
Day 30													
S4.3-MQR	320.77 <sup>a</sup>	94.73 <sup>ab</sup>	85.96 <sup>bc</sup>	116.28 <sup>c</sup>	123.67 <sup>c</sup>	43.49 <sup>c</sup>	1.80 <sup>a</sup>	0.42 <sup>ab</sup>	0.43 <sup>de</sup>	0.15 <sup>ab</sup>	0.63 <sup>a</sup>	0.19 <sup>b</sup>	0.17 <sup>c</sup>
S4.3-HQR	248.97 <sup>ab</sup>	69.53 <sup>c</sup>	78.61 <sup>c</sup>	126.82 <sup>c</sup>	168.94 <sup>c</sup>	53.81 <sup>bc</sup>	1.14 <sup>b</sup>	0.36 <sup>b</sup>	0.48 <sup>cd</sup>	0.16 <sup>ab</sup>	0.62 <sup>ab</sup>	0.18 <sup>b</sup>	0.2 <sup>bc</sup>
S5.1-MQR	320.20 <sup>a</sup>	85.49 <sup>bc</sup>	96.77 <sup>ab</sup>	217.24 <sup>b</sup>	515.10 <sup>b</sup>	102.63 <sup>a</sup>	0.60 <sup>cd</sup>	0.26 <sup>c</sup>	0.62 <sup>ab</sup>	0.12 <sup>bc</sup>	0.64 <sup>a</sup>	0.17 <sup>b</sup>	0.19 <sup>bc</sup>
S5.1-HQR	241.39 <sup>b</sup>	110.71 <sup>a</sup>	112.61 <sup>a</sup>	248.69 <sup>ab</sup>	740.70 <sup>a</sup>	125.40 <sup>a</sup>	0.42 <sup>d</sup>	0.23 <sup>c</sup>	0.66 <sup>a</sup>	0.11 <sup>c</sup>	0.52 <sup>c</sup>	0.24 <sup>a</sup>	0.24 <sup>a</sup>
S4.3	105.85 <sup>c</sup>	36.25 <sup>d</sup>	40.84 <sup>d</sup>	126.64 <sup>c</sup>	87.60 <sup>c</sup>	40.48 <sup>c</sup>	0.73 <sup>c</sup>	0.49 <sup>a</sup>	0.34 <sup>e</sup>	0.16 <sup>a</sup>	0.58 <sup>b</sup>	0.20 <sup>b</sup>	0.22 <sup>ab</sup>
S5.1	290.03 <sup>ab</sup>	74.75 <sup>c</sup>	82.16 <sup>bc</sup>	271.22 <sup>a</sup>	398.80 <sup>b</sup>	74.87 <sup>b</sup>	0.61 <sup>cd</sup>	0.36 <sup>b</sup>	0.54 <sup>bc</sup>	0.1 <sup>c</sup>	0.65 <sup>a</sup>	0.17 <sup>b</sup>	0.18 <sup>c</sup>
P-level (trt)	***	***	***	***	***	***	***	***	***	***	***	***	***
P-level (SD)	***	***	***	***	***	***	*	***	***	***	***	***	***

P-level (trt*SD)	***	***	***	***	***	***	***	***	***	***	***	***	***
LSD value	78.28	17.08	17.52	44.31	117.47	22.81	0.27	0.08	0.08	0.03	0.06	0.03	0.04
Day 45													
S4.3-MQR	341.35a	76.67c	92.85abc	190.86b	159.11d	47.25c	1.29a	0.48a	0.40c	0.12bc	0.66a	0.15b	0.18a
S4.3-HQR	238.06b	92.62c	85.82bc	114.69c	180.43d	49.90c	1.21a	0.33b	0.52b	0.15ab	0.57b	0.22a	0.21a
S5.1-MQR	329.24a	112.21b	100.69ab	197.45b	586.68b	104.45ab	0.61bc	0.22c	0.66a	0.12bc	0.60b	0.21a	0.19a
S5.1-HQR	363.10a	141.40a	107.12a	255.18a	739.40a	121.69a	0.55c	0.23c	0.66a	0.11c	0.59b	0.23a	0.18a
S4.3	119.96c	52.63d	43.56d	119.41c	85.58d	43.29c	0.87b	0.48a	0.35c	0.17a	0.56b	0.24a	0.20a
S5.1	241.27b	86.01c	79.65c	230.50ab	370.06c	89.28b	0.59c	0.33b	0.54b	0.13bc	0.59b	0.21a	0.20a
P-level (trt)	***	***	***	***	***	***	***	***	***	***	***	***	NS
P-level (SD)	***	***	***	***	***	***	*	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	***	***	***	***	***	***
LSD value	78.28	17.08	17.52	44.31	117.47	22.81	0.27	0.08	0.08	0.03	0.06	0.03	0.04

Table S2 (continued)

Treatments	BGL	BCL	BXL	LAP	AAP	SAA	EEAC/E EAN	LAP/ EEAN	AAP/ EEAN	SAA/ EEAN	BGL/ EEAC	BCL/E EAC	BXL/ EEAC
Day 60													
S4.3-MQR	303.55ab	110.18a	81.79bc	142.77bc	255.32b	55.65bc	1.09b	0.31abc	0.56a	0.12c	0.61bc	0.23a	0.17c
S4.3-HQR	256.25bc	98.20ab	71.30cd	143.78b	187.15bc	61.57abc	1.13b	0.37a	0.46b	0.17b	0.60bc	0.23a	0.17c
S5.1-MQR	290.89ab	56.29cd	104.06a	225.82a	407.64a	79.788a	0.63c	0.32abc	0.57a	0.11c	0.65ab	0.13c	0.23ab
S5.1-HQR	345.82a	62.11c	98.77ab	200.54a	485.40a	78.72a	0.68c	0.27bc	0.63b	0.11c	0.68a	0.13c	0.20bc
S4.3	138.75d	40.36d	62.91d	56.43d	73.24c	38.99c	1.44a	0.34ab	0.43b	0.23c	0.57cd	0.17b	0.26a
S5.1	196.89cd	81.24b	86.62abc	98.77cd	239.31b	78.40ab	0.88bc	0.24c	0.57a	0.19b	0.54d	0.22a	0.24ab
P-level (trt)	***	***	***	***	***	***	***	***	***	***	***	***	***
P-level (SD)	***	***	***	***	***	***	*	***	***	***	***	***	***
P-level (trt*SD)	***	***	***	***	***	***	***	***	***	***	***	***	***
LSD value	78.28	17.08	17.52	44.31	117.47	22.81	0.27	0.08	0.08	0.03	0.06	0.03	0.04

P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; Different letters show significant differences between means at P<.0001

## **Chapter 5**

### **General discussion**

This chapter discusses the most important findings of the chapters 2 to 4 whether our results confirm and/or against the findings in the literature.

## 5 General Discussions

Soil fertility variability is a profound problem hindering the successful implementation of integrated soil fertility management (ISFM) in smallholder farmers of Eastern and Central Africa (Chikowo et al., 2014). The most noticeable biophysical factors responsible for soil fertility variability in sub-Saharan African (SSA) are topographical factors (e.g. soil type, elevation, slope and aspect as well as climatic characteristics) (Tittonell et al., 2005a). Furthermore, Stewart et al. (2020) identified a lack of quality soil testing and site-specific fertilizer recommendations, limited opportunities to increase soil organic matter (SOM), and unequal opportunity of smallholder farmers to access inorganic fertilizer and retention of crop residues in the soil are other sources of soil fertility variability. To address these bottlenecks, for example in Ethiopia, the Ethiopian Soil Information System (EthioSIS) under Agricultural Transformation Agency (ATA) has developed a nation-wide soil nutrient mapping. This is an initiative to provide policy advice on the use of fertilizer for smallholder farmers (Amare et al., 2018). In addition to biophysical factors and socioeconomic drivers, farmers resource endowments are other sources of soil fertility variability in East and Central Africa (Hailelassie et al., 2006; Nyamangara et al., 2011; Tittonell et al., 2005a). Moreover, there is only limited data available yet that considered the inter-related effects of both biophysical (e.g., agro-ecology defined based on elevation, climate conditions, and cropping system) and socio-economic factors (e.g., farmers' resource endowments, market access and farmers' indigenous knowledge) on soil fertility variability on a detailed farm scale. This is because not only the individual drivers but also the synchronization of both biophysical and socio-economic factors contributed for soil fertility variability beyond the individual factors (Balume et al., 2020; Agumas et al., 2021). To address these issues, generic and harmonized soil surveying procedures have to be developed, allowing direct comparisons of different agro-ecologies and associated farming systems across regions or countries. Among the different soil survey approaches, the use of midDRIFTS is a robust and more efficient survey approach with minimum cost and time investment. MidDRIFTS is not only analyzing quantitative soil physical and chemical properties but it is also used to quantify the soil organic carbon (SOC) qualitative groups (soil organic carbon functional groups). Therefore, this study developed different models for predicting selected soil physicochemical properties in the two countries (Ethiopia and DRC) as a case study using midDRIFTS-partial least square regression (PLSR). Furthermore, the study also considered SOC

functional groups as additional soil fertility indicators as well as carbon use efficiency (CUE) as the most important sensitive soil fertility indicative parameters considered in this PhD study.

Based on the generated predicted data sets of the two countries, country-specific research hypotheses were tested. Chapter 2 assessed the inter-related effect of different agro-ecology, farm typology classes and farmers' indigenous knowledge on soil fertility status while chapter 3 assessed the interacted impact of market distance, farm typology classes and farmers' indigenous knowledge on soil fertility status. Chapter 4 dealt with the contributing factors for microbial CUE to enhance soil fertility status taking organic residue quality and soil pH as factors.

### **5.1 Inter-related effect of agro-ecology, farm typology and market access present heterogeneous soil fertility status in smallholder farming systems**

Heterogeneity in soil fertility status presents a major challenge to the successful implementation of ISFM strategies in SSA, including, but not limited to, Ethiopia and DRC (Vanlauwe et al., 2015). This is because soil fertility status across different locations varied not because of isolated factor such as agro-ecology (defined based on geological, climatic and farming system variations), farm typology and market access (distance from the nearest market) but also the combination of inter-related factors (chapters 2 & 3).

In both studies (chapters 2 & 3), the effect of site specificity was more pronounced in available phosphorus ( $P_{av}$ ) and total nitrogen (TN). The differences in  $P_{av}$  due to the factors site and agro-ecology may be attributed to the influence of soil mineralogy, which originated from the geology of the soils. The Ethiopian case study sites showed higher pH and  $P_{av}$  values recorded in the limestone bedrock in the highlands contrary to the basaltic bedrock found in the low and midlands (Ali & Goshu, 2017; Hamza & Raghuvanshi, 2017) (chapter 2). Similarly, the case study in the DRC indicated that differences between soil texture,  $P_{av}$ ,  $Ca_{ex}$  and  $Mg_{ex}$  in the two study sites (Bushumba versus Mushinga) were due to geological variations (chapter 3). In this respect, the Madoka sub-site (Mushinga site) was dominated by a sandy soil texture with the typical reddish color originating from basaltic rocks (van Engelen et al., 2006). On the other hand, there were no clear differences in available potassium  $K_{av}$  values between DRC case study sites contrary to that of Ethiopia. This may be due to variation in elevation, climatic conditions and the agricultural farming systems between the two country study sites. The average elevation of the Ethiopian study sites varied from 1,281 meters above sea level (m.a.s.l) in the lowland

agro-ecological study site where fallowing, burning of organic residues and application of farmyard manure is taken as major soil fertility management option to an elevation of 2,911 m.a.s.l in the highland agro-ecological sites where a combination of organic and inorganic fertilizer is taken as a viable option to soil management. On the contrary, elevation between the DRC case study sites did not vary widely (1,604-1,740 m. a.s.l.) which might not lead to diversified cropping systems. These diversified elevation ranges in the Ethiopian study sites shapes the climatic conditions that control the farming system types of smallholder farmers. For example, the average annual rain fall amount range from 2,037 mm (lowland kola agro-ecology study site) to 938 mm (high land dega agro-ecological study sites). Furthermore, the farming systems in the Ethiopian study sites is dominated by integrated mixed crop livestock farming. Therefore, these differences may be responsible for the pronounced variations in soil fertility indicators between the Ethiopian sites. The farming system in DRC is dominated by slash and burn crop cultivation (Kane et al., 2004), and this may be responsible for the absence of clear variations in most of the soil fertility indicators ( $K_{av}$ , pH and total organic carbon (TOC) between the different case study sites.

The effect of resource endowment in the Ethiopian case study sites was visible compared to the DRC sites. In the kola agro-ecological case study sites of Ethiopia for instance, higher values for  $K_{av}$  were found on wealthy farmers' fields as compared to poor farmers' fields. In addition, there were distinct differences of TOC content and labile SOC functional groups, being higher in the fields of the wealthy than less wealthy farmers (chapter 2). There was also a higher SOC stability index (ratio of peak 1620 to 2930) in poor than wealthy farmers' fields in Ethiopian case study farm typologies (wealthy versus poor). On the contrary, there were no differences in TOC and SOC functional groups as well as their stability indexes between farm typology groups in DRC. This might be attributed to the differences in soil management decisions between Ethiopian farm typology case study groups (wealthy versus poor). For example, in the lowland case study sites, wealthy farmers fallow their field, burn organic residue and apply farmyard manure obtained from higher livestock numbers, while the management differences in the highlands might be explained by the option of wealthy farmers to combine organic and inorganic fertilizer inputs as compared to using only inorganic fertilizer by poor farmers. On the contrary, there were no differences in TOC and SOC functional groups as well as their stability indexes between farm typology groups in DRC. Contrary to the high variability in the management decisions between

the different farm typology in the Ethiopian case study farmers, there was no clear soil management differences between the different farm typology groups in DRC except that farmers with <1 ha and >2 ha near to the market buy and apply farmyard manure and household waste such as ash. This might be on the one hand due to farmers' practice of only slash and burn cultivation system (Kane et al., 2004). On the other hand, the use of inorganic fertilizer by all farm typology groups in DRC was very small and sometimes nonexistent (Kane et al., 2004) compared to the Ethiopian wealthy farmers (chapters 2 & 3).

It must be noted that all these results and conclusions were from the use of similar and harmonized science-based research methodology in the two case studies (chapters 2 & 3). Most importantly, both studies used more robust, time and cost efficient midDRIFTS spectral analysis to analyze bulk soil samples collected from spatially different sites of Ethiopia and DRC. The midDRIFTS-PLSR models were developed to predict selected soil physico-chemical properties that were used to tackle different research questions and objectives in the two countries. Furthermore, the study approach in both case studies used similar and harmonized research methodology such as focused group discussion and semi-structure questioner to categorize farm typologies. Similarly, in the field of both countries study sites, a Y-shaped scheme was used for soil sample collection in the field. Therefore, it is evident that midDRIFTS as well as the use of similar and harmonized research methodology enables the evaluation of soil fertility status across a large spatial scale which ranges from east Africa (Ethiopia) to central Africa (DRC). Hence, these findings confirmed the claim raised by Shepherd and Walsh (2007) and Seybold et al. (2019) that infrared spectroscopy is a rapid, low cost and highly reproducible soil survey tool that can be used in large spatial scale. Furthermore, midDRIFTS provides various physical, chemical, and biological soil properties (such as enzymatic activities, microbial biomass and microbial abundance) from a single spectrum (Seybold et al., 2019; Rasche et al., 2014).

A study used to assess the influence of market access on soil fertility status conducted in the DRC case study sites showed that, soil fertility, as displayed by TOC and TN concentrations, decreased with increasing market distance. This showed an innovative approach revealing the impact of poor market access on recurrent soil fertility problems in SSA. Further study on this need to be extended to other SSA countries such as Ethiopia. This is because Ethiopia is endowed with a wide range of agro-ecologies and farming systems (e.g. integrated crop livestock mixed farming) which might be difficult to translate directly the findings of market access on

soil fertility found in DRC. The type and cost of inputs needed for soil fertility improvement as well as the type of crops produced in highland agro-ecology is different from the lowland agro-ecology in Ethiopia. For example, lime and acid free synthetic fertilizers are more important in the highlands where soil acidity is high than in the low land agro-ecology. Furthermore, the agricultural extension systems and extent of infrastructure development (road construction) of Ethiopia might be different from DR-C. Hence, further understanding of how market accesses affect soil fertility status across the different agro-ecologically distinct sites in Ethiopia is required.

## **5.2 SOC functional groups and stability indexes as indicators of soil fertility assessment**

To assess soil fertility status, sensitive indicators such as SOC functional groups and stability indexes calculated from relative peak areas of selected midDRIFTS peaks are important in addition to soil nutrients and TOC. This is based on the premise that labile SOC functional groups may change more easily due to tillage, manuring, fertilization, crop rotation and other interventions than total organic matter (Bongiovanni & Lobartini, 2006; Duval et al., 2018; Heitkamp et al., 2009). A larger pool of labile SOC along with a lower SOC stability index (i.e., peak area ratio of 1620:2930, 1530:2930 and 1159:2930) indicated higher soil fertility as compared to higher C=C aromatic and a higher SOC stability index (chapter 2), because the labile SOC pool is acknowledged to increase important soil functions, such as soil aggregate formation, nutrient supply and can serve as a microbial energy source (Gmach et al., 2020; Haynes, 2005; Strosser, 2010). On the contrary, C=C aromatic pools are known to increase soil C stabilization (Haynes, 2005). Thus, claims that soils with higher labile SOC functional groups are fertile than those with higher C=C aromatic groups were confirmed with higher  $P_{av}$  and soil pH in the fertile than less fertile soils in our case study (chapter 2). Similar results were reported by Demyan et al. (2012), who also found higher C-H aliphatic SOC groups in plots of the Bad Lauchstädt long-term field experiment (Germany) treated with both chemical and organic fertilizers for more than 100 years (suggested as fertile) than in plots receiving only farm yard manure (less fertile). In contrast, the higher 1159  $cm^{-1}$  relative peak area and stability index (i.e., peak area ratio of 1620:2930) in the less fertile as compared to fertile soils (chapter 2) may be indicative that more stabilized SOC functional groups were found in less fertile soils due to the

absence of regular organic resources amendments. Furthermore, we found an increasing ratio of 1530:2930 with increasing market distance in medium and poor farmers' field plots, implying a lower SOC quality due to limited or absence of organic inputs. The assumption "SOC functional groups can be used as soil fertility indicators" was confirmed with the existence of relationships with other soil physico-chemical properties in our studies. For example, in the Ethiopian case study, significant positive correlations of pH and TOC with C-H aliphatic SOC ( $r^2=0.39$ ,  $r^2=0.51$ ) ( $P<0.001$ ) were noted, while negative relationships were found for C=C aromatic SOC ( $r^2=0.39$ ,  $r^2=0.47$ ) ( $P<0.001$ ), indicating that fertile soils had more labile SOC functional groups and less stabilized SOC functional counterparts. Furthermore, this assumption was corroborated by the negative correlation between the ratio of 1530:2930 and organic C content in the DR-C case study (chapter 3). Therefore, in addition to application of inorganic fertilizers, SOC management and application of organic resources such as plant residues and farmyard manure or compost is a useful strategy to enhance SOC labile functional groups (Demyan et al., 2012). This is because the addition of organic inputs to soils increases the labile pools in the short term and accumulates SOC in the long term due to mineralization (Margenot et al., 2015). Hence, understanding the extent of labile and aromatic SOC functional groups can be used as key indicator to guide short and/or long-term soil fertility management strategies.

However, the extent of labile SOC pools and accumulation of more stable aromatic SOC pools is controlled by two factors, namely organic C input and SOC degradation rates (Deng et al., 2019). Organic input degradation rates are closely related to the quality of C inputs, environmental constraints and the efficiency of microbial decomposers called CUE. However, future work may be extended to include additional more sensitive soil microbiological properties (e.g. soil enzymatic activities, microbial biomass and microbial abundance) as indicators for soil fertility assessment. This is because, these properties are more sensitive and can mediate the biochemical transformations of organic matter that reinforces essential ecosystem functions, including decomposition, mineralization of plant available nutrients and nutrient retentions (Bowles et al., 2014). Therefore, understanding the relationship between soil chemical properties specifically SOC functional groups and soil microbial processes can provide better insight into understanding the extent of soil fertility status in small holder farming systems.

### **5.3 Could microbiome CUE be an indicator for soil fertility status?**

To assess the extent of soil fertility status, researchers and agricultural experts used different indicators such as plant nutrients and TOC. Even though CUE is an important ecological characteristic of microbial metabolism and soil C cycling (Manzoni et al., 2008; Spohn et al., 2016), however, its use as indicator for soil fertility assessment is not common. To test whether CUE could be used as one of the soil fertility indicators, we conducted a microcosm experiment under laboratory conditions (chapter 4), considering that infertile soils have either stress problems (soil acidity) or nutrient limitations. To address this issue, we used strongly (described as less fertile) and medium acidic (described as fertile) soils amended with medium quality (resource-limited) and high-quality residue (resource unlimited) soils as a case study (chapter 4). This is because, CUE is increasingly gaining attention as an important factor governing the fate of metabolized C, and thus SOM formation, nutrient dynamics, and release of C to the atmosphere (Arcand et al., 2017; Blagodatskaya et al., 2014; Manzoni et al., 2012). Hence, understanding CUE in different soils could provide a better insight not only about the extent of soil fertility status but also linking soil biochemical process (e.g. soil mineralization, organic matter decompositions) with ecological soil functions (such as nutrient cycling, soil C storage and soil aggregate stabilization). This is due to the fact that the efficiency of the soil microbiome (i.e communities of soil microorganisms consisting of bacteria, fungi and protozoa associated with various soils habitats (Lakshmanan et al., 2014)) controls soil C decomposition and sequestration potential which is a responsible parameter to guide major soil ecological functions such as nutrient cycling (Spohn et al., 2016). Based on these premises, we found lower CUE in the more acidic soils amended with medium quality residues in which mineralization process has been hampered than less acidic soils amended by high-quality residues (chapter 4). A similar finding by Schimel et al. (2007) revealed that lower soil microbial CUE was observed in drought stressed soils than non-stressed soils. This was probably due to higher energy investment to tolerate both soil acidity as well as drought stress (Rousk et al., 2009) and the accessibility of less decomposable residues (Johnson et al., 2007). This inefficient CUE, in more acidic soils amended with relatively more recalcitrant residues (less fertile), may exacerbate CO<sub>2</sub> emission than returning C to the soil via soil microbial biomass (Janzen, 2015). Furthermore, in less fertile soils, the stoichiometry requirement of microbial biomass for nutrients may further decrease the

CUE of microorganisms (Rui et al., 2016), because of the limited amount of nutrients in these type of soils. The existence of lower CUE in the less fertile soils thus, indicated that the soil is limited in nutrient provision to plants as well as nutrients and energy to soil microorganisms. On the contrary, fertile soil possess higher CUE values (chapter 4; Schimel et al., 2007) due to the availability of C for microorganisms growth and to increase microbial biomass. This in turn enhanced the labile soil pools such as microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and fastens the turnover of the SOC which might also increase the availability of soil nutrients (chapter 4). According to Rui et al. (2016) soils with low labile C (e.g. MBC) displayed lower CUE of soil microorganisms, because the limited available C is used to satisfy the energy demands for cell maintenance with little left for cell growth and division. Thus, the high value of CUE in fertile than less fertile soils is indicative that CUE could be used as an important indicator to monitor the extent of soil fertility status in agricultural soils. However, these findings were based on laboratory incubation studies and may not be directly translated to field conditions to assess soil fertility status. Therefore, I recommend further research to unravel the effect of different soil fertility level on CUE in more heterogeneous soil conditions considering not only soil pH but also different levels of nutrients (e.g.  $P_{av}$ ,  $K_{av}$ ) and SOC contents as factors. This is because our study was constrained by a very narrow difference in soil pH and further evidence might be required to understand how soils with different status of macro nutrients and SOC content affect microbial CUE. This could be done using midDRIFTS, because it is confirmed that most important soil microbiological data important for CUE calculations (e.g. microbial biomass, enzymatic activities and microbial DNA) could be predicted in cost and time efficient way (Rasche et al., 2014). This approach will permit easier estimation of CUE and to be used as an important soil fertility indicator under a larger spatial regional scale in smallholder farming systems of SSA.

#### **5.4 Could multi-enzymatic stoichiometry be a better approach for microbial CUE?**

Accurate quantification of CUE in terrestrial ecosystems has become one of the challenges in soil ecology. Different assumptions are employed for different methods and to capture different aspects of microbial metabolism. CUE calculation based on direct C-balance method assumed net increase in microbial biomass while enzymatic stoichiometry modeling (STM) assumes that

the imbalance of microbial C/N and labile pool substrate C/N ratio, as well as a ratio between C and N (P) acquiring enzymes, are a direct control of microbial CUE. To understand the different principles and hence CUE estimation approaches, a controlled experiment was conducted (chapter 4) using plant residue contrary to Geyer et al. (2019) who used only glucose as a substrate.

In this experiment, we tested the direct C-balance method and the indirect stoichiometry (multi-enzymes and single enzymes) approaches (chapter 4). We found different CUE values using the direct C-balance, multi-enzymes,  $\beta$ -D-glucosidase (BGL),  $\beta$ -D-cellobiohydrolase (BCL) and  $\beta$ -D-xylosidase (BXL) enzymatic activities as a proxy for C-cycling enzymes, confirming that CUE values are shaped by applied methods (chapter 4). The lowest CUE values were recorded in multi-enzymes followed by BGL enzymatic activities; this might be due to nutrient and microbial biomass strict stoichiometry principles. On the other hand, the higher CUE in direct C-balance method, which might be due to MBC estimation by fumigation extraction method, did not account for any C lost through microbial enzyme and metabolite excretion (Hagerty et al., 2018; Manzoni et al., 2012; Sinsabaugh et al., 2013). Similar results were earlier reported, confirming that the direct method gave higher CUE values as compared to the STM method (Blagodatskaya et al., 2014; Geyer et al., 2019; Spohn et al., 2016).

We could not calculate CUE using the C-balance method after the first seven days of incubation because of microbial turn over effects. Instead, we calculated  $q\text{CO}_2$  for longer incubation periods creating difficulty for direct comparison between  $q\text{CO}_2$  and CUE values. This was one of the limitations of this method as compared to the stoichiometry approaches. Also, this method demands monitoring of  $\text{CO}_2$ -C to calculate CUE during the whole incubation period.

The main advantage of stoichiometry modeling approaches as compared to C-balance method is that it utilizes common soil analysis for CUE calculation, and can be applied at various spatio-temporal scales (Sinsabaugh et al., 2016). The principal difference between the direct calculation of CUE by C-balance method and STM approaches is that the latter considers the effect of nutrient limitation on CUE. As detailed in chapter 4, enzymatic STM considers the C to nutrient (N, P) ratios of microbial biomass and detrital organic matter and the ratio between C and nutrient (N) acquiring enzymatic activities (Sinsabaugh & Follstad Shah, 2012; Spohn et al., 2016). However, it was not clear so far whether single enzymatic or multi-enzymatic stoichiometry is an appropriate approach to quantify CUE for long term plant residue

decomposition. Again, in this study we compared single versus multi-enzymatic stoichiometry and found lower CUE using multi-enzymes as compared to single STM. Since BCL and BXL activities are rate limiting in plant residue decomposition (Voříšková et al., 2011), involvement of these enzymatic activities in addition to BGL will give better insight for CUE estimation in multi-enzymes approach. Even though, multi-enzymatic stoichiometry approach could be suggested as an alternative option for CUE estimation, it must be noted that involvement of lignin degrading enzymes activities in to the calculation of CUE might be beneficial. This is because; lignin constitutes 15-25% of the total dry matter of plant residues and farmyard manure (Palm et al., 2001). This could be even more important in small-holder farming systems where the use of organic resources for soil fertility replenishment is common.

## **5.5 Future research work**

Over all, this PhD study provides evidence-based drivers of soil fertility variability in SSA using more efficient and robust approaches in case studies in Ethiopia and DRC. A harmonized midDRIFTS-PLSR model development and peak area integration analysis were employed for the two countries. Even though, the applied approach is robust and efficient to analyze large data sets, this study covered only major plant nutrients (NPK), total C, pH and SOC pools. Thus, it would be a further advantage to use midDRIFTS to develop a model that predicts other macro (e.g., Sulphur, calcium and magnesium) and soil texture for soil fertility assessment in the study areas. This is because, these macro nutrients as well as soil texture are important soil fertility indicators and determination of these parameters using conventional method (e.g. wet chemistry) demands more time and cost. However, accurate predictions on available phosphorous and exchangeable potassium using midDRIFTS were not successful because of midDRIFTS prediction depends on soil mineralogy whereas availability of these nutrients depend not only on the inherent soil mineralogy but also environmental conditions such as soil pH.

As shown in this study, more sensitive SOC pools are used as indicator for soil fertility assessment, although this is a good start, further studies would be required to use soil microbial communities and their functional diversity (e.g. microbial enzymatic activities and soil respiration) as indicators of soil fertility assessment. This is because microbial functions (i.e enzymatic activities) play a key role in decomposing organic matter and provision of plant nutrients. Furthermore, microbial functional diversity has been found very sensitive to

environmental changes and affect nutrient cycling process in the soil (Neiendam and Winding, 2002). Similarly, microbial abundance and diversity controls the actual microbial functions (enzymatic activities, respirations) and influences soil fertility status. This could help to understand how soil microbial activities would shape soil biochemical process (mineralization and decomposition) to give a deeper insight on soil ecological functions (nutrient cycling, C storage, aggregate stability).

Interestingly, this work explained the linkage between CUE and soil fertility status and showed how to use it as a proxy for soil fertility. This study also addressed the impact of estimation methods and environmental variability on CUE. However, evaluating of the entire available CUE estimation methods including  $^{13}\text{C}$  and  $^{18}\text{O}$  isotope labeling approaches using organic residues as substrate in terrestrial ecosystems needs to be considered for future research.  $^{13}\text{C}$  isotope labeling could help to trace the amount of C partitioned in to different C pools from the applied substrate (e.g. MBC, DOC,  $\text{CO}_2$ ) that could be useful for accurate estimations of CUE (Muller et al., 2016; Geyer et al., 2019). By doing this, it could be possible to disentangle the CUE of bacteria community from fungi. This might be a useful approach to confirm the claim that the efficiency of fungi community in degrading organic inputs in general and lignin in particular is suspected to be higher than bacterial community. To address this critical research gaps, linking fungal and bacterial gene abundance with microbial biomass carbon (MBC) and nitrogen (MBN) on the one hand and quantification of  $\text{CO}_2\text{-C}$  from the individual community on the other hand in soil ecological study should be considered for future research. Quantification of  $\text{CO}_2\text{-C}$  could be done using selective inhibition technique to measure the contribution of the bacterial and fungal community to  $\text{CO}_2\text{-C}$  production. Thus, the quantification of individual communities' abundance (e.g bacteria; gram positive and gram negative and saprotrophic fungi) can be done using stable isotopes in combination with biomarker molecule analyses such as the phospholipid fatty acids (PLFA) technique (Müller et al., 2016). By doing this, it might be possible to quantify C gain and loss in each individual community (bacteria and fungi) that will provide important steps for estimation of CUE for fungal and bacterial individual community in terrestrial ecosystems. On the other hand,  $^{18}\text{O}$  isotope labelling uses the incorporation of  $^{18}\text{O}$ -labeled and unlabeled natural water into soil to measure DNA gross growth (Gerey et al., 2019). Thus, combining molecular and biochemical techniques may be necessary for the estimation of CUE in individual fungal and bacterial community and to better understand carbon dynamics in terrestrial ecosystems. Lack of

lignin degrading enzymes in the multi-enzymes model in this study is also another limitation which demands further investigation because of 15-25% of the compositions in plant residues and farmyard manure are lignin (Palm et al., 2001), and these organic resources are the main sources of fertilization for small holder farmers.

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## 6 Summary

The main drivers of soil fertility variability across Sub-Saharan Africa (SAA) must be understood to develop tailor-made integrated soil fertility management (ISFM) strategies, considering agro-ecological zones, smallholder farmers' resource endowment and their indigenous knowledge of soil fertility. Moreover, most soil fertility indicators including, but not limited to total soil organic carbon (SOC) content, lack in sensitivity and accuracy. The insensitivity and inaccuracy of these indicators impedes their application for soil fertility surveys in smallholder farming systems across larger spatial scales. Hence, the verification of novel soil fertility indicators, such as SOC functional groups and microbial carbon use efficiency (CUE) as influenced by environmental factors (e.g. soil pH, organic input quality), become paramount important to overcome this constraint. The implementation of such methodological innovation would help to better understand the extent of regional soil fertility variability and subsequently design niche-based ISFM strategies for smallholder farming systems in SSA.

Therefore, the first aim of this study was to explore the interrelated effects of biophysical and socio-economic factors on soil fertility variability, as reflected by soil nutrient contents as well as SOC content and quality parameters (i.e., SOC functional groups). The second aim was to evaluate soil microbial CUE as an additional proxy to assess soil fertility considering the influence of environmental and methodological variations on CUE calculation.

The specific objectives of this PhD study were to:

- verify that soil fertility variability across two model regions in Central and Western Ethiopia with four distinct agro-ecological zones could be determined by the inter-related effects of agro-ecology and farmers' resource endowment (“wealthy” versus “poor” farmers).
- confirm this approach of local soil fertility assessment in Ethiopia by including “market distance” as an additional factor for soil fertility variability, as exemplified in the Democratic Republic of Congo (DRC).
- test whether farmers' indigenous knowledge on soil fertility status is driven by inter-related effects of agro-ecology, market distance and farm typology, considering the continuous knowledge transfer among farmers within and across agro-ecological zones.

- evaluate the potential of SOC functional groups and soil microbial CUE as promising indicators of soil fertility status influenced by physico-chemical soil properties and organic input management.
- to modify the existing single C-cycling enzymatic stoichiometry (SCE-STM) through proposing novel “multi”-C-cycling enzymatic stoichiometry (MCE-STM) methods for soil microbial CUE estimation.

To tackle objectives 1-3 of the presented PhD study, two local field-based soil fertility surveys were conducted in Ethiopia and DRC. A lab-based incubation study was implemented for objectives 4 and 5. For the soil fertility surveys, mid-infrared spectroscopy coupled to partial least squares regression (midDRIFTS-PLSR) and wet-lab analyses were used to assess the soil fertility (i.e., soil pH, total soil carbon (TC), total soil nitrogen (TN), plant-available phosphorous ( $P_{av}$ ) and potassium ( $K_{av}$ ), exchangeable calcium ( $Ca_{ex}$ ) and magnesium ( $Mg_{ex}$ )) across four agro-ecological zones in Ethiopia. MidDRIFTS peak area analysis of spectral frequencies (2930 (aliphatic C-H), 1620 (aromatic C=C), 1159 (C-O poly-alcoholic and ether groups)  $cm^{-1}$ ) were applied to characterize SOC quality and to calculate the SOC stability index (1620:2930, 1530:2930). While in DRC, both techniques were employed to assess soil fertility proxies across market distances (defined as walking time) in distinct regions. For the lab-based incubation study (60 days), two soils differing mainly in acidity level mixed with two specimens of plant residues differing mainly in lignin (L) and polyphenol (PP) content were used. For estimating soil microbial CUE during plant residue decomposition in the different soils, single C-cycling enzymatic stoichiometry (SCE-STM) and the newly proposed “multi”-C-cycling enzymatic stoichiometry (MCE-STM) methods were validated against the conventional C-balance method.

MidDRIFTS-PLSR and peak area analysis results of the Ethiopian case study showed that the inter-related effects of agroecology and farmers’ resource endowment determined the observed soil fertility variability across four agro-ecological zones. Resource endowment dependent soil fertility management options revealed higher TC in the high altitude agro-ecological zone, while higher TN and  $K_{av}$  was found in the lower agro-ecological zones in the fields of wealthy farmers. Similarly, SOC of higher quality was found in soils of wealthy than poor farms in higher altitude zones. Thus, agro-ecological zone distinctions contributed to these differences in soil fertility variability. It was deduced that this difference in soil fertility status between wealthy and poor

farmers' fields across agro-ecological zones has been due to the high variability in landholding size per capita, livestock population and amount of fertilizer used per unit area.

Complementary, the results of the DRC case study revealed that “market distance” and “farm typology” were key determinants of soil fertility variability, both with contrasting trends in the study sites. Decreasing soil fertility status was noted across all farm typologies with increasing market distance. A significant influence of “farm typology” was found for  $Ca_{ex}$  and  $Mg_{ex}$ , while factor “site” resulted in a significant difference of  $P_{av}$ . For SOC quality indices (i.e., ratio 1530:2930), factor “site” was decisive, as reflected in its interaction with “market distance”. However, the effect of market distance became obvious in the medium wealthy and poor farmers fields, where an increasing SOC quality index of 1530:2930 with increasing market distance implied a lower SOC quality in remote farms.

Soil depth and soil color were the most frequently used soil fertility indicators by farmers across agro-ecologies, market distances, and farm typologies. Concerning farmers' indigenous knowledge across the study regions in Ethiopia and DRC, fertile and less fertile fields were distinguished visually by soil color. Higher pH and  $P_{av}$  were found in fertile (brown/black) than less fertile (red) soils in most agro-ecological zones of the Ethiopian case study. Furthermore, higher peak areas of  $1159\text{ cm}^{-1}$  and SOC stability indices were observed in less fertile compared to fertile soils in Ethiopia. In close agreement with farmers' indigenous knowledge in the DRC study region, soil fertility levels were higher in deep than shallow soils, which was reflected in higher nutrient stocks in deep soils receiving organic amendments. Accordingly, site-specific soil management strategies with the integration of farmers' indigenous knowledge will be a feasible option to overcome the low adoption of ISFM.

This PhD study suggested the use of more sensitive indicators, such as soil microbial CUE, to accurately assess soil fertility status for the design of niche-based soil fertility management decisions. Furthermore, the PhD study showed that higher CUE was recorded in more fertile and less acidic (pH 5.1) soils amended with residues of higher quality than the other three combinations. It was deduced that microorganisms invested more energy to support growth in more acidic (pH 4.3) soil to tolerate soil acidity, which, in turn, suppressed N-acquiring enzymatic activities and further decreased CUE. Lower CUE values were recorded from multi-C cycling enzymatic stoichiometry modeling (MCE-STM) as compared to the CUE values

obtained from C-balance and single-C cycling enzymatic stoichiometry modeling (SCE-STM) methods. The modification of the MCE-STM method for CUE determination proposed in this dissertation work was capable to quantify the combined effect of soil pH and plant residue quality on the efficiency of microbial metabolism. As a result, it improved the original stoichiometric modeling approach (SCE-STM), which relied only on the concept of nutrient availability.

In conclusion, for regional soil fertility assessment, midDRIFTS-PLSR predictions along with midDRIFTS peaks representing SOC functional groups proved to be a sensitive as well as more efficient and robust approach as compared to the existing approaches relying on classical soil properties (e.g., SOC content) assessed by wet lab analyses. Based on the generated data using midDRIFTS, the main drivers of soil fertility variability have been revealed, considering specifically the interrelated effects of agro-ecology, resource endowment, market distance and farmers' indigenous knowledge. Furthermore, integration of soil microbial CUE (e.g. MCE-STM) in soil fertility assessments does not only provide a clearer picture of soil fertility status. It also serves for better understanding of ecological processes in soils in general. With his, this PhD study fostered the knowledge of soil fertility drivers across spatial scales and laid the scientific basis for the furthering of novel soil fertility indicators based on soil microbial CUE. This outcome will benefit the design of niche-based soil fertility management strategies, which are of paramount importance to secure the livelihoods of SSA smallholder farming systems.

## 7. Zusammenfassung

Die Hauptfaktoren für die Variabilität der Bodenfruchtbarkeit in Subsahara-Afrika (SAA) müssen verstanden werden, um maßgeschneiderte Strategien für ein integriertes Bodenfruchtbarkeitsmanagement (ISFM) zu entwickeln, die die agro-ökologischen Zonen, die Ressourcenausstattung der Kleinbauern und ihr indigenes Wissen über die Bodenfruchtbarkeit berücksichtigen. Darüber hinaus mangelt es den meisten Bodenfruchtbarkeitsindikatoren, einschließlich, aber nicht beschränkt auf den Gesamtgehalt an organischem Kohlenstoff (SOC) im Boden, an Empfindlichkeit und Genauigkeit. Die Unempfindlichkeit und Ungenauigkeit dieser Indikatoren erschwert ihre Anwendung für die Erfassung der Bodenfruchtbarkeit in kleinbäuerlichen Systemen auf größeren räumlichen Skalen. Daher ist die Überprüfung neuartiger Bodenfruchtbarkeitsindikatoren, wie z.B. funktionelle SOC-Gruppen und mikrobielle Kohlenstoffnutzungseffizienz (CUE), die von Umweltfaktoren (z.B. pH-Wert des Bodens, Qualität des organischen Inputs) beeinflusst werden, von größter Bedeutung, um diese Einschränkung zu überwinden. Die Umsetzung einer solchen methodischen Innovation würde helfen, das Ausmaß der regionalen Bodenfruchtbarkeitsvariabilität besser zu verstehen und anschließend nischenbasierte ISFM-Strategien für kleinbäuerliche Anbausysteme in SSA zu entwickeln. Daher war das erste Ziel dieser Studie, die wechselseitigen Auswirkungen biophysikalischer und sozioökonomischer Faktoren auf die Variabilität der Bodenfruchtbarkeit zu untersuchen, wie sie sich in den Nährstoffgehalten des Bodens sowie im SOC-Gehalt und den Qualitätsparametern (d.h. den SOC-Funktionsgruppen) widerspiegelt. Das zweite Ziel war die Bewertung des CUE als zusätzlicher Proxy zur Beurteilung der Bodenfruchtbarkeit unter Berücksichtigung des Einflusses von Umwelt- und methodischen Variationen auf die CUE-Berechnung.

Die spezifischen Ziele dieser PhD-Studie waren,:

- zu verifizieren, dass die Variabilität der Bodenfruchtbarkeit in zwei Modellregionen in Zentral- und West-Äthiopien mit vier unterschiedlichen agro-ökologischen Zonen durch die miteinander verbundenen Effekte der Agrarökologie und der Ressourcenausstattung der Landwirte ("reiche" versus "arme" Landwirte) bestimmt werden kann.

- diesen Ansatz der lokalen Bewertung der Bodenfruchtbarkeit in Äthiopien zu bestätigen, indem die "Marktdistanz" als zusätzlicher Faktor für die Variabilität der Bodenfruchtbarkeit einbezogen wird, wie es in der Demokratischen Republik Kongo (DRC) vorgemacht wurde.
- zu testen, ob das indigene Wissen der Landwirte über den Zustand der Bodenfruchtbarkeit durch interdependente Effekte der Agrarökologie, der Marktdistanz und der Betriebstypologie bestimmt wird, unter Berücksichtigung des kontinuierlichen Wissenstransfers zwischen den Landwirten innerhalb und zwischen den agro-ökologischen Zonen.
- das Potenzial der funktionellen SOC-Gruppen und des mikrobiellen CUE im Boden als vielversprechende Indikatoren für den Zustand der Bodenfruchtbarkeit zu bewerten, die von den physikalisch-chemischen Bodeneigenschaften und dem Management organischer Inputs beeinflusst werden.
- die bestehende Single-C-Cycling-Enzym-Stöchiometrie (SCE-STM) zu modifizieren, indem neuartige "Multi"-C-Cycling-Enzym-Stöchiometrie (MCE-STM) Methoden zur CUE-Abschätzung vorgeschlagen werden.

Um die Ziele 1-3 der vorgestellten PhD-Studie anzugehen, wurden zwei lokale feldbasierte Bodenfruchtbarkeitsuntersuchungen in Äthiopien und der DRC durchgeführt. Für die Ziele 4 und 5 wurde eine laborbasierte Inkubationsstudie durchgeführt. Für die Erhebungen der Bodenfruchtbarkeit wurden die Mid-Infrarot-Spektroskopie gekoppelt mit der Partial Least Squares Regression (midDRIFTS-PLSR) und Nasslaboranalysen verwendet, um die Bodenfruchtbarkeit (d.h. pH-Wert des Bodens, Gesamtkohlenstoff (TC), Gesamtstickstoff (TN), pflanzenverfügbare Phosphor ( $P_{av}$ ) und Kalium ( $K_{av}$ ), austauschbares Kalzium ( $Ca_{ex}$ ) und Magnesium ( $Mg_{ex}$ )) in vier agro-ökologischen Zonen in Äthiopien zu bewerten. Die MidDRIFTS-Peakflächenanalyse der Spektralfrequenzen (2930 (aliphatische C-H), 1620 (aromatische C=C), 1159 (C-O polyalkoholische und Ether-Gruppen)  $cm^{-1}$ ) wurde zur Charakterisierung der SOC-Qualität und zur Berechnung des SOC-Stabilitätsindex angewendet. In der DRC wurden beide Techniken eingesetzt, um die Bodenfruchtbarkeit über Marktentfernungen (definiert als Gehzeit) in verschiedenen Regionen zu bewerten. Für die laborbasierte Inkubationsstudie (60 Tage) wurden zwei Böden, die sich hauptsächlich im

Säuregrad unterscheiden, mit zwei Proben von Pflanzenresten gemischt, die sich im Lignin- (L) und Polyphenolgehalt (PP) unterscheiden. Zur Abschätzung der mikrobiellen CUE im Boden während des Abbaus von Pflanzenresten in den verschiedenen Böden wurden die Methoden der Single-C-Cycling-Enzym-Stöchiometrie (SCE-STM) und der neu vorgeschlagenen "Multi"-C-Cycling-Enzym-Stöchiometrie (MCE-STM) gegenüber der herkömmlichen C-Bilanz-Methode validiert.

Die Ergebnisse der MidDRIFTS-PLSR- und Peak-Flächen-Analyse der äthiopischen Fallstudie zeigten, dass die miteinander verbundenen Effekte der Agrarökologie und der Ressourcenausstattung der Landwirte die beobachtete Variabilität der Bodenfruchtbarkeit in vier agroökologischen Zonen bestimmten. Von der Ressourcenausstattung abhängige Optionen des Bodenfruchtbarkeitsmanagements zeigten eine höhere TZ in der hochgelegenen agroökologischen Zone, während in den niedrigeren agroökologischen Zonen auf den Feldern der wohlhabenden Landwirte eine höhere TN und  $K_{av}$  gefunden wurde. In ähnlicher Weise wurde eine höhere SOC-Qualität in den Böden von wohlhabenden als von armen Betrieben in den höher gelegenen Zonen gefunden. Somit trugen agro-ökologische Zonenunterschiede zu diesen Unterschieden in der Variabilität der Bodenfruchtbarkeit bei. Es wurde abgeleitet, dass dieser Unterschied im Bodenfruchtbarkeitsstatus zwischen den Feldern wohlhabender und armer Landwirte in den verschiedenen agro-ökologischen Zonen auf die hohe Variabilität in der Pro-Kopf-Größe des Landbesitzes, des Viehbestandes und der Menge des pro Flächeneinheit verwendeten Düngers zurückzuführen ist. So legen wohlhabende Landwirte im Tiefland ihr Land brach und bringen organische Reststoffe aus, während die Landwirte im Hochland den Einsatz von chemischen Düngemitteln und Hofdünger in größerem Umfang in Betracht ziehen.

Ergänzend zeigten die Ergebnisse der DRC-Fallstudie, dass die "Marktdistanz" und die "Betriebstypologie" wichtige Determinanten für die Variabilität der Bodenfruchtbarkeit sind, beide mit gegensätzlichen Trends in den Untersuchungsgebieten. Ein abnehmender Bodenfruchtbarkeitsstatus wurde bei allen Betriebstypologien mit zunehmender Marktentfernung festgestellt. Ein signifikanter Einfluss der "Betriebstypologie" wurde für Caex und Mgex gefunden, während der Faktor "Standort" zu einem signifikanten Unterschied von Pav führte. Für die SOC-Qualitätsindizes (d.h. das Verhältnis 1530:2930) war der Faktor "Standort"

entscheidend, was sich in seiner Interaktion mit der "Marktdistanz" widerspiegelte. Der Effekt der Marktdistanz wurde jedoch auf den Feldern der mittelreichen und armen Landwirte deutlich, wo ein steigender SOC-Qualitätsindex von 1530:2930 mit zunehmender Marktdistanz eine geringere SOC-Qualität in den abgelegenen Betrieben implizierte.

Bodentiefe und Bodenfarbe waren die von den Landwirten am häufigsten verwendeten Indikatoren für die Bodenfruchtbarkeit, unabhängig von der Agrarökologie, der Marktentfernung und der Betriebstypologie. Was das indigene Wissen der Landwirte in den Untersuchungsregionen in Äthiopien und der Demokratischen Republik Kongo betrifft, wurden fruchtbare und weniger fruchtbare Felder visuell durch die Bodenfarbe unterschieden. In den meisten agro-ökologischen Zonen der äthiopischen Fallstudie wurden höhere pH-Werte und Pav-Werte in fruchtbaren (braun/schwarz) als in weniger fruchtbaren (rot) Böden gefunden. Außerdem wurden höhere Peakflächen von  $1159 \text{ cm}^{-1}$  und SOC-Stabilitätsindizes in weniger fruchtbaren im Vergleich zu fruchtbaren Böden in Äthiopien beobachtet. In enger Übereinstimmung mit dem einheimischen Wissen der Landwirte in der DRC-Studienregion war die Bodenfruchtbarkeit in tiefen Böden höher als in flachen Böden, was sich in höheren Nährstoffvorräten in tiefen Böden widerspiegelte, die organische Ergänzungen erhielten. Dementsprechend sind standortspezifische Bodenbewirtschaftungsstrategien mit der Integration des indigenen Wissens der Landwirte eine machbare Option, um die geringe Akzeptanz von ISFM zu überwinden.

Diese PhD-Studie schlug vor, empfindlichere Indikatoren, wie z.B. den mikrobiellen CUE-Wert des Bodens, zu verwenden, um den Zustand der Bodenfruchtbarkeit genau zu beurteilen und Entscheidungen für ein nischenbasiertes Bodenfruchtbarkeitsmanagement zu treffen. Darüber hinaus zeigte die PhD-Studie, dass in fruchtbareren und weniger sauren (pH 5,1) Böden, die mit Rückständen höherer Qualität ergänzt wurden, ein höherer CUE-Wert gemessen wurde als in den anderen drei Kombinationen. Daraus wurde gefolgert, dass die Mikroorganismen mehr Energie zur Unterstützung des Wachstums in saureren (pH 4,3) Böden investierten, um die Bodensäure zu tolerieren, was wiederum die N-akquirierenden enzymatischen Aktivitäten unterdrückte und den CUE weiter reduzierte. Niedrigere CUE-Werte wurden von der Multi-C-Cycling-Enzym-Stöchiometrie-Modellierung (MCE-STM) im Vergleich zu den CUE-Werten aufgezeichnet, die

von den C-Balance- und Single-C-Cycling-Enzym-Stöchiometrie-Modellierungsmethoden (SCE-STM) erhalten wurden. Die in dieser Dissertationsarbeit vorgeschlagene Modifikation der MCE-STM-Methode zur CUE-Bestimmung war in der Lage, den kombinierten Effekt von Boden-pH und Pflanzenrückstandsqualität auf die Effizienz des mikrobiellen Stoffwechsels zu quantifizieren. Dadurch verbesserte sie den ursprünglichen stöchiometrischen Modellierungsansatz (SCE-STM), der sich nur auf das Konzept der Nährstoffverfügbarkeit stützte.

Zusammenfassend lässt sich sagen, dass sich die midDRIFTS-PLSR-Vorhersagen zusammen mit den midDRIFTS-Peaks, die die funktionalen SOC-Gruppen repräsentieren, für die regionale Bewertung der Bodenfruchtbarkeit als sensibler sowie effizienter und robuster Ansatz erwiesen haben, verglichen mit den bestehenden Ansätzen, die sich auf klassische Bodeneigenschaften (z. B. den SOC-Gehalt) stützen, die durch Nasslaboranalysen ermittelt werden. Basierend auf den mit midDRIFTS generierten Daten wurden die Haupttreiber für die Variabilität der Bodenfruchtbarkeit aufgedeckt, wobei insbesondere die zusammenhängenden Effekte von Agrarökologie, Ressourcenausstattung, Marktdistanz und indigenem Wissen der Landwirte berücksichtigt wurden. Darüber hinaus liefert die Integration der mikrobiellen CUE (z.B. MCE-STM) in die Bewertung der Bodenfruchtbarkeit nicht nur ein klareres Bild des Zustands der Bodenfruchtbarkeit. Sie dient auch dem besseren Verständnis ökologischer Prozesse in Böden im Allgemeinen. Damit förderte diese Doktorandenstudie das Wissen über Bodenfruchtbarkeitstreiber über räumliche Skalen hinweg und legte die wissenschaftliche Basis für die Förderung neuartiger Bodenfruchtbarkeitsindikatoren, die auf mikrobiellen CUE im Boden basieren. Dieses Ergebnis wird der Entwicklung von Nischen-basierten Bodenfruchtbarkeits-Management-Strategien zugute kommen, die von größter Bedeutung für die Sicherung der Lebensgrundlage von kleinbäuerlichen Systemen in SSA sind.

## 8. Curriculum Vitae

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### Personal information

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### Educational information

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Sept 2015- present	PhD candidate. Agronomy in the tropics and subtropics, Faculty of Agricultural Science at University of Hohenheim, Stuttgart, Germany
Sep 2006-Mar 2009	MSc. Environmental science at Addis Ababa University, Addis Ababa, Ethiopia. 2009.
Thesis:	Status of Soil Acidity Problems and Comparison of Lime Requirement Determination Methods in Different Land Use Systems in the High Lands of Gojjam: The Case of Fagetalekoma Woreda, Awi Zone.
Sep 1999-July 2002	BSc. Plant Science at Alemaya University, Dire Dawa, Ethiopia. 2002.

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Sep1995- Jun 1998	High School Certificate, Debremarkos Comprehensive Senior Secondary School Debre Markos, Ethiopia
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**Work Experience**

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Oct 2015-present	PhD Candidate
Feb 2015-Oct 2015	Soil fertility researcher at Amhara Regional Agricultural Research Institute (ARARI)
Jan 2012-Jan 2015	Head of soil and water research directorate at Adet Agricultural Research center, ARARI
Jun 2010-Dec 2012	Soil fertility researcher and regional coordinator of acid soil management research in Amhara Region
Nov 2009-Jun 2010	Director of Gambella Regional Agricultural Research Institute
Feb 2009-Oct 2009	soil fertility researcher at Gambella Regional Agricultural Research Institute
Jan 2004-Sep 2006	Head of Agronomy and physiology division at Abobo Agricultural Research Center, Gambella
Feb 2003-Jan 2004	Agronomy researcher at Abobo Agricultural Research Center, Gambella

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**Publications**

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**09/02/2021**

**Date**

**Birhanu agumas -----**

**Name and signature**

**Annex 3**

**Declaration in lieu of an oath on independent work**

**according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences**

1. The dissertation submitted on the topic  
Impact of environmental and socio-economic factors on soil fertility variability and  
microbial carbon use efficiency in tropical smallholder farming systems

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Bahar Day, 09/02/2021

Place, Date

  
\_\_\_\_\_  
Signature