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**Abundance and diversity of total and nitrifying prokaryotes as influenced  
by biochemical quality of organic inputs, mineral nitrogen fertilizer and  
soil texture in tropical agro-ecosystems**

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This work is dedicated to my departed father. Your final words before your last breath are still very fresh in my mind. God has and will continue to honor your wishes towards my education, career and life.



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## Acronyms and abbreviations

ABI = Applied Biosystems

AIC = Akaike information criterion

ANOSIM = Analysis of similarity

ANOVA = Analysis of variance

AOA = Ammonia-oxidizing archaea

AOB = Ammonia-oxidizing bacteria

a.s.l = Above the sea level

°C = Degree celcius

C = Carbon

CA = California, USA

CaNH<sub>4</sub>NO<sub>3</sub> = Calcium ammonium nitrate

CAP = Canonical Analysis of Principal coordinates

CC = *Calliandra calothyrsus*

CIAT = International Center for Tropical Agriculture

cm = Centimetre

COR = Correlations

DAAD = Deutscher Akademischer Austausch Dienst

DISTLIM = Distance-based linear models

DNA = Deoxyribonucleic acid

EC30 = Young plants growth stage

EC60 = Flowering stage.

FAM = Carboxyfluorescein dye

FAO = Food and Agriculture Organization of the United Nations

FastDNA™ SPIN = A Kit that contains all the components needed for isolation of soil DNA.

FSC = Food Security Centre

GDP = Gross Domestic Product

GE = General Electric

GLIMMIX = generalized linear mixed models

GmbH = Gesellschaft mit beschränkter Haftung

ha = Hactare

HEX = Carboxyhexachlorofluorescein dye

HIDI = The Highly Deionized Formamide

HWEC = Hot water extractable carbon

HWEN = Hot water extractable nitrogen

ICRAF = World Agroforestry Centre

LR = Long rains

LSMEANS = Least square means

K = Potassium

Km = Kilometre

m = metre

MA = Massachusetts, USA

MC = Moisture content

MP = Milan Panic the Founder, President and Chief Executive of MP Biomedicals  
N = Nitrogen  
n.s = Not significant  
NC = North Carolina, USA  
NEB = New England Biolabs  
 $\text{NH}_3^-$  = Ammonia  
 $\text{NH}_4^+$  = Ammonium  
 $\text{NO}_3^-$  = Nitrates  
 $\text{N}_t$  = Total nitrogen  
P = Phosphorus  
PDIFF = Pairwise differences  
PERMANOVA = Permutation multivariate analysis of variance  
PRB = Population Reference Bureau  
PROC = Procedure  
QIAGEN = A trade name for a company that provides kits with all components needed for the isolation of genetic materials.  
qPCR = Quantitative Polymerase Chain Reaction  
Q-Q = Quantile plots  
REG = Regression  
RNA = Ribosomal nucleic acid  
ROX = carboxy-x-rhodamine dye  
SAS = Statistical Analysis Software  
SED = Standard error of the difference  
SOC = Soil organic carbon  
SR = Short rains  
SOM = Soil organic matter  
SSA = Sub-Saharan Africa  
SYBR Green = An asymmetrical cyanine dye (Fluorescent DNA binding dye)  
TAMRA = tetramethylrhodine azide  
T4 gene 32 protein = A nucleic acid binding protein that destabilizes the DNA helical structure  
TC = Total carbon  
TD = *Tithonia diversifolia*  
TRFs = Terminal Restriction Fragments  
TRFLP = Terminal Restriction Fragment Length Polymorphism  
USA = United States of America  
U = Units  
WI = Winsconsin, USA  
ZM = *Zea mays*

## **Chapter 1 General introduction**

### **1.1. Persistent soil nutrients mining and proposed measures**

In Africa, particularly in Sub-Saharan Africa (SSA) agriculture and agro-industry sectors form the backbone of economic development. Agriculture contributes to more than 25 % of the Gross Domestic Product (GDP) and is rated the main source of income and employment of at least 65 % of Africa's population (Henao and Baanante, 2006; World Bank, 2012). Consequently, it is directly linked to soil nutrients mining (Sanchez, 1997) through crops harvesting, leaching, erosion or volatilization in form of greenhouse gases (Hai et al., 2009; Kibunja, 2007). Soil degradation has therefore significantly and continuously suppressed soil productivity in SSA region.

The two viable options of increasing crop production are either through intensive production per unit of land or by increasing the area under cultivation (Henao and Baanante, 2006). Nevertheless, SSA has experienced a steady population pressure with up to 2.5 to 4 % growth per annum (Population Reference Bureau (PRB), 2015; World Bank, 2012). This rapid population growth has constrained the arable land as more land has been used for settlements and infrastructure. Consequently, shifting cultivation which was traditionally used to replenish soil fertility is no longer possible. This strategy used to leave the land under cultivation fallow for an adequate period to regain fertility and opening virgin areas. Unfortunately, the disappearance of shifting cultivation has not been matched with sufficient use of inorganic and organic (FAO, 2012) fertilizers to maintain soil quality and productivity. Substantial increases in use of inorganic fertilizers was viewed a viable means to increase crop production per unit of land (Minot and Benson, 2009). However, according to Vanlauwe et al. (2010), inorganic fertilizers should be considered in the framework of integrated soil fertility management (ISFM) approach or in a holistic cropping management approach like conservation agriculture (CA) (FAO, 2008). Nevertheless, this option has been faced with limited quantities of inorganic fertilizers available to small scale farmers, i.e., very low intensity ( $< 10$  nutrients  $\text{kg ha}^{-1}$ ) of use, and slow rates of growth in fertilizer consumption (FAO, 2012). This is mainly due to high poverty levels among smallholder farmers in SSA and high costs of inorganic fertilizers while organic fertilizers face challenges of competing uses like animal fodder, but inadequate manure production in return (Vanlauwe et al., 2010).

Use of organic residues or manures to improve soil fertility is facilitated by the activities of soil microbial communities (Rasche and Cadisch, 2013). There is therefore a need to understand the basic processes underlying the decomposition and mineralization of organic residues by soil microbial communities. This is because microbial mediated processes avail nutrients held in organic to inorganic forms like ammonium ( $\text{NH}_4^+$ ) and nitrates ( $\text{NO}_3^-$ ) that are accessible to plants eventually improving soil productivity (Zhang et al., 2013).

## **1.2. Case studies of integrated soil fertility management approach**

### **1.2.1. Long-term trials in Africa**

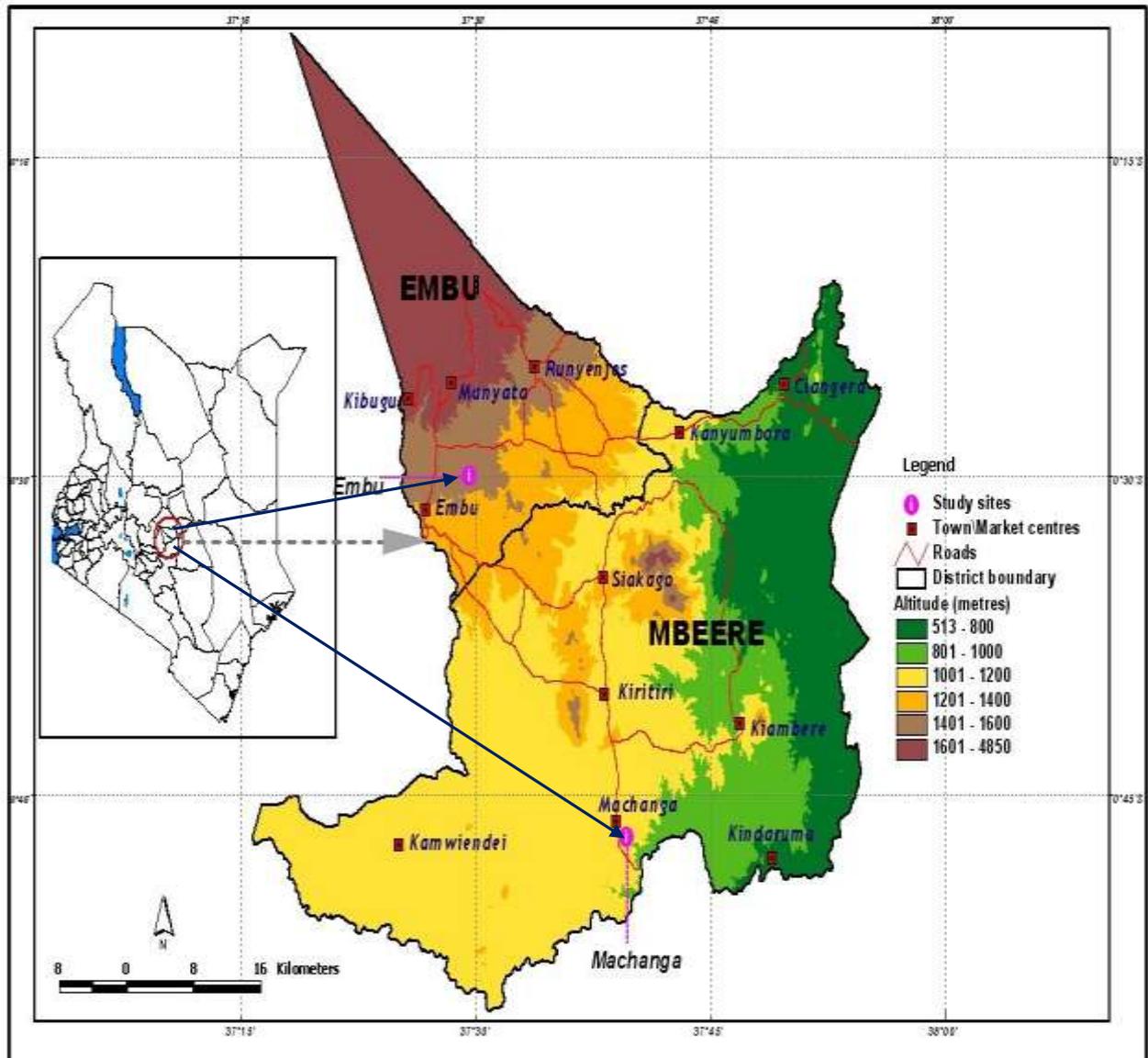
Integrated soil fertility management (ISFM) strategy is defined as application of inorganic fertilizers and organic inputs with improved germplasm combined with knowledge of how to adapt this practice to local conditions (Vanlauwe et al., 2010). The aim is to maximize agronomic use efficiency of the applied nutrients to improve crop productivity. This approach relies heavily on immense contribution of organic resources classification for smallholder farmers earlier developed by Palm et al. (2001b). Accordingly, organic resources were classified either as high, intermediate or low quality. Further details including the management of the mentioned categories are found in section 1.3 of this thesis. In this respect, a long-term (20 years) trial in Burkina-Faso showed a significant improvement in sorghum grain yield and soil organic matter (SOM) accumulation upon combination of low quality sorghum straw residue with urea fertilizer over sole use of either input (Mando et al., 2005). This was attributed to a better synchrony of supply and demand of nutrients by combination of mineral fertilizer and low quality inputs. Low quality inputs slowly decompose and mineralize availing nutrients to plants during demand. This improves crop productivity and prevents nutrient losses via leaching (Chivenge et al., 2009; Gentile et al., 2009; Mtambanengwe et al., 2006). Similar observations on crop grain yields were reported in Niger in a 17 years old trial as well as in Kenya, Kabete after 18 years and Chuka after 6 years (Bationo et al., 2012). These positive interaction effects were also attributed to provision of other nutrients like P and K by residue inputs influencing the uptake of N (Partey et al., 2013; Sanchez and Jama, 2002). In addition, organic inputs influence other physical, chemical or biological properties like soil aggregation that might have positively impacted on soil productivity (Fonte et al., 2009; Kunlanit et al., 2014; Puttaso et al., 2011).

### 1.2.2. Soil organic matter (SOM) trials in Kenya and Zimbabwe

In 2002 ISFM trials were set up in Kenya (Embu and Machanga sites, Fig. 1.1) and in Zimbabwe in two contrasting environments (clayey and sandy soils). The goal of the trials was to investigate the effect of different qualities of organic resources and combination with mineral N fertilizer on management of N availability and losses, soil stabilization and carbon sequestration as well as crop productivity (Chivenge et al., 2009; Gentile et al., 2009; Mtambanengwe et al., 2006). In Zimbabwe, Mtambanengwe et al. (2006) observed up to seven-fold increases in maize grain yields upon the combination of low quality inputs and mineral N compared to sole use of organic residues in the nutrient depleted sandy soil during the first year of the trial. The sandy clay-loam soil on the other hand did not show significant treatment differences in maize grain yields. This could be attributed to the high nutrient levels of the sandy clay-loam compared to the sandy soil (Mtambanegwe et al., 2006), as well as high N losses via leaching associated with high quality inputs masking treatments effects on crop yields (Gentile et al., 2009; Mapfumo et al., 2007).

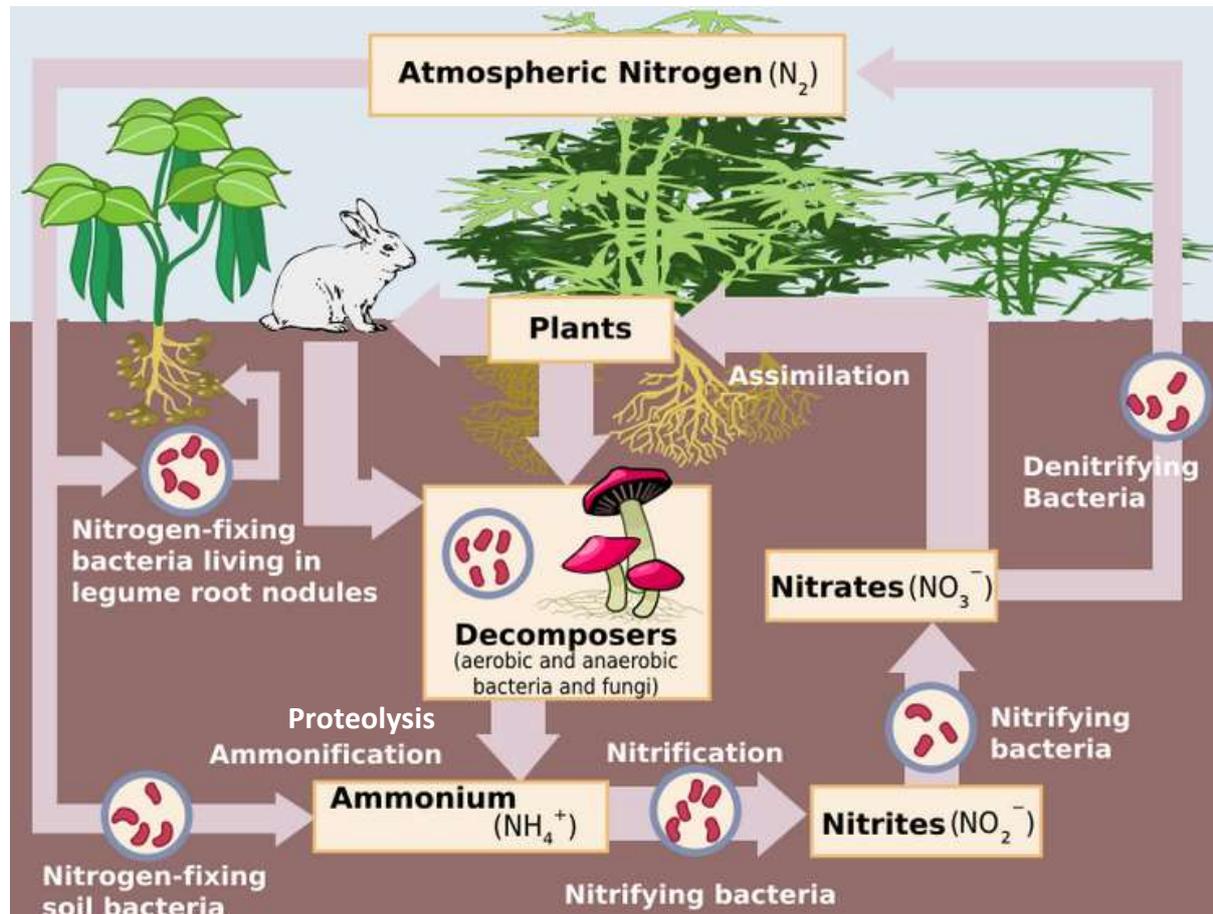
At the Kenyan site, high quality organic inputs (i.e., *Tithonia diversifolia*) and combinations of intermediate (*Calliandra calothyrsus*) and low quality (*Zea mays*) inputs with mineral N averaged across ten seasons increased maize productivity (Chivenge et al., 2009). These findings were attributed to the influence of soil N dynamics by residues quality during the growing season, both when applied alone and in combination with mineral N fertilizer. Combination of low quality maize stover residue with mineral fertilizer reduced N losses and resulted in positive interactive effects on crop N uptake and high maize yields (Chivenge et al., 2009; Gentile et al., 2009; Mapfumo et al., 2007; Mtambanengwe et al., 2006). The reduced N leaching was as a result of induced N immobilization by microbes, followed by slow decomposition and mineralization later in the growing season which coincided with plants demand, thus affecting productivity (Gentile et al., 2009). Provision of inorganic soil nutrients as demonstrated by increased crop productivity was associated with accumulated SOM through continued application of organic inputs. SOM has effects on the physico-chemical functions of the soil since it promotes good soil structure which in turn improves the tilth, aeration, retention of soil moisture as well as increased buffering and exchange capacity of soils (Vanlauwe et al., 2001b). For example, *Zea mays* stover with low N content increased soil aggregate stability, soil organic C and total soil N (Chivenge et al., 2011). Biologically, SOM provide resource substrates for the metabolic activities of soil microbial communities which include decomposition and mineralization of organic inputs. In turn, microbial communities influence

soil structure by their by-products and nutrient fluxes (Lucas, 2013; Six et al., 2004). These multiple benefits to soil resource derived from SOM via the incorporation of organic residues through the activities of microbial communities, coupled with the development of advanced molecular tools, raised the need to understand the interactions between soil microbial communities and organic resources and implications to sustainable soil resource management.



**Figure 1.1:** Map of the study sites, Embu (0° 30' S, 37° 30' E) and Machanga (0° 47' S, 37° 40' E) in Kenya, GIS ICRAF (2007).

### 1.3. Dynamics of microbial communities as shaped by SOM

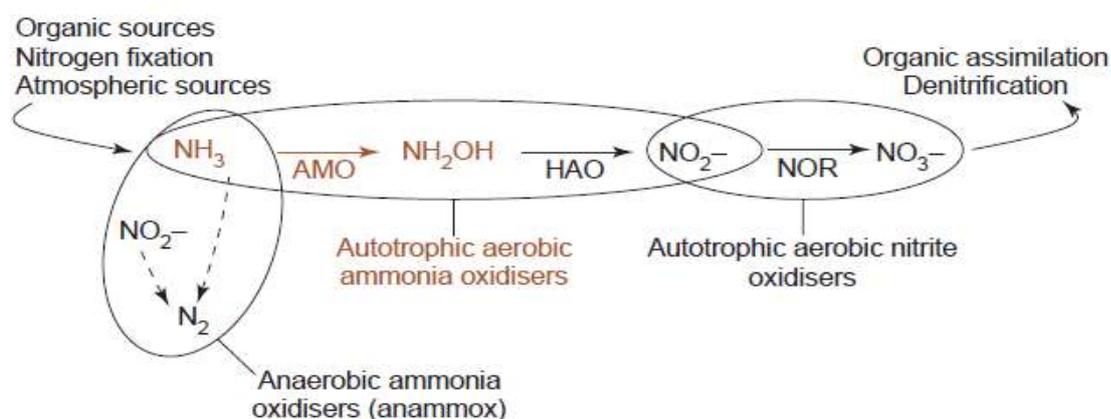


**Figure 1.2:** Schematic representation of the flow of nitrogen through the environment. Microbial communities are key in the cycle, providing different forms of nitrogen compounds.

Source: [https://en.wikipedia.org/wiki/Nitrogen\\_cycle](https://en.wikipedia.org/wiki/Nitrogen_cycle)

Generally, soil microbial communities are catalysts of global C and N cycles during decomposition and mineralization of SOM (Fontaine et al., 2003). This influences fluxes of nutrients like N (Fig. 1.2), SOC as well as greenhouse gases like CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>. SOM therefore act as a habitat for microbial communities which in the event contribute to binding of soil particles into aggregates through the release of their by-products (Chivenge et al., 2011; Fonte et al., 2009; Gentile et al., 2011a, 2011b; Kunlanit et al., 2014). As a result, dynamics of soil microbial communities during SOM decomposition and mineralization has widely been studied. For example, Kamaa et al. (2011) showed contrasting variations in diversity of fungal and bacterial communities between a fallow and farm yard manure treated plots in the tropics. Accordingly, the fallow showed lower fungal but higher bacterial diversity but vice versa for the farm yard manure. Regulation of fungal community abundance and activities by quality of

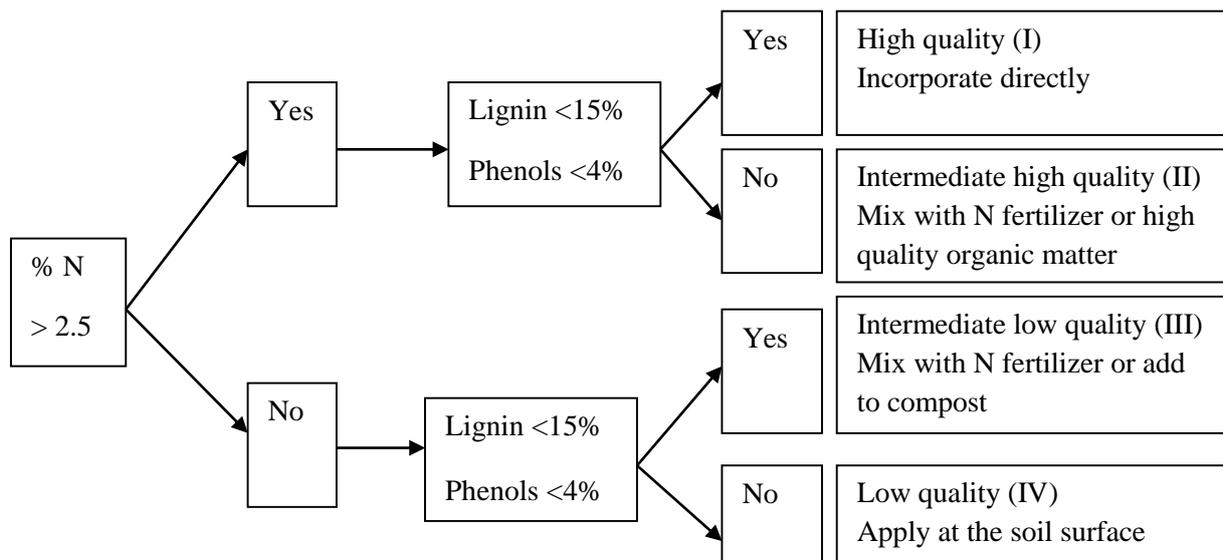
organic residues in a sandy soil in tropics was reported by Kamolmanit et al. (2013). In their findings, the most recalcitrant (high polyphenol content) residues depressed fungal community abundance but promoted the activity of specific functional groups as marked by increased polyphenol oxidase activity. Focusing on N-cycle, Magdalena and Stefania (2011), Mrkonjic et al. (2008), Okur et al. (2009), Rasche et al. (2014) and Sakurai et al. (2007) studied the initial step of N mineralization on different types of organic residues by assessing the dynamics and functions of bacterial proteolytic communities. These authors demonstrated that type of organic resources which intern influence the fluxes of organic C and N in the soil control the dynamics and activities of bacterial communities. Hai et al. (2009) and Hallin et al. (2009) demonstrated manure treatment as the main determinant of nitrogen fixation (*nifH*), nitrate reduction (*narG*) as well as denitrification (*nirK*, *nirS* and *nosZ*) genes abundance compared to cereals straw treatments. Additionally, Chèneby et al. (2010) reported promotion of abundance and alteration of diversity of nitrate reducing communities (*narG* and *napA*) genes with use of wheat, rape and alfalfa residues compared to no input treatment. Several other studies focused on nitrification process (Di et al., 2010, 2009; Levičnik-Höfferle et al., 2012; Rasche et al., 2011; Strauss et al., 2014; Wessén et al., 2010).



**Figure 1.3:** Autotrophic ammonia oxidation during nitrification. Ammonia-oxidizing organisms convert ammonia to nitrite through hydroxylamine using ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Autotrophic nitrite oxidizers subsequently use the enzyme nitrite oxidoreductase (NOR) to convert nitrite to nitrate, which can be assimilated, lost through leaching or subjected to denitrification processes. In anaerobic environments, ammonia can be converted to molecular nitrogen by the ‘anammox’ process by several enzymatic steps (represented by dashed arrows).

**Source:** Hyman and Arp (1992), Nicol and Schleper (2006).

Emphasizing on ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities by focusing on ammonia monooxygenase (AMO) (Fig.1.3), these authors showed distinct responses of AOB and AOA to organically mineralized and fertilizer N. SOM therefore regulates biogeochemical soil processes via the activities of microbial communities with consequent impact on soil productivity. The complexity and variability of SOM influence biogeochemical processes by modifying specific microbial communities. Its wide range includes green manures, crop and plant residues as well as animal remains (Fig.1.2). These make it a substance of different qualities due to variations in biochemical compounds like cellulose, hemicelluloses, starches, proteins, lipids and polyphenols consequently influencing rates of decomposition (Kunlanit et al., 2014; Kononova, 1966; Palm et al., 2001b; Partey et al., 2013). The biochemical properties mainly the organic N (proteins), lignin and polyphenol contents as well as the recently reported cellulose create interspecific differences and play a major role in regulation of N dynamics in soils (Kunlanit et al., 2014; Palm et al., 2001b; Partey et al., 2013). In this regard, a decision guide manual was developed for organic N management in tropical agro-ecosystems (Palm et al., 2001b). The organic materials were differentiated into four quality classes (I to IV) (Fig.1.4). Resources containing high lignin and polyphenol contents (intermediate high quality) classified as class II, induce N limitation through the formation of protein-polyphenol complexes (PPCs) which are resistant to microbial decomposition (Kraus et al., 2003; Millar and Baggs, 2004; Talbot and Finzi, 2008). In contrast, high quality resources with high N content but low lignin and polyphenol contents (class I) are rapidly decomposed and mineralized. Organic nutrients thus released, particularly  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are either available for plants uptake, lost through leaching in the case of  $\text{NO}_3^-$  or immobilized by the microbes (Kaewpradit et al., 2008; Kanerva et al., 2006).



**Figure 1.4:** Decision tree for biomass transfer of plant materials for soil fertility management. **Source:** Palm et al. (2001b).

Vanlauwe et al. (2005) and Kaewpradit et al. (2008) confirmed the potential of *Tithonia diversifolia* and groundnut residues classified as high quality resource to rapidly release mineralized N particularly at the beginning of the vegetation period. An intermediate-high quality resource, (*Calliandra calothyrsus*), was proved to slowly release N during decomposition, which is however available for plants uptake over a vegetative growth season including the succeeding crop (Cadisch et al., 1996). Such a delayed N release is associated with formation of PPCs which inhibit immediate microbial decomposition and consequent mineralization (Millar and Baggs, 2004; Mutabaruka et al., 2007; Partey et al., 2013; Talbot and Finzi, 2008). Maize stover is composed of low N, lignin and polyphenol contents (Chivenge et al., 2011; Gentile et al., 2011; Shepherd et al., 2003; Tian et al., 1995), falling under intermediate-low quality group (Palm et al., 2001b). Intermediate-low quality materials have N immobilization similar to the intermediate-high quality organic materials. However, maize stover was recently revealed to increase microbial abundance of some specific groups compared to *Calliandra calothyrsus* (Muema et al., 2015). This observation was associated with the high cellulose content in maize stover which is accessible to microbial decomposition (Kunlanit et al., 2014; Puttaso et al., 2011). Consequently, maize stover increased soil aggregate stability, soil organic carbon (SOC) and N compared to the intermediate-high quality inputs (Chivenge et al., 2011). Therefore, high quality inputs were proposed to be used as substitutes, while the intermediate and low quality resources as complements to mineral fertilizers (Partey et al., 2013; Palm et al., 2001a, 2001b). From this overview, it is clear that

quality of organic resources and mineral fertilizers have impacts on soil resources in several aspects. Firstly, by improving soil productivity as proved by increased crop grain yields (Chivenge et al., 2009; Mtambanegwe et al., 2006; Partey et al., 2013). Secondly, by influencing soil C and N dynamics (Chivenge et al. 2011; Gentile et al., 2011a, 2011b) and finally these inputs influence dynamics of microbial communities (Chèneby et al., 2010; Hai et al., 2009; Kamolmanit et al., 2013; Rasche et al., 2014). However, the effects of integration of quality of organic inputs, mineral N fertilizer, seasonality and soil texture on nitrogen cycle prokaryotes in arable tropical conditions particularly in SSA is not fully understood.

#### **1.4. Environmental factors affecting N-cycling microbial communities**

##### **1.4.1. Nutrient substrates**

N-cycling microbial communities have revealed contrasting responses to N addition in soils depending on N source, whether organic or inorganic. For example, Hallin et al. (2009) demonstrated a reduction of *narG*, *nirK*, *nirS* and *nosZ* genes abundance by ammonium fertilizer compared to cattle manure. N fixation (*nifH*) and organic N mineralization (*chiA*) genes showed contrasting responses to N fertilizer availability. Specifically, these genes showed high competitiveness when N was limiting, increasing in abundance but their abundance decreased when N was high due to reduced competitiveness (Zhang et al., 2013). Chèneby et al. (2010) reported a promotion of abundance and changes in diversity of nitrate-reducing community (*narG* and *napA*) genes with N addition from wheat, rape and alfalfa residues compared to no input treatment. In another study, variations in the amount of organic N content in different organic residues had different impacts on fungal community abundance in a sandy soil. Accordingly, high and low quality inputs showed significantly lower fungal abundance compared to the intermediate quality inputs (Kamolmanit et al., 2013). Several other studies have also reported promotion of AOB by high levels of  $\text{NH}_3$  (de Gannes et al. 2014; Jia and Conrad, 2009; Levičnik-Höfferle et al. 2012; Schleper, 2010; Zhalnina et al., 2012; Zhang et al., 2013). On the other hand, mixed reactions exist concerning the influence of  $\text{NH}_4^+$  on the growth or activities of AOA. For example, Di et al. (2009) and Tourna et al. (2010) reported inhibition of AOA nitrification under high  $\text{NH}_4^+$  concentrations. On the contrary, Treusch et al. (2005) reported growth and activity of AOA in soil to be promoted by high  $\text{NH}_4^+$  concentration. Moreover, substrate concentrations (i.e., low, medium or high) levels of  $\text{NH}_3$  were reported not to influence the growth or activity of AOA (Di et al., 2009; Ke and Lu, 2012; Stopnišek et al, 2010; Verhamme et al., 2011). These contradictions were associated with a

high affinity of AOA for  $\text{NH}_3/\text{NH}_4^+$  (de Gannes et al. 2014; Levičnik-Höfferle et al. 2012; Martens-Habbena and Stahl, 2011; Martens-Habbena et al., 2009). This observation suggests that AOA can obtain their energy even under low levels of substrate, masking the effects of  $\text{NH}_3$  concentrations. Sources of  $\text{NH}_3$  or  $\text{NH}_4^+$  have also been reported to greatly influence both AOB and AOA (Jia and Conrad, 2009; Zhang et al., 2010). In this respect, while AOB are highly promoted by mineral N, AOA have been shown to thrive best on organically mineralized  $\text{NH}_4^+$  (Di et al., 2009; Hofferle et al., 2010; Ke and Lu, 2012; Stopnisek et al., 2010; Verhamme et al., 2011).

#### **1.4.2. Soil pH**

The influence of soil pH on N-cycling communities is linked to  $\text{NH}_3$  availability in soils (Nicol et al., 2008; Zhalnina et al., 2012). Reduction in soil pH was found to reduce the abundance of *nifH*, *chiA*, *nirS*, *nirS* and *nosZ* genes (Hallin et al., 2009; Zhang et al., 2013). AOA have high affinity for  $\text{NH}_3$  and that is why they dominate acidic soils compared to AOB (Gubry-Rangin et al., 2010; Martens-Habbena et al., 2009; Nicol et al., 2008). As for AOB, their low affinity for  $\text{NH}_3$  explains why their abundance and activities are low in acidic soils as  $\text{NH}_3$  is limiting (de Boer and Kowalchuk, 2001; Zhalnina et al., 2012). Previous studies have reported increased abundance and activity of AOA in acidic (pH = 3.6 - 4.0) soils (Leininger et al., 2006; Gubry-Rangin et al., 2010; He et al., 2007; Zhang et al., 2011; Isobe et al., 2012), while increase in pH increased AOB abundance and its activities (Che et al., 2015; Nicol et al., 2008). Moreover, Shen et al. (2008) reported a positive correlation for AOB with soil pH. On the other hand, Yao et al. (2011) revealed AOA driven nitrification compared to AOB in the acidic tea grown soils. Nevertheless, AOA have also been reported in alkaline soils (Bates et al., 2011; Bru et al., 2011; Shen et al., 2008; Wessén et al., 2010; Zhang et al., 2010). However, it is clear that AOB dominate and are more active in alkaline conditions while AOA dominate and drive nitrification in acidic conditions.

#### **1.4.3. Soil moisture and temperature**

Soil moisture content and temperature are critical in N-cycle processes like SOM decomposition and mineralization (Belnap, 2001; Kirschbaum, 1995; Norton and Stark, 2011; Zheng et al., 2000; Zhang et al., 2013). Soil water is a medium upon which nutrients are transported while temperature is critical in controlling microbial enzymatic activities. Heavy precipitation can cause leaching of  $\text{NO}_3^-$  which could lead to a decrease in denitrifiers. However, wetting of soils could potentially buffer acidification effects of N additions and promote denitrifiers. Moreover, the buffered soil pH combined with increased watering can

create an anaerobic environment which could stimulate denitrifiers (Zhang et al., 2013). Soil moisture content was reported to control the activities of proteolytic communities i.e., neutral metalloproteases and serine proteases (npr; sub) genes with accelerated activities recorded in autumn compared to summer (Mrkonjic et al., 2009, 2008). Decreased potential activity of proteolytic activity with reduction in soil moisture content was also observed by Hofmockel et al. (2010) and Sardans and Penuela (2005). Rasche et al. (2011) revealed dynamics of N-cycle prokaryotes groups as shaped by seasonality in a beech forest in temperate regions. For example, AOB increased with increased moisture content while AOA increased with decreased temperatures. Similarly, AOB and AOA were reported to bear different responses to different water filled pore spaces (WFPS) and temperature with AOB being much more sensitive than AOA (Avrahami and Bohannan, 2007; Gleeson et al., 2010; Szukics et al., 2010). Accordingly, 65 % was the approximate optimum WFPS for AOB while AOA remained rather stable over a wide range from 25 to 95 %. In addition, AOB were abundant and active at 20-25 °C while AOA were active in low temperatures (< 15 °C) but reduced at temperatures greater than 25 °C. Szukics et al. (2012) reported high abundance of AOA in dry soil condition (i.e., 40 %) WFPS compared to wet soil condition (70 %) WFPS. Angel et al. (2010) found differences in composition of archaeal and bacterial communities between arid, semiarid and Mediterranean regions which were associated to differences in precipitation.

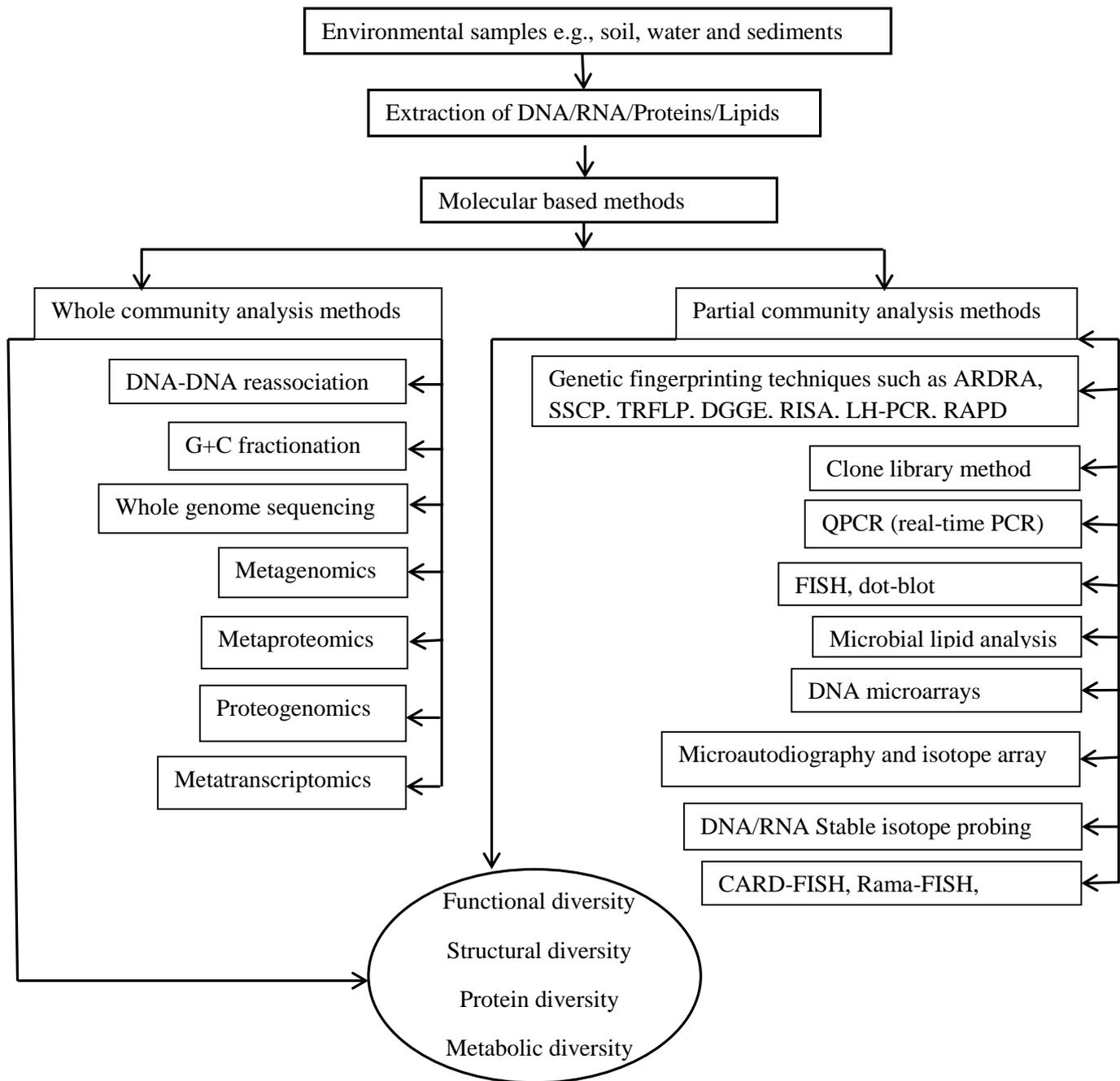
#### **1.4.4. Soil texture**

Soil texture is a physical property described by proportions of different solid fractions (clay, silt, sand and organic matter) and provide microbial habitats (Garbeva et al., 2004). Variations in these fractions particularly the organic matter is responsible for the fertility and productivity of soil resource as an ecosystem (Chivenge et al., 2009; Mtambanengwe et al., 2006; Rasche and Cadisch, 2013). This is due to the ability of SOM to provide nutrients to crops and microorganisms as well as regulation of soil aggregation (Bossuyt et al., 2001; Chivenge et al., 2011; Fonte et al., 2009; Gentile et al., 2011a, 2011b). For example, high abundance and activities of proteolytic genes were measured in the clayey soil associated with high organic C and N compared to the sandy soil in southern Germany (Mrkonjic et al., 2009, 2008). In addition, proportions of mineral fractions regulate other factors like O<sub>2</sub> levels. For instance, high clay content has a likely hood of reducing O<sub>2</sub> levels favoring AOA compared to AOB (Morimoto et al., 2011). Evidence has shown that soil type is an important determinant of microbial populations and composition in the bulk and rhizosphere soils. For example, Gelsomino et al. (1999) showed distinct variations in patterns of bacterial profiles across 16

different types of soils of the Netherlands using denaturing gradient gel electrophoresis technique (PCR-DGGE). These findings revealed soil type as a determinant of bacterial structures and that similar soil types contained similar communities. Girvan et al. (2003) observed soil type as the main driver of separation of total bacterial and functional communities over management practices. Clayey soil texture promoted the composition of total bacterial communities compared to soil organic amendments (Sessitsch et al., 2001). Rasche et al. (2014) determined distinct differences in abundance and composition of total and proteolytic bacteria between clayey and sandy textured soils. In addition, soil texture dependent responses of total and ammonia-oxidizing prokaryotes were reported by Musyoki et al. (2015) and Neumann et al. (2013). Clayey soils have a high SOC background and a strong soil structure particularly protecting freshly incorporated SOM from microbial access whereas sandy soils are generally nutrient limiting and have a coarse soil structure which exhibit less protection to SOM ( Kögel-Knabner et al., 2008; Puttaso et al., 2011). These soil type specific properties result in distinct responses of specific microbial communities (**chapter four** of this thesis, Musyoki et al., 2015; Rasche et al., 2014).

### **1.5. Molecular approaches to assess microbial communities**

Culture-based approaches are vital in microbial ecology but are limited since they present < 1% of the total number of microbial species present in any given sample (Hugenholtz, 2002). In addition, cultivable microbial species are numerically hardly abundant or functionally significant in environments they are cultured (Rastogi and Sani, 2011). This limitation has been overcome by development of molecular techniques which are able to assess the uncultured but viable microbial species based on direct isolation and analysis of nucleic acids, proteins and lipids from environmental samples including soil and water sediments. These approaches are able to reveal community structures, functions and dynamics of microbial communities and help to understand their interactions with biotic and abiotic environmental factors (Rastogi and Sani, 2011). Overall, these techniques are classified into two main categories following their capacity to portray microbial structure, function and diversity as follows: 1) whole community analyses approaches and 2) partial community analyses approaches (Fig. 1.5).



**Figure 1.5:** Culture-independent molecular toolbox to characterize the structural and functional diversity of microorganisms in the environment. **Source:** Rastogi and Sani (2011).

### **1.5.1. Whole community analysis approaches**

These techniques are based on analysis of whole-genome of microbial communities present in the total DNA extracted from an environmental sample (Rastogi and Sani, 2011). They thus overcome the limitation of use of 16S rRNA genes (partial analysis techniques) which is highly conserved and therefore has limited resolution at species and strain level (Konstantinidis et al., 2006). PCR-based approaches also target single or few genes limiting the genetic information contained in the isolated total DNA. Whole community molecular analyses approaches are however more expensive compared to partial analysis techniques. Different approaches used for whole community studies are shown in figure 1.5 and their descriptions are provided by Rastogi and Sani (2011) and Singh et al. (2010).

### **1.5.2 Partial community analysis approaches**

These techniques employ polymerase chain reaction (PCR)-based approaches such that DNA/RNA isolated from environmental samples is used as a template for characterization of microbes. Basically, PCR generated product is a mixture of microbial genes signatures present in a sample including those viable but non-cultured (VBNC) proportion (Rastogi and Sani, 2011). The advantages of these methods are that the conserved region (16S rRNA) used for PCR amplification is: i) present in all prokaryotes, ii) structurally and functionally conserved, and iii) contain variable highly conserved regions (Hagenholz, 2002). Specific methods used are shown in figure 1.5 and further descriptions can be retrieved from Rastogi and Sani (2011).

#### **1.5.2.1 Principle of qPCR**

Quantitative PCR (qPCR or real-time PCR) is a standard PCR with the advantage of detecting the amount of DNA formed after each cycle with either fluorescent dyes or fluorescently-tagged oligonucleotide probes. This technique is used to measure the abundance and expression of taxonomic and functional gene markers (Bustin et al., 2005; Smith and Osborn, 2009). The quantity of the detected DNA can either be an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. Its procedure follows the principle of PCR but its main feature is that, the amplified DNA is detected as a reaction progresses in real time. Two common methods for detection of products in real-time PCR are: i) non-specific fluorescent dyes that intercalate with any double stranded DNA (SYBR green-based), and ii) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only after hybridization of the probe with

its complementary DNA target (probe-based) (Rastogi and Sani, 2011). QPCR technique is robust, highly reproducible and sensitive.

### **1.5.2.2 Principle of terminal restriction fragment length polymorphism (TRFLP)**

TRFLP is a rapid fingerprinting technique used for studying diversity, structure and dynamics of microbial communities (Rastogi and Sani, 2011; Smaller et al., 2007). It is a method that profiles mixed populations of a homologous amplicon which is a diverse sequence of a single gene. Isolated DNA from environmental sample is amplified using fluorescent universal or specific primers (Liu et al., 1997; Schütte et al., 2008). The amplified PCR products are digested using restriction enzymes. Labelled terminal fragments are detected and sized by capillary with automated DNA sequencer or using gel electrophoresis. One of the primers is labeled at a 5' terminus with a fluorescent dye, so that the terminal restriction fragments (TRFs) of the digested amplicon can be detected and quantified by the automated DNA sequencer (Liu et al., 1997; Schütte et al., 2008). From the DNA sequencer electropherograms are generated (i.e., the visual profile of the community). Simultaneously, digital data in a table format is generated whereby in principle, peak heights and areas are representative of community abundance. In TRFLP technique, results are reproducible and it is a relatively faster way of monitoring community dynamics which produces digital data that can be used for further analyses e.g cluster analysis, calculation of diversity indices, and correlations with environmental data like soil chemical properties. TRFLP data can also be linked to clone libraries (Deutzmann *et al.*, 2002). However, this technique is challenged with underestimation of microbial populations through the following challenges;

i) Choice of primers. This is so because although primers may potentially amplify relatively high microbial 16S rRNA gene sequences in Ribosomal Database Project (RDP), this does not take into account that the sequence databases contain only a fraction of the surviving/extant bacterial diversity. Labeling of one primer underestimates the microbial diversity in a sample because different bacterial populations can share the same terminal restriction fragment length for a particular enzyme combination. This problem can be reduced if the two primers are labelled.

ii) Choice of restriction enzymes. Use of one restriction enzyme can underestimate the microbial diversity because different bacterial populations can share the same terminal restriction fragment length for a particular primer-enzyme combination. This can be resolved by using more than one restriction enzyme to facilitate resolution of microbial population.

iii) Differences in migration speed of labeled DNA as a result of differences in molecular weights of different labeling dyes (Pandey et al., 2007; Schütte et al., 2008). Different molecular weight can modify the mass of individual DNA fragments which may lead to differential migration. For example fluorescent dye like carboxyfluorescein (FAM) is lighter than carboxyhexachlorofluorescein (HEX). This means that DNA labeled with FAM can migrate faster than DNA labeled with HEX. Internal standard dyes such as tetramethylrhodine (TAMRA) azide, carboxyl-x-rhodamine (ROX) also have different molecular weights. TRFs from lighter labeling dyes could thus be underestimated since it is not easy to adjust for differences as the magnitude of discrepancy is not constant across fragment sizes (Pandey et al., 2007; Shütte et al., 2008).

### **1.5.2.3 Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE)**

For DGGE, DNA is amplified using primers for specific molecular marker (e.g., 16S rRNA gene) and electrophoresed on a polyacrylamide gel containing a linear gradient mixture of chemical denaturants (e.g., urea and formamide) (Muyzer et al., 1999, 1993). The same applies for TGGE but instead a temperature rather than chemical gradient is used. Since fragment sequence differences dictate the melting point of a sample DNA, both techniques are able to separate DNA fragments of the same size but different sequences depending on their melting points. They use a 5'-GC clamp (30-50 nucleotides) forward primer during the PCR step which prevents two DNA strands from complete dissociation into single strands during electrophoresis (Rastogi and Sani, 2011). Their disadvantages include: i) limited sequence information (< 500 base pairs (bp)) obtained for phylogenetic analysis from DNA bands, ii) possibility of different DNA fragments sharing similar melting points, iii) several varied DNA fragments, which can be separated by polyacrylamide gel electrophoresis, and iv) sequence heterogeneity between multiple rRNA eperons of single bacterium can lead to multiple bands causing overestimation of the diversity. More fingerprinting techniques are discussed in Rastogi and Sani (2011).

Studies that have compared TRFLP and DGGE techniques provide a consistent evidence that both approaches give similar outputs (Enwall and Hallin, 2009; Smalla et al., 2007), suggesting the two approaches to be alternatives. The choice of TRFLP approach for this study therefore was based on the availability of the equipment at the laboratory, the large sample size and the desire to broaden knowledge to other approaches as was already conversant with DGGE

technique. In my own experience however, DGGE demands extra care when particularly handling the polyacrylamide gels as they are very fragile.

### **1.5.3. Limitations and proposed measures of molecular community analysis techniques**

- 1) Biases during DNA extraction. According to Feinstein et al. (2009), this setback could be overcome by isolation of DNA using several validated protocols and pooling it together prior to PCR performance.
- 2) Biases encountered during PCR like inhibition of PCR by co-extracted humic acids. Purification of DNA should be performed although it may lead to loss of DNA during purification. Dilution of DNA can be applied although also interferes with PCR efficiency.
- 3) Efficiency of hybridization and primers specificity can cause preferential amplification of certain templates interfering with the quantitative assessment of microbial diversity.
- 4) Formation of PCR artifacts like primer dimers may also provide misleading results. Therefore primer pairs that generate primer-dimers should be avoided. For the qPCR technique, extensive optimization of primer concentrations especially with the SYBR green fluorescent dye should be conducted to ensure that only the target product is formed. In addition, melting curve (post-PCR dissociation) analysis should be carried out to confirm that fluorescence signals generated are from the target templates but not from non-specific amplifications.

### **1.6. Focus of current work and link to food security**

The role of microbial communities in decomposition and mineralization processes underscores their importance in nutrient recycling and geochemical processes which governs the overall functioning and health of soils. This places a need to understand at a community level the spatial and temporal variability of microbial populations in response to agricultural practices. The emphasis of ISFM strategy particularly in SSA to increase soil productivity in terms of grain yields (Vanlauwe et al., 2010) therefore laid the foundation for this work. This was due to the use of organic inputs locally available to smallholder farmers in addition to inorganic fertilizers to replenish soil nutrients. Importantly, N is one of the macronutrients whose inadequate supply in soils limits crops growth and productivity (Ågren et al., 2012). Its adverse effects have however, mainly been evident in the old weathered soils of the tropics (Bationo et al., 2012). This drove my interest to understand at a community level the dynamics of nitrogen cycle prokaryotes specifically those involved in the nitrification process, as this has not been

reported in Kenya. Nitrification is a two-step process in nitrogen cycle whereby ammonia ( $\text{NH}_3$ ) is oxidized to nitrite ( $\text{NO}_2^-$ ) and eventually nitrates ( $\text{NO}_3^-$ ) which are available to microorganisms and plants (Petersen et al., 2012; Zhang et al., 2013). Nitrification is catalyzed by the activities of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities (Arp et al., 2002). Ammonia oxidizers' critical role in N regulation has been underscored (Di et al., 2010, 2009; Erguder et al., 2009; Jia and Conrad, 2009; Leininger et al., 2006; Musyoki et al., 2015; Prosser and Nicol, 2008, 2012; Schleper, 2010; Schleper et al., 2005; Zhahnina et al., 2012; Zhang et al., 2010). In addition, reports have shown the associated improved soil productivity through increases in grain yields upon incorporation of organic resources either solely or in combination with inorganic fertilizers particularly in nutrients limited soils (Chivenge et al., 2009; Mapfumo et al., 2007; Mtambanengwe et al., 2006). On the basis of the prior introduced medium to long-term SOM trials established in Kenya in 2002, the abundance and composition of total and ammonia-oxidizing prokaryotes governing the decomposition and mineralization of SOM were assayed in two contrasting environments.

### **1.7. Problem statement and justification**

Soil degradation remains a topical issue which negatively affects food security particularly in SSA where population pressure is constantly increasing while the land factor remains constant or even reducing with increased infrastructure. With regard to the two proposed mechanisms of improving soil productivity, i.e. increasing the land area under cultivation or by intensive production from the already existing arable land, the latter has widely been adopted. From the presented overview, the significance of the ISFM strategy with specific emphasis on the role of the biochemical quality of organic resources in improving crop productivity has been confirmed (Bationo et al., 2012; Chivenge et al., 2009; Gentile et al., 2009; Mando et al., 2005; Mapfumo et al., 2007; Mtambanengwe et al., 2006; Partey et al., 2013). Moreover, its role in improving soil aggregation, chemical and biological soil properties by controlling dynamics of SOC and SON was reported (Bossuyt et al., 2001; Chivenge et al., 2011; Fonte et al., 2009; Gentile et al., 2011a, 2011b; Kunlanit et al., 2014; Puttaso et al., 2001, 2013; Samahadthai et al., 2010; Six et al., 2006). The biological properties as indicators of soil quality have been extended to studying the dynamics of soil microbial communities (Hai et al., 2009; Jia and Conrad, 2009; Musyoki et al., 2015; Wessén et al., 2010; Rasche et al., 2014). In addition, abiotic factors that affect populations and activities of soil microbial communities like soil pH, soil moisture content and temperature as well as soil texture have been discussed (Che et al.,

2015; Girvan et al., 2003; Szukics et al., 2012, 2010; Yao et al., 2011; Zhelnina et al., 2012). Nevertheless, most of field reports on soil processes through the study of the dynamics of soil microorganisms at a community level as shaped by the biochemical quality of organic inputs and inorganic fertilizers particularly in tropical arable systems have been based on one occasion or single season soil sampling (Hai et al., 2009; Rasche et al., 2014). Since the tropics are characterized by erratic rainfall between seasons within and across years, it is still not well understood, and has not been reported in the framework of ISFM strategy in SSA, how seasonality shapes the effects of quality of organic resources on dynamics of microbial communities at the community level. Hence, consideration of two consecutive years (2012 and 2013) during long rains seasons in SOM field trials (that were established in Kenya in 2002 in the framework of ISFM strategy) was a unique factor in this study. Soil samplings were conducted prior to the incorporation of soil inputs and after soil amendments at distinct maize growth stages in two contrasting soil textures (clayey and sandy). This was vital to distinguish the effect of the buildup of organic matter over time from that of freshly incorporated organic inputs on dynamics of soil microbial communities. Accordingly, generated data were used to firstly understand how 10 years of continuous application of different quality organic resources, to restore the depleted SOM and provide nutrients to crops, shaped the abundance and diversity of nitrogen cycling prokaryotes. Secondly, the dynamics of microbial communities in response to interactions between seasonality and quality of organic inputs given the competition of nutrients from the crops, were elucidated. The third component considered the interrelations of soil texture and quality of organic residues on dynamics of soil microbial communities. This knowledge is critical in assessing soil quality as well as understanding the dynamics and functions of the soil ecosystem. Such information can be used to sensitize key stakeholders like farmers and particularly policy makers to incorporate the aspect of organic fertilizers in decision making for site-adapted management practices which would ensure improved food production.

## 1.8. Hypotheses

1. Continuous application of high quality organic inputs, due to their high content of organic N whose decomposition releases high amounts of  $\text{NH}_4^+$  as opposed to low (limited N) and intermediate (high polyphenol and lignin contents induced N limitation) quality inputs, promotes the abundance and diversity of total bacterial and archaeal as well as ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities.
2. Combination of mineral N fertilizer with low and intermediate quality inputs promotes the abundance and diversity of total and nitrifying prokaryotes because of its compensation effect for the limiting N. This is facilitated through the direct provision of  $\text{NH}_4^+$ , a source of energy for AOB and AOA, as well as a source of N for N-demanding processes of total microbial communities (e.g., protein synthesis).
3. Increased microbial abundance is expected under high quality inputs at young plants growth stage (EC30) due to the faster decomposition of organic N leading to a high availability of  $\text{NH}_4^+$  as opposed to intermediate quality inputs with high polyphenol and lignin contents and low quality inputs with low organic N contents inducing  $\text{NH}_4^+$  limitation. On the other hand, a reduced microbial abundance at EC60 with high quality inputs was presumed due to negligible remnants of decomposable organic N as compared to the intermediate and low quality inputs with their delayed and thus gradual decomposition of N.
4. A clayey soil, regardless of quality of organic inputs, promotes the abundance and diversity of total and ammonia-oxidizing communities due to its native nutrient rich status. This is specifically because of the availability of organic N derived from the strong soil organic matter background, a source of substrates for soil microorganisms. In addition, the hypothesized high microbial abundance is supported by clay-sized soil particles providing a large surface area for microbial and residue attachment. A sandy soil, on the other hand, promotes the abundance of microbial communities and alters their composition upon application of high quality inputs early in the growing season. This is due to the high content of accessible decomposable organic N in high quality inputs availing  $\text{NH}_4^+$ . Similar responses of microbial communities were expected for the intermediate and low quality inputs but later in the season as a result of their gradual N decomposition. Such expectations were anticipated since sandy soils are natively nutrient limited and characterized by a coarse soil texture rendering less protection of organic inputs to microbial access.

## **1.9. Objectives**

### **1.9.1. Overall objective**

The overall goal of this work was to understand the impact of long and short-term use of organic and inorganic inputs on the population dynamics of total and functional microbial communities involved in the nitrification process in two contrasting environments in the tropics.

### **1.9.2. Specific methodological objectives**

1. To study the effects of continuous application of different quality organic inputs and their combinations with mineral N on the DNA-based functional potential abundance and diversity of total bacteria and archaea as well as AOB and AOA after 10 years.
2. To study the dynamics of total bacterial and archaeal as well as AOB and AOA DNA-based functional potential abundance as shaped by the biochemical quality of organic inputs combined with mineral N fertilizer at young growth (EC30) and flowering (EC60) stages of maize during two long rain seasons.
3. To determine the response of abundance (DNA-based functional potential abundance) and diversity of total bacterial and archaeal as well as AOB and AOA communities to the interactions between quality of organic inputs and soil texture (clayey and sandy).

### **1.10. Structure of the thesis**

This thesis is composed of five chapters. Chapter 1 provides a general overview of soil fertility depletion in Africa and suggested options which are linked to microbial mediated processes on SOM turnover. It introduces organic inputs quality and inorganic soil inputs which are components of integrated soil fertility management technology (ISFM). Chapter 2 reports on impacts of long-term (10 years) application of biochemically contrasting organic resources and their combination with mineral nitrogen (N) fertilizer on the abundance and composition of total and ammonia-oxidizing prokaryotes using quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (TRFLP) techniques respectively in a clayey soil in Kenya. Chapter 3 discusses dynamics of the abundance of total and nitrifying prokaryotes as influenced by quality of organic inputs and mineral N fertilizer at distinct crop growth stages over two consecutive seasons in a clayey soil in Kenya. This was vital to understand the short-term effects of freshly incorporated organic inputs on dynamics of microbial decomposer communities. It was also crucial to understand the input quality seasonality related effects on microbial communities. Chapter 4 investigates the effects of interrelations of soil type (clayey and sandy textures) and quality of organic residues on the abundance and composition of ammonia-oxidizing bacteria (AOB) and archaea (AOA). Such knowledge is useful to develop site adapted organic management options as tropics are characterized by varied and distinct soil types. Chapter 5 forms the general discussion. Here, an overview and feedback on implications of long and short-term application of organic and inorganic inputs in consideration of distinct soil textures is reported on the following aspects; i) soil quality and sustainability, ii) dynamics of soil microbial communities and soil productivity, and iii) future directions. This thesis proceeds with a summary in English and a German translation with additional information in the appendices.

## **Chapter 2 Response of ammonia oxidizing bacteria and archaea to biochemical quality of organic inputs combined with mineral nitrogen fertilizer in an arable soil<sup>1</sup>**

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## Abstract

There exists a considerable knowledge gap about the effect of biochemical quality of organic inputs and their combination with inorganic N on abundance and community composition of ammonia-oxidizing archaea (AOA) and bacteria (AOB). Here, we investigated in a Humic Nitisol of 10-year old field experiment in Kenya the effect of contrasting organic inputs (i.e., *Tithonia diversifolia* (TD; C/N ratio: 13, lignin: 8.9 %; polyphenols: 1.7 %), *Calliandra calothyrsus* (CC; 13; 13; 9.4) and *Zea mays* (ZM; 59; 5.4; 1.2)); rate of 4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) combined with mineral N fertilizer (120 kg CaNH<sub>4</sub>NO<sub>3</sub> ha<sup>-1</sup> growing season<sup>-1</sup>) on *amoA* gene-based abundance (i.e., functional potential) and community composition of AOB and AOA. AOB abundance was significantly lower in CC and ZM compared to TD, whereas AOA abundance was significantly lower in CC compared to ZM and TD. This reduction was attributed to a considerable N stress induced by limited organic N availability in ZM and polyphenol-protein complexes in CC. High abundance of AOA under ZM was attributed to their affinity to ammonium under N limiting conditions. Abundance shifts matched observed community composition differences between TD versus ZM (AOB) as well as TD versus ZM and CC (AOA). Mineral N irrespective of organic input type depressed abundance of AOA, but not AOB. This implied utilization of ammonium from fertilizer and organic N by AOB, while AOA mainly utilized ammonium from organic N. Our findings suggested input type dependent effects on AOB/AOA abundance and community composition, but influence of other factors such as soil type, seasonality and crop growth stages remain uncertain. These factors should not only be studied on basis of the functional potential of ammonia oxidizing prokaryotes as given in this study, but also by rRNA analyses to capture the active proportion of existent AOB and AOA.

**Keywords:** Ammonia-oxidizing bacteria; Ammonia-oxidizing archaea; Abundance; Community composition; Biochemical organic input quality; Mineral nitrogen fertilizer.

## 2.1 Introduction

Combined and particularly site-adapted use of mineral fertilizers and organic inputs (i.e., crop residues as well as prunings from shrubs and trees) has been recommended for soil fertility and crop productivity enhancement in resource limited smallholder tropical cropping systems (Partey et al., 2013; Rasche and Cadisch, 2013; Vanlauwe et al., 2010). To maximize the beneficial effects of organic inputs on crop nutrition and soil organic matter build-up, an

explicit decision support system for smallholder farmers was developed by Palm et al. (2001b). This defines high quality organic inputs (rich in organic nitrogen (N) (> 2.5 %), but low in polyphenols (< 4 %)) to be applied primarily as substitution of mineral fertilizers, while intermediate (high contents of organic N (> 2.5 %) and polyphenols (> 4 %)) and low quality (low contents of organic N (< 2.5 %) and polyphenols (< 4 %)) inputs should be used as complement to mineral fertilization.

On basis of this decision support system, long-term field experiments were initiated in Kenya and Zimbabwe in 2002 to evaluate the effect of contrasting biochemical quality organic inputs with and without mineral N fertilizer applications on crop performance and soil carbon (C) and N dynamics (Chivenge et al., 2009; Gentile et al., 2011a; Mtambanengwe et al., 2006). At the Kenyan site, located in the central highlands, high quality organic inputs (i.e., *Tithonia diversifolia*) and combination of intermediate (*Calliandra calothyrsus*) and low quality (*Zea mays*) inputs with mineral N improved soil fertility and increased maize production (Chivenge et al., 2009). Moreover, *Z. mays* stover with low N content increased soil aggregate stability, soil organic C and total soil N compared to the polyphenol rich inputs of *C. calothyrsus* in the short-term. However, this positive effect was inversed when *Z. mays* inputs were combined with mineral N fertilizer (Chivenge et al., 2011).

In contrast to this knowledge gain on geochemical dynamics and crop performance, the effect of long-term use of biochemically contrasting organic inputs in combination with mineral N fertilizers on soil microbial communities remain uncertain. A critical knowledge gap within the frame of these long-term field experiments is left, although it is acknowledged that organic input quality is critically regulating microbial decomposition and mineralization including nitrification (Partey et al., 2013; Wardle and Giller, 1996) and proteolysis (Rasche et al., 2014). Consequently, soil microbial communities have been recognized to control the synchrony of crop available nutrients including ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) with crop demand (Vanlauwe et al., 2010).

Microbial nitrification plays a central role in the terrestrial N cycle through ammonia-oxidizing bacteria (AOB) and archaea (AOA) using a  $\alpha$ -subunit of monooxygenase (*amoA* gene) as catalyzing enzyme (Hyman and Arp, 1992; Nicol and Schleper, 2006). Extensive studies have shown that prokaryotic ammonia oxidation is partially controlled by organic inputs and mineral fertilization (Wessén et al., 2010; Wu et al., 2011). In this sense, de Gannes et al. (2014) and Levičnik-Höfferle et al. (2012) evidenced distinct community differences between AOA and AOB with respect to contrasting N availabilities. In their studies, it was reported that AOA adapted successfully to low  $\text{NH}_4^+$  environments, while AOB showed opposite tendencies.

Furthermore, Jia and Conrad (2009) reported the dominance of AOB over AOA in soils receiving N fertilizer, while an opposite effect was assessed by Zhang et al. (2010). Wessén et al. (2010) showed consistently higher AOA abundance than AOB with use of straw and peat inputs in combination with mineral N soil amendments although AOB was higher in peat input without N fertilizer. It is worthwhile emphasizing that these long-term studies were restricted to temperate environments and most of them apart from Wessén et al. (2010) focused on the sole use of either organic inputs or mineral fertilizer. Contrastingly, AOB and AOA community dynamics in tropical soils under agricultural use have been hitherto underestimated with respect to their exposure to both biochemically contrasting organic inputs and mineral N fertilizer.

The primary aim of this study was to assess the effects of ten years continuous application of biochemically contrasting organic inputs in combination with mineral N fertilizer on the functional potential (DNA-based abundance quantification) of AOB and AOA and their respective community composition (terminal restriction fragment length polymorphism analyses) in a tropical soil under agricultural use. We hypothesized a higher AOB and AOA abundance along with altered community composition with the use of high quality inputs due to a higher accessibility of organic C and N substrates as opposed to low quality inputs. Furthermore, with respect to the combination of mineral N with low and intermediate quality organic inputs, we hypothesized a higher AOB abundance than AOA in response to an enhanced degradation of organic inputs to alleviate N stress.

## **2.2 Materials and methods**

### **2.2.1 Site description and experimental design**

The experimental site was located in Embu (0° 30' S, 37° 30' E; 1380 m above sea level) in the central highlands of Kenya (130 km northeast of Nairobi). The site has an annual mean temperature of 20 °C and a mean annual rainfall of 1200 mm. Rainfall is bimodal with long rains received from mid-March to June and short rains from mid-October to December. The soil is defined as a Humic Nitisol (FAO, 2006) dominated by Kaolinite minerals derived from basic volcanic rocks. The texture of the topsoil layer (0-15 cm) was characterized by 17 % sand, 18 % silt and 65 % clay and contained 29.4 g kg<sup>-1</sup> organic C, 2.7 g kg<sup>-1</sup> total N and a pH of 5.8 (H<sub>2</sub>O) at start of experiment in 2002 (Gentile et al., 2009).

The soil organic matter (SOM) long-term field experiment was established to study primarily the effect of continuous annual application of biochemically contrasting organic inputs with and without inorganic mineral N fertilizer on crop performance as well as soil C and N

dynamics (Chivenge et al., 2011, 2009; Gentile et al., 2011a). The experiment was laid out in a split plot design with three replicates per treatment with organic inputs as main plots (size, 12 m x 5 m) and mineral N fertilizer as sub-plots (size, 6 m x 5 m) located in the organic input plots. Three organic input types were considered in this study: high quality *Tithonia diversifolia* (C/N ratio: 13, Lignin: 8.9 %; Polyphenols: 1.7 %), intermediate quality *Calliandra calothyrsus* (13; 13 %; 9.4 %) and low quality *Zea mays* (59; 5.4 %; 1.2 %) inputs (Gentile et al., 2011b). At the onset of long rains in each year, organic inputs (leaves, petioles and small branches for *C. calothyrsus* in addition to stems for *T. diversifolia* and *Z. mays* stover) were collected and analyzed for dry matter and total C and N content. Dry matter and total C data were then used to determine the amount of each organic material to be applied amounting to 4 Mg C ha<sup>-1</sup> a<sup>-1</sup> (Chivenge et al., 2009; Gentile et al., 2011a). Prior to maize sowing, organic inputs were chopped into small pieces, broadcast and hand-incorporated at a soil depth of 0-15 cm (Chivenge et al., 2009; Gentile et al., 2011a). In the sub-plots of each plot, mineral N fertilizer was applied as calcium ammonium nitrate (CaNH<sub>4</sub>NO<sub>3</sub>) at a rate of 0 and 120 kg N ha<sup>-1</sup> growing season<sup>-1</sup> (Chivenge et al., 2009; Gentile et al., 2011a, 2011b). One third of the mineral N fertilizer was applied 3 weeks after sowing of *Z. mays* (test crop) and the remainder 8 weeks later by broadcasting and incorporating into soil (Chivenge et al., 2009). Mineral N fertilizer was recommended as a complement for low and intermediate quality organic inputs to compensate the presumed N limitation (Palm et al., 2001b; Partey et al., 2013). For treatment comparison purposes in the presented study, the mineral N fertilizer was also combined with the high quality organic input. All plots received a blanket basal application of 60 kg P ha<sup>-1</sup> season<sup>-1</sup> and 60 kg K ha<sup>-1</sup> season<sup>-1</sup> before sowing. Further details of the field experiment set-up can be retrieved from Chivenge et al. (2009) and Gentile et al. (2009).

### **2.2.2 Soil sampling**

In March 2012, at onset of long rains and prior to incorporation of fresh organic inputs, soil samples were collected from the plots designated for the crop (*Zea mays*) (Gentile et al., 2011a). This sampling time point allowed us to assess the targeted long-term effects of organic and mineral inputs on alteration of the functional potential (abundance) and community composition of AOB and AOA, while avoiding interference of short-term microbial community dynamics due to initialized decomposition and mineralization of fresh inputs. The following 8 treatments (each replicated three times) were considered: 4 treatments without mineral N fertilizer including the no input control (CON) and 3 organic input treatments (i.e., *C. calothyrsus* (CC), *Z. mays* (ZM) and *T. diversifolia* (TD)). The other 4 treatments were their

respective counterparts receiving mineral N fertilizer. In each plot, 10 soil sub-samples were randomly obtained at a depth of 0-15 cm using a soil auger. These 10 sub-samples were bulked to 1 composite and representative sample per plot sieved (2-mm mesh) and stored at 4 °C. In total, 24 soil samples were obtained. Prior to analysis, each composite soil sample was split into 3 proportions. A fresh sample was used for immediate extraction of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The second proportion was air-dried for analysis of pH, hot water extractable C and N (HWEC/N), total C (TC) and total N ( $\text{N}_t$ ). The third proportion was freeze-dried to be shipped to Germany for microbial community analyses.

## **2.2.3 Microbiological soil analysis**

### **2.2.3.1 Soil DNA extraction**

Total soil DNA was isolated from 0.5 g of each freeze dried soil sample using the FastDNA<sup>TM</sup> SPIN Kit for Soil (MP Biomedicals, Solon, Ohio, USA) according to the manufacturer's instructions. Quality of isolated DNA was checked on 1.5 % (w/v) agarose gels. DNA was quantified photometrically (NanoDrop<sup>TM</sup> 2000/2000c spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). For each isolated total DNA sample, 2 consistent NanoDrop readings, whose variation was less than 5 %, were considered for subsequent determination of DNA concentration. Quantified DNA was stored at -20°C until further analysis.

### **2.2.3.2 Microbial abundance**

For DNA-based quantification of target genes (total bacteria and archaea (16S rRNA gene), ammonia-oxidizing bacteria (bacterial *amoA* gene; AOB) and archaea (archaeal *amoA* gene; AOA)), plasmid standards were prepared (Table 2.1), purified (Invisorb Fragment CleanUp kit, Stratec Molecular GmbH, Berlin, Germany) and quantified according to Rasche et al. (2011). The qPCRs (3 analytical replicates per DNA sample) were conducted in a 25 µl reaction cocktail containing 12.5 µl Power SYBR green master mix (Applied Biosystems, Foster City, CA, USA), 0.4 µM of each primer (Table 2.1), 0.25 µl T4 gene 32 protein (500 µg ml<sup>-1</sup>, MP Biomedicals) and 5 ng DNA template (except for AOB gene (10 ng)) from the prior prepared DNA working stock of 5 ng µl<sup>-1</sup> concentration. The optimal dilution of DNA extracts was tested before to compensate any reaction inhibition by humic acids co-extracted during DNA isolation (data not shown). Reactions were run on a StepOne Plus Real-time PCR detection system (Applied Biosystems) starting with an initial denaturation step at 95 °C for 10 min, followed by amplification cycles specific for each target gene (Table 2.1). Melting curve analysis of amplicons was conducted to ensure that fluorescence signals originated from specific

amplicons and not from primer dimers or other artifacts. Gene copy numbers and reaction efficiencies (total bacteria 106 %, total archaea 71%, AOB 96 %, AOA 63 %) were calculated using StepOne Software version 2.2.2 (Applied Biosystems) and presented as gram of dry soil.

### **2.2.3.3 Microbial community composition**

For terminal restriction fragment length polymorphism (TRFLP) analysis, the total (16S rRNA gene) and ammonia-oxidizing (*amoA* genes) prokaryotic communities were amplified as described previously (Rasche et al., 2011) (Table 2.1). All forward primers were labeled with 6-carboxyfluorescein at their 5' ends. Amplicons (both 16S rRNA genes, AOA) were purified (Invisorb Fragment CleanUp kit) prior to digestion. For AOB, 3 replicate PCRs were pooled, precipitated (Rasche et al., 2006b), and purified (QIAGEN® Gel Extraction Kit, Hilden, Germany). Two hundred ng of each purified amplicon were digested with 5 U *AluI* (New England Biolabs (NEB), Ipswich, USA) for bacterial and archaeal 16S rRNA gene amplicons, while a 5 U combination of *AluI* and *RsaI* (NEB) was used for AOB and AOA. Reactions were incubated at 37 °C for 4 hours (bacterial 16S rRNA genes, AOB, AOA) and overnight (archaeal 16S rRNA genes). The latter was necessary to gain a more efficient digestion. Digested products were purified (Sephadex G-50, GE Healthcare Biosciences, Waukesha, WI, USA) (Rasche et al., 2006a) and an aliquot of 2 µl was mixed with 17.75 µl HiDi formamide (Applied Biosystems) and 0.25 µl internal 500 ROX™ size standard (Applied Biosystems). Labeled terminal restriction fragments (TRFs) were denatured at 95°C for 3 min, chilled on ice and detected on an ABI 3130 automatic DNA sequencer (Applied Biosystems). Peak Scanner™ software package (version 1.0, Applied Biosystems) was used to compare relative lengths of TRFs with the internal size standard and to compile electropherograms into numeric data set, in which fragment length and peak height >50 fluorescence units were used for profile comparison. TRFLP profiles used for statistical analyses were normalized according to Dunbar et al. (2001).

### **2.2.4 Geochemical properties**

Soil pH values were measured in water suspensions at a solid: liquid ratio of 1:2.5. Hot water extractable C (HWEC) and N (HWEN) analyses were determined according to Schulz (2004) as well as Schulz and Körschens (1998). C content of hot water extracts was analyzed using potassium dichromate in sulfuric acid milieu (Multi N/C analyzer, Analytik Jena, Jena, Germany), while HWEN was analyzed by a modified Kjeldahl digestion. Total C (TC) and total N (N<sub>t</sub>) of soils were quantified by dry combustion (Flash EA 1112 Elemental Analyzer,

Thermo Fisher Scientific). Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) were calorimetrically measured spectrophotometrically on field fresh soils (ICRAF, 1999).

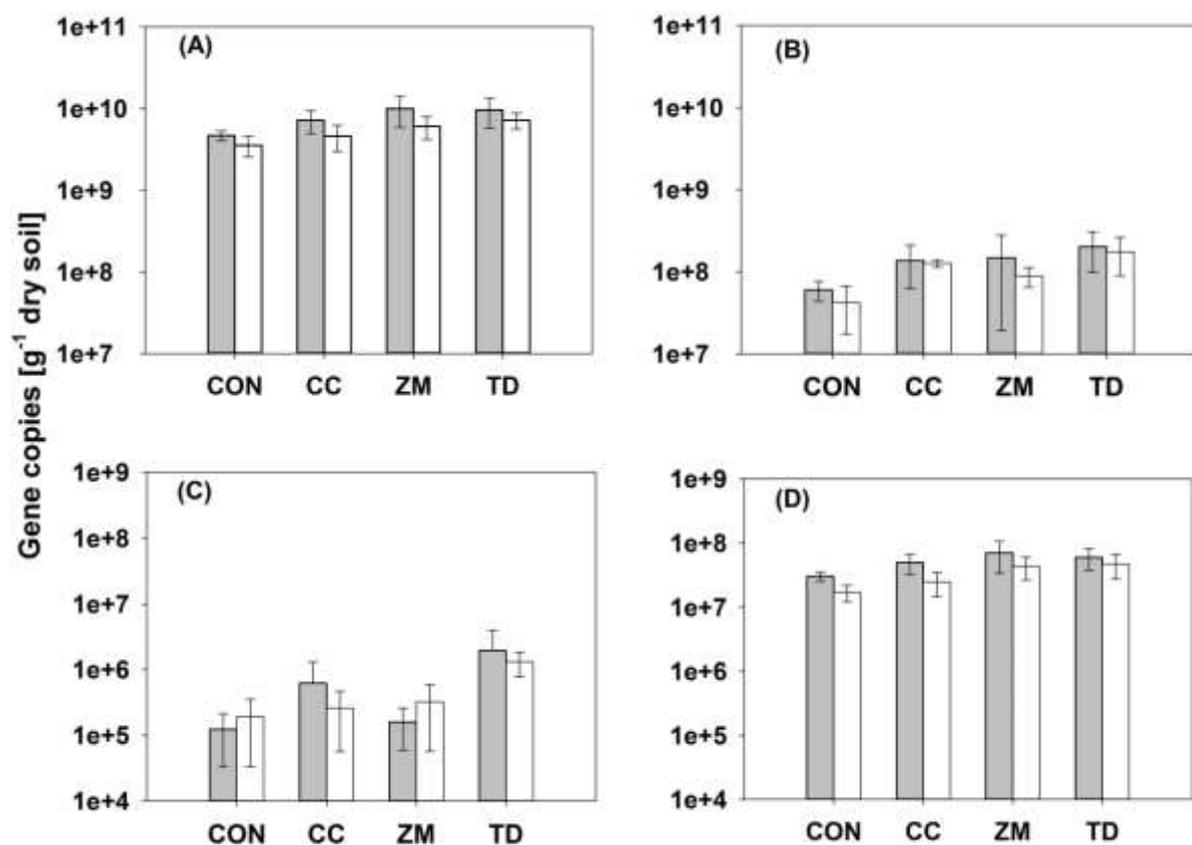
**Table 2.1:** Description of primer sets, PCR ingredients and amplification details used for quantitative PCR (qPCR) and TRFLP analyses.

Target gene	Primer (reference)	qPCR	TRFLP
All bacteria	Eub338f (Lane, 1991)	95 °C 5 min; 40 cycles: 95 °C 30s, 55 °C 35s, 72 °C 45s	95 °C 5 min; 40 cycles: 95 °C 1 min, 58 °C 30s, 72 °C 1 min; 72 °C 10 min
	Eub518r (Muyzer et al., 1993)		
All archaea	8f (Weisburg et al., 1991)	95 °C 5 min; 40 cycles: 95 °C 30s, 52 °C 35s, 72 °C 45s, 78 °C 20s	95 °C 5 min; 35 cycles: 95 °C 1 min, 52 °C 30s, 72 °C 1 min; 72 °C 10 min
	1520r (Edwards et al. 1989)		
Ammonia oxidizing bacteria (AOB)	Ar109f (Lueders and Friedrich, 2000)	95 °C 5 min; 45 cycles: 95 °C 30s, 57 °C 45s, 72 °C 45s, 78 °C 20s	95°C 5 min; 40 cycles: 94°C 30s, 53°C 30s, 72°C 1 min; 72°C 10 min
	Ar912r (Lueders and Friedrich, 2000)		
Ammonia oxidizing archaea (AOA)	AmoA-1f (Rotthauwe et al., 1997)	95 °C 5 min; 45 cycles: 95 °C 30s, 53 °C 45s, 72 °C 45s, 78 °C 20s	94 °C 5 min; 35 cycles: 94 °C 30s, 53 °C 45s, 72 °C 10 min; 72 °C 1 min
	AmoA-2r (Rotthauwe et al., 1997)		
Ammonia oxidizing archaea (AOA)	Arch-amoAf (Francis et al., 2005)	95 °C 5 min; 45 cycles: 95 °C 30s, 53 °C 45s, 72 °C 45s, 78 °C 20s	94 °C 5 min; 35 cycles: 94 °C 30s, 53 °C 45s, 72 °C 10 min; 72 °C 1 min
	Arch-amoAr (Francis et al., 2005)		

### 2.2.5 Statistical analysis

Analyses of variance (ANOVA) were conducted using Statistical Analysis Software program SAS (SAS Institute, 2014). For the abundance data of the four studied genes, a generalized linear mixed model with a negative binomial distribution error and a log link function was used. The model was fitted by restricted log pseudo-likelihood with expansion about the solution for the random effects in the SAS GLIMMIX procedure. For the geochemical data, linear mixed models were fitted in the SAS MIXED procedure. The data were checked for normality and homoscedasticity on model residuals using quantile-quantile (Q-Q) plots, histograms and studentized residual plots. Different transformations were tried in an attempt to satisfy the assumption underlying the ANOVA model (Piepho, 2009). A full factorial model was specified with the effects of organic inputs (the 3 contrasting organic inputs (ZM, TD, CC) and the no input control (CON)), mineral N fertilizer (0 and 120 kg N ha<sup>-1</sup> rates) and their interactions as fixed effects on the response variables (i.e., abundance of the 4 target genes and geochemical properties). Statistical significance of all effects was assessed at a significance level of 0.05. Blocks in the described factorial model were considered as both fixed and random effects and model selected accordingly using the Akaike Information Criterion (AIC). PDIFF option (i.e., table of *P* values for all possible pairwise comparisons of least square means (LSMEANS)), as well as the letters (letter display) from the SAS generalized linear mixed models procedure were used to separate treatment means/medians. Twenty-four observations (8 treatments, replicated 3 times as described in section 2.2) and presented in descriptive statistics (Fig. 2.1; Table 2.2) were used for all statistical analyses. It is however, worth mentioning that for abundance of all 4 genes no significant interactions between the 2 factors “Organic input” and “Mineral N fertilizer” were determined (Table 2.3). This was also the case for geochemical data except HWE C (Table 2.5). Therefore, treatment codes in figure 2.2 and table 2.5 (i.e., CON, CC, ZM, TD) were irrespective of the mineral N fertilizer levels (i.e., 0 N, 120 N) and those of mineral N levels irrespective of organic input type. Graphs were created in Systat software program using SigmaPlot package version 12.5 (San Jose, CA, USA). Linear regression analyses were conducted in the SAS REG procedure to relate the abundance (dependent variables) of target genes to the geochemical properties (independent variables). Soil treatment main and interaction effects on TRFLP data sets generated for each gene were tested using permutation multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Treatment effects were further assayed based on Bray-Curtis similarity coefficients (Rasche et al., 2011; Rees et al., 2005). A similarity matrix was generated for all possible pairs of samples

for each target gene. The similarity matrix was used for analysis of similarity (ANOSIM) to test if composition of soil microbial communities was altered by different quality organic inputs and combinations with mineral N fertilizer. ANOSIM is based on rank similarities between the sample matrix and produces a test statistic called ‘*R*’ (Rees et al., 2005). A ‘global’ *R* was first calculated in ANOSIM, which evaluated the overall effect of each factor in the data set. This step was followed by a pairwise comparison, whereby the magnitude of *R* indicated the degree of separation between two tested communities. An *R* score of 1 indicated a complete separation, while 0 indicated no separation (Rees et al., 2005).



**Figure 2.1:** Gene copy numbers of the four assayed genes in the different treatments (descriptive means  $\pm$  standard deviation,  $n=3$ ): Total number of observations = 24. (A) total bacteria (bacterial 16S rRNA gene), (B) total archaea (archaeal 16S rRNA gene), (C) ammonia-oxidizing bacteria (AOB, bacterial *amoA* gene), and (D) ammonia-oxidizing archaea (AOA, archaeal *amoA* gene) after 10 years of application of different biochemical quality organic inputs and combination with mineral N. Effect of quality of organic input treatments (grey bars); Effect of mineral N treatments (white bars): 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>: CON = control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.

**Table 2.2:** Geochemical properties of the Embu soil treated with different quality organic inputs and combinations with (+) and without (-) N fertilizer. Values are descriptive means ( $n = 3 \pm$  standard deviation) for soils sampled in respective treatments. Total number of observations = 24. Values followed by different superscript letters (<sup>a-f</sup>) in the same column are significantly different ( $P < 0.05$ ).

Treatments	Geochemical properties						
	Soil pH	HWEC [mg kg <sup>-1</sup> ]	HWEN [mg kg <sup>-1</sup> ]	TC [%]	N <sub>t</sub> [%]	NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]
CON-N	5.09 ± 0.19 <sup>cd</sup>	402.98 ± 29.4 <sup>c</sup>	30.02 ± 2.46 <sup>c</sup>	2.28 ± 0.1 <sup>b</sup>	0.20 ± 0.01 <sup>c</sup>	11.7 ± 2.1 <sup>c</sup>	3.8 ± 0.5 <sup>b</sup>
CON+N	4.64 ± 0.16 <sup>f</sup>	511.03 ± 72.1 <sup>abc</sup>	61.45 ± 14.39 <sup>a</sup>	2.28 ± 0.09 <sup>b</sup>	0.21 ± 0.01 <sup>c</sup>	48.3 ± 10.7 <sup>a</sup>	25.1 ± 13.8 <sup>a</sup>
CC-N	5.17 ± 0.20 <sup>bc</sup>	498.18 ± 63.8 <sup>bc</sup>	38.07 ± 5.10 <sup>bc</sup>	2.66 ± 0.23 <sup>a</sup>	0.23 ± 0.02 <sup>b</sup>	11.7 ± 1.6 <sup>c</sup>	7.6 ± 0.8 <sup>b</sup>
CC+N	4.73 ± 0.22 <sup>ef</sup>	487.72 ± 48.98 <sup>bc</sup>	62.82 ± 6.41 <sup>a</sup>	2.67 ± 0.23 <sup>a</sup>	0.24 ± 0.03 <sup>ab</sup>	34.1 ± 3.7 <sup>b</sup>	26.9 ± 10.2 <sup>a</sup>
ZM-N	5.44 ± 0.31 <sup>ab</sup>	593.42 ± 160.6 <sup>ab</sup>	42.63 ± 9.43 <sup>b</sup>	2.92 ± 0.37 <sup>a</sup>	0.26 ± 0.03 <sup>a</sup>	11.5 ± 2.0 <sup>c</sup>	7.0 ± 1.4 <sup>b</sup>
ZM+N	4.89 ± 0.13 <sup>def</sup>	544.95 ± 108.6 <sup>ab</sup>	64.34 ± 11.65 <sup>a</sup>	2.70 ± 0.31 <sup>a</sup>	0.24 ± 0.04 <sup>ab</sup>	35.8 ± 10.2 <sup>ab</sup>	15.1 ± 8.1 <sup>ab</sup>
TD-N	5.48 ± 0.20 <sup>a</sup>	622.60 ± 72.8 <sup>a</sup>	46.05 ± 1.65 <sup>b</sup>	2.86 ± 0.15 <sup>a</sup>	0.25 ± 0.02 <sup>ab</sup>	11.9 ± 0.4 <sup>c</sup>	8.1 ± 0.5 <sup>b</sup>
TD+N	4.97 ± 0.05 <sup>cde</sup>	620.82 ± 65.8 <sup>a</sup>	64.88 ± 16.71 <sup>a</sup>	2.80 ± 0.20 <sup>a</sup>	0.25 ± 0.03 <sup>ab</sup>	33.9 ± 14.2 <sup>b</sup>	24.0 ± 13.0 <sup>a</sup>

Soil property definition: HWEC/N = Hot water extractable carbon/nitrogen, TC = Total carbon, N<sub>t</sub> = Total nitrogen, NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrate. Treatment codes: CON - N = Control + 0 kg N ha<sup>-1</sup>, CON + N = Control + 120 kg N ha<sup>-1</sup>, CC - N = *Calliandra calothyrsus* + 0 kg N ha<sup>-1</sup>, CC + N = *Calliandra calothyrsus* + 120 kg N ha<sup>-1</sup>, ZM - N = *Zea mays* + 0 kg N ha<sup>-1</sup>, ZM + N = *Zea mays* + 120 kg N ha<sup>-1</sup>, TD - N = *Tithonia diversifolia* + 0 kg N ha<sup>-1</sup>, TD + N = *Tithonia diversifolia* + 120 kg N ha<sup>-1</sup>.

**Table 2.3:** Statistical evaluation of gene abundance and community composition by full factorial analysis of variance (ANOVA) and permutation multivariate analysis of variance (PERMANOVA) respectively

Microbial groups		Effects/Source of variation		
		Organic input	Mineral N	Input x Mineral N
qPCR				
Total bacteria		**	**	n.s
Total archaea		**	*	n.s
Ammonia-oxidizing (AOB)	bacteria	**	n.s	n.s
Ammonia-oxidizing (AOA)	archaea	**	**	n.s
TRFLP				
Total bacteria		n.s	*	n.s
Total archaea		n.s	**	n.s
Ammonia-oxidizing (AOB)	bacteria	**	*	n.s
Ammonia oxidizing (AOA)	archaea	n.s	n.s	n.s

Significance levels: n.s:  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ .

For graphical visualization of the relationship between compositions of prokaryotic communities with soil chemical data, Canonical Analysis of Principal coordinates (CAP) was performed on resemblance matrix data generated based on Bray-Curtis similarity coefficients (Anderson and Ter Braak, 2003; Anderson and Robinson, 2003; Clarke, 1993). In addition, regression analyses were performed using Distance-based linear models (DISTLM) (Boj et al., 2011; Legendre and Anderson, 1999; McArdle and Anderson, 2001). These analyses were used to test the relationship between the composition of AOB, AOA and total communities based on Shannon-Weaver indices ( $H'$ , Shannon and Weaver, 1949) (predicted variables) and soil chemical properties (predictor variables). DISTLM is a routine for analyzing the relationship between a multivariate data, based on resemblance matrix, and one or more predictor variables (Legendre and Anderson, 1999; McArdle and Anderson, 2001). It partitions variation in a data set (i.e., community composition of target genes, soil chemical data) according to regression models. PERMANOVA, ANOSIM, CAP and DISTLM analyses were conducted using Primer6 for Windows (version 6.1.13, Primer-E Ltd., Plymouth, UK) with (PERMANOVA+ version 1.0.6) add-on for Primer 6 software (Anderson, 2001).

## 2.3 Results

### 2.3.1 Microbial abundance

Copy numbers of bacterial 16S rRNA genes ranged from  $4.21 \times 10^9$  to  $8.10 \times 10^9$  per gram dry soil in CON and TD plots, respectively (Fig. 2.2A). With regard to organic input quality, TD yielded significantly higher total 16S rRNA gene copy numbers compared to CC ( $P < 0.05$ ). Use of mineral N reduced bacterial 16S rRNA gene copy numbers compared to the zero rates ( $P < 0.05$ ).

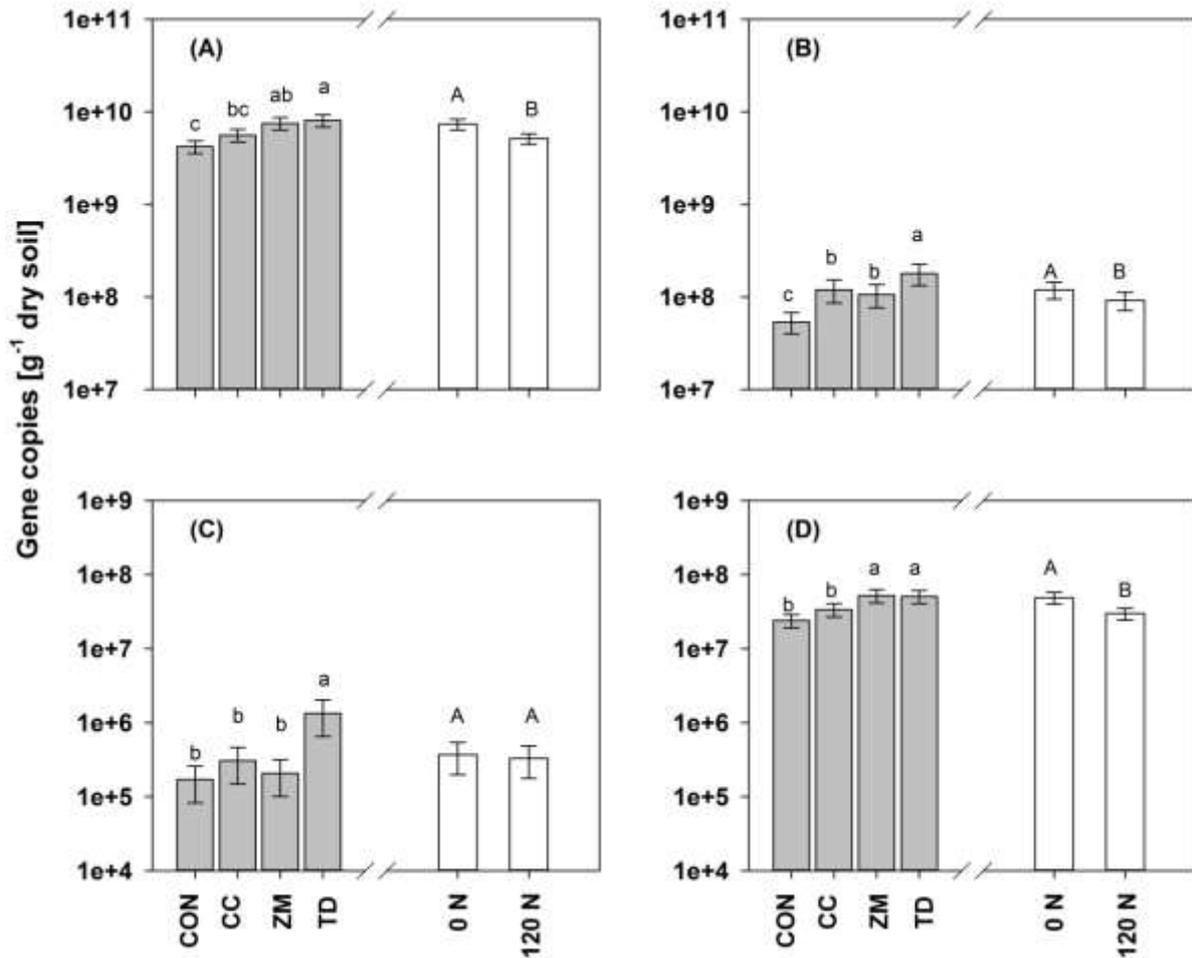
Copy numbers of archaeal 16S rRNA genes ranged from  $5.37 \times 10^7$  to  $1.79 \times 10^8$  per gram dry soil in CON and TD plots, respectively (Fig. 2.2B). Considering the effects of organic input quality, CC and ZM yielded significantly lower gene copy numbers compared to TD ( $P < 0.05$ ). Similar to bacterial 16S rRNA genes, mineral N irrespective of organic input quality reduced abundance of archaeal 16S rRNA genes compared to the zero rates ( $P < 0.05$ ).

Copy numbers of AOB gene ranged from  $1.71 \times 10^5$  to  $1.35 \times 10^6$  per gram of dry soil in CON and TD, respectively (Fig. 2.2C). Regarding the effect of quality of organic inputs, AOB gene copy numbers under TD were significantly higher than those under CC and ZM ( $P < 0.01$ ). Application of mineral N irrespective of organic input did not influence AOB compared to the zero rates ( $P > 0.05$ ).

Copy numbers of AOA gene ranged from  $2.40 \times 10^7$  to  $5.20 \times 10^7$  per gram of dry soil in CON and ZM treatments, respectively (Fig. 2.2D). Focusing on the effect of organic input quality, ZM and TD yielded significantly higher AOA gene copies compared to CC ( $P < 0.05$ ). Similar to abundance of total communities, application of mineral N yielded lower AOA gene copies compared to the zero rates ( $P < 0.05$ ).

### 2.3.2 Microbial community composition

Canonical analysis of principal coordinates (CAP) (Fig. 2.3 and 2.4) plots revealed distinct patterns for all the studied prokaryotic communities apart from the AOA between treatments which had received mineral N fertilizer from those which did not. Interestingly, the fertilizer treated soils were located in the direction of increasing  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and HWEN particularly for total communities. On the other hand, distinct patterns with regard to quality of organic inputs were revealed for AOB and AOA but not for total communities.



**Figure 2.2:** Gene copy numbers of the four assayed genes in the different treatments (means  $n = 6$ ): Total number of observations = 24. (A) total bacteria (bacterial 16S rRNA gene), (B) total archaea (archaeal 16S rRNA gene), (C) ammonia-oxidizing bacteria (AOB, bacterial *amoA* gene), and (D) ammonia-oxidizing archaea (AOA, archaeal *amoA* gene) after 10 years of application of different biochemical quality organic inputs and combination with mineral N. Different letters (a-c) above the bars indicate treatments with significant differences ( $P < 0.05$ ). Effect of quality of organic input treatments irrespective of mineral N fertilizer levels (grey bars): CON = control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*. Effect of mineral N treatments irrespective of organic inputs (white bars): 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>.

PERMANOVA results did not reveal significant interactions between organic inputs and mineral N fertilizer on composition of prokaryotic communities (Table 2.3) ( $P > 0.05$ ).

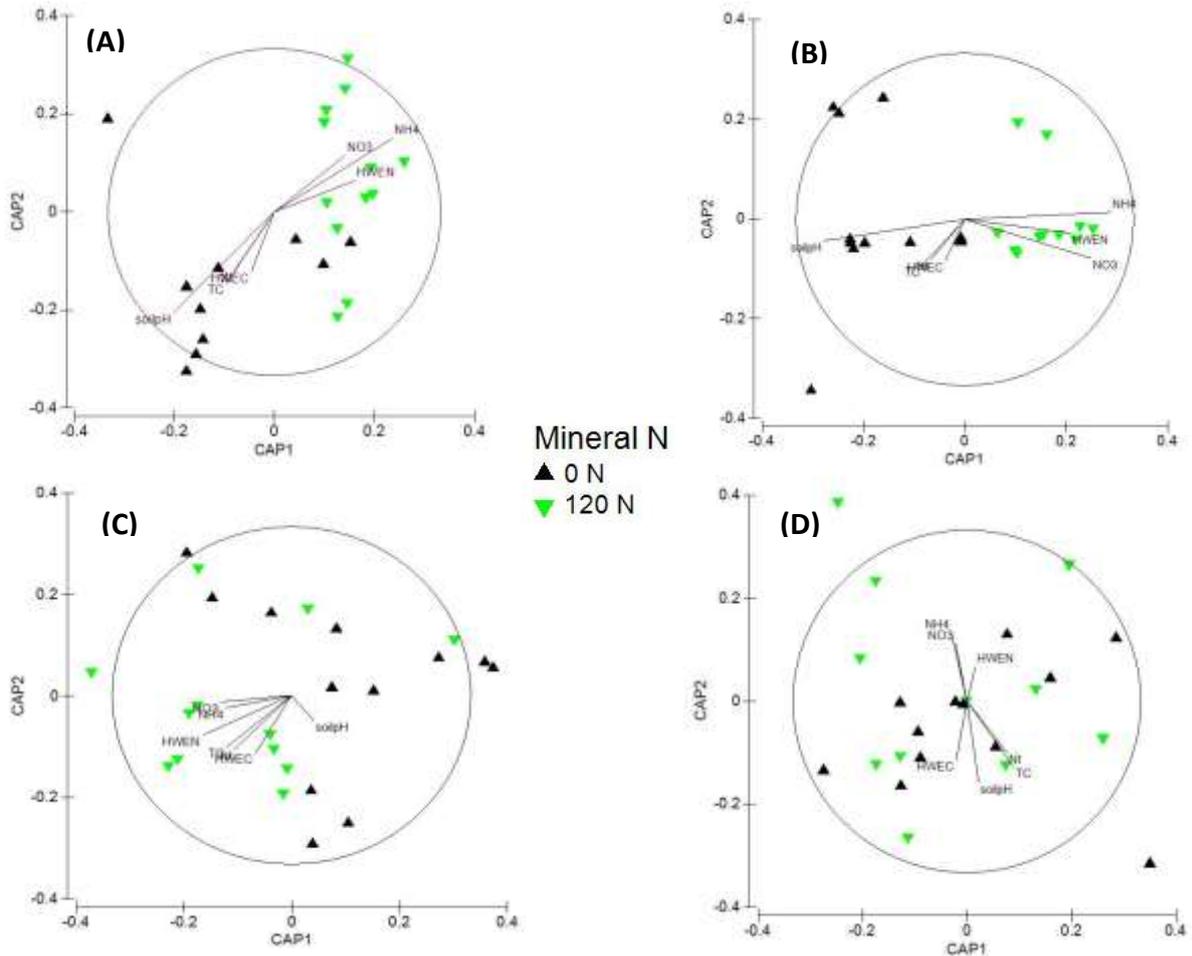
Total archaeal, total bacterial and AOB communities were significantly influenced by mineral N fertilizer, whereas quality of organic inputs revealed significant effects on AOB ( $P < 0.01$ ). Analyses of similarity (ANOSIM) of TRFLP fingerprints revealed shifts of community composition of bacterial *amoA* (AOB) (Global  $R = 0.257$ ) and archaeal *amoA* (AOA) (Global  $R = 0.189$ ) genes due to quality of organic inputs ( $P < 0.05$ ), while no significant effect on composition of total communities was revealed (Table 2.4). For pairwise comparisons between organic input treatments irrespective of mineral N fertilizer, the highest community composition separation of AOB was shown between CON and TD ( $R = 0.535$ ). TD versus ZM showed for AOB an  $R = 0.343$ , while an  $R = 0.320$  was calculated between CON and ZM. CON versus CC presented an  $R = 0.231$ , but no separation of AOB was found between TD versus CC and CC versus ZM. For AOA, pairwise comparisons showed an  $R = 0.346$  between CON and ZM, while an  $R = 0.285$  and  $R = 0.209$  was calculated between TD and ZM as well as TD and CC, respectively. Meanwhile, irrespective of organic inputs, mineral N fertilizer addition altered the composition of total bacterial (Global  $R = 0.202$ ) and total archaeal (Global  $R = 0.404$ ) communities ( $P < 0.05$ ), but AOB and AOA were not affected (data not shown). ANOSIM results were to a larger extent in agreement with CAP ordination plots and PERMANOVA results.

### 2.3.3 Geochemical properties

No interaction between combination of organic inputs and mineral N on geochemical properties was determined except for HWEC ( $P > 0.05$ ). Therefore, the main effects of organic inputs and mineral N results only were displayed (Table 2.5). ZM (pH = 5.18) and TD (5.23) showed higher soil pH values compared to CON (4.87) and CC (4.96) ( $P < 0.05$ ). Lower pH values were recorded in all plots which received mineral N fertilizer compared to those that did not ( $P < 0.001$ ). All pH values decreased in comparison to the start of experiment in 2002 (5.81) (Gentile et al., 2011a). TD increased HWEC values compared to CON and CC ( $P < 0.05$ ). On the other hand, mineral N did not influence HWEC contents ( $P > 0.05$ ). HWEN was not influenced by organic input quality ( $P > 0.05$ ). However, mineral N increased HWEN values ( $P < 0.001$ ).

TD treated plots had higher TC values compared to CON and CC ( $P < 0.05$ ).  $N_t$  values were increased by application, but not quality of organic inputs compared to CON ( $P < 0.05$ ). Application of mineral N did not influence TC and  $N_t$  contents ( $P > 0.05$ ). TC and  $N_t$  remained

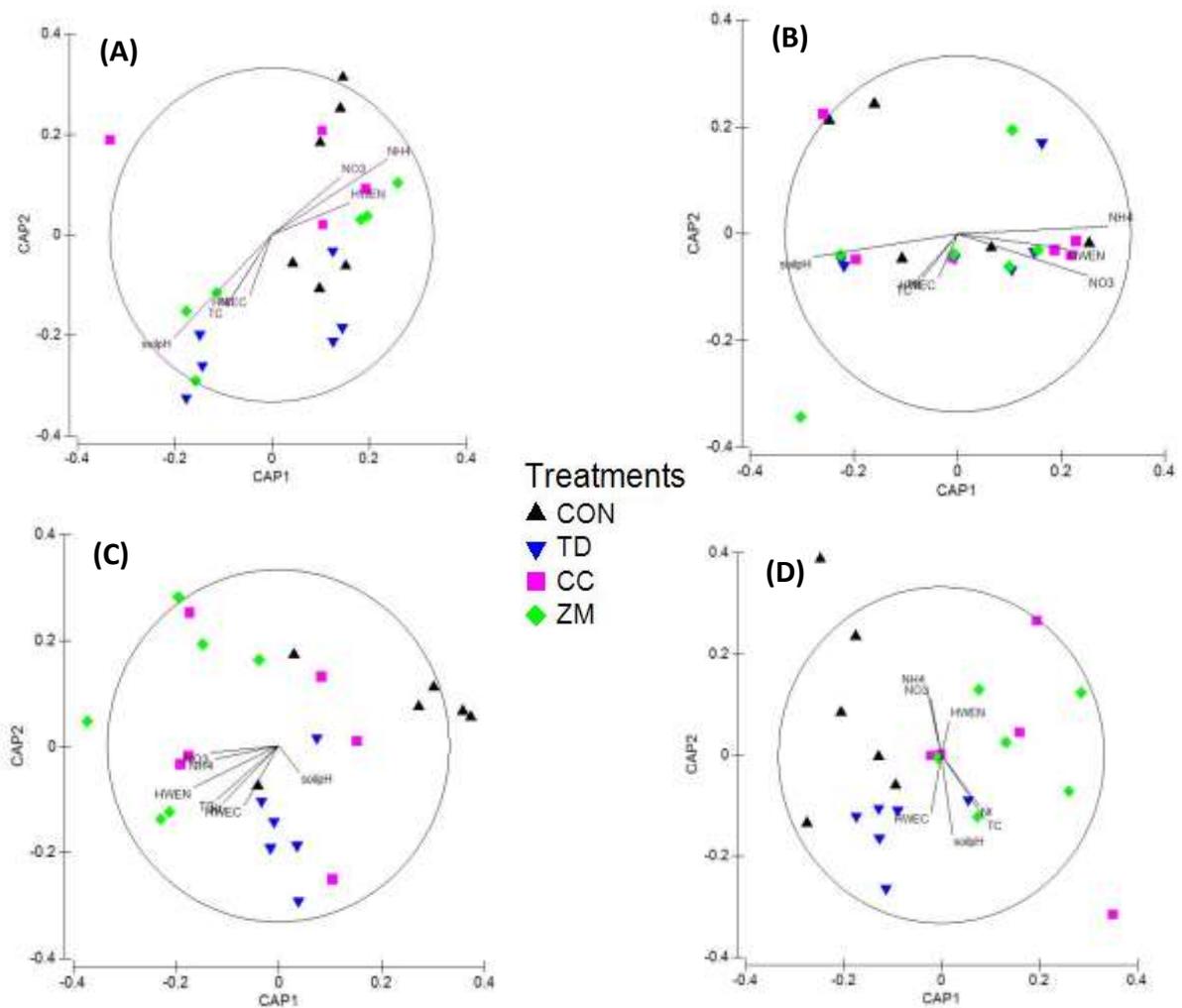
relatively constant in all treatments, but were reduced in CON compared to the start of experiment in 2002 (Gentile et al., 2011a). CON showed highest  $\text{NH}_4^+$  over all the other treatments ( $P < 0.05$ ).  $\text{NO}_3^-$  was not influenced by quality or application of organic inputs ( $P > 0.05$ ). Mineral N received treatments increased  $\text{NH}_4^+$  and  $\text{NO}_3^-$  compared to sole use of organic inputs or the no input control ( $P < 0.001$ ).



**Figure 2.3:** Canonical Analysis of Principal coordinates (CAP) for visual presentation of patterns of composition of prokaryotic communities as shaped by mineral N and their relationship with soil chemical properties. Prokaryotic communities: A = Total bacteria, B = Total archaea, C = Ammonia-oxidizing bacteria and D = Ammonia-oxidizing archaea. Treatments codes: 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>.

#### 2.3.4 Regression analysis

Soil pH, HWEC, TC and  $N_t$  were the strongest predictors of shifts in abundance of archaeal and bacterial 16S rRNA as well as archaeal *amoA* genes (Table 2.6). An exception was AOB abundance, where HWEC, TC and  $N_t$  had generally a weak influence. Specifically, up to 80 %, 75 % and 60 % increase in abundance of total bacteria, AOA and total archaea respectively was explained by soil pH. Up to 60 %, 57 % and 52 % increase in abundance of archaeal communities (i.e., total archaea and AOA), total bacteria and AOB respectively was accounted for by HWEC. Up to 70 % increase in abundance of AOA, total bacteria, total archaea and 45 % for AOB was explained by TC. Up to 60 % increase in abundance of AOA, total archaea, total bacteria and 40 % for AOB was accounted for by  $N_t$ . Up to 50 % and 47 % decrease in abundance of AOA and total bacterial respectively was attributed to  $NH_4^+$ , while up to 36 % decrease in total bacterial abundance was attributed to  $NO_3^-$ . On the microbial composition results (Table 2.6) up to 60 %, 50 % and 33 % change in total bacterial, total archaeal and AOA communities' composition respectively was explained by soil pH. Up to 17 % and 25 % change in composition of AOB was explained by HWEC and TC respectively. Approximately 28 %, 20 % and 17 % shift in total bacteria, AOA and AOB compositions respectively was attributed to  $N_t$ . Up to 48 % and 33 % change in total archaea was attributed to  $NH_4^+$  and  $NO_3^-$  respectively.



**Figure 2.4:** Canonical analysis of principal coordinates (CAP) for visual presentation of patterns of composition of prokaryotic communities as shaped by contrasting biochemical organic inputs and their relationship with soil chemical properties. Prokaryotic communities: A = Total bacteria, B = Total archaea, C = Ammonia-oxidizing bacteria and D = Ammonia-oxidizing archaea. Treatments codes: CON = Control, TD = *Tithonia diversifolia*, CC = *Calliandra calothyrsus*, ZM = *Zea mays*.

**Table 2.4:** Analysis of similarity (ANOSIM) to determine the influence of biochemical quality of organic inputs on the community structure of total and ammonia-oxidizing prokaryotes in the SOM field experiment.

	Bacterial 16S rRNA	Archaeal 16S rRNA	AOA	AOB
Global <i>R</i>	0.089	-0.053	0.189	0.257
Significance level across groups	n.s	n.s	*	*
	Total bacteria	Total archaea	AOA	AOB
Pairwise comparisons	<i>R</i> statistics			
CON vs. TD	0.237	-0.048	0.109	0.535
CON vs. CC	0.270	-0.067	0.178	0.231
CON vs. ZM	0.041	-0.069	0.346	0.320
TD vs. CC	0.065	-0.013	0.209	0.081
TD vs. ZM	-0.05	-0.081	0.285	0.343
CC vs. ZM	-0.011	-0.041	-0.074	0.052

Global *R* indicates the degree of separation of communities within each microbial group.

Significance levels: n.s:  $P > 0.05$ ; \* $P < 0.05$ .

Treatment codes: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*.

*R* statistics indicates the degree of separation of communities between two treatments, with a score of 1

indicating complete separation and 0 indicating no separation.

**Table 2.5:** Geochemical properties of the Embu soil showing main effects of different quality organic inputs and mineral N treatments. Values ((means or medians) n = 24) followed by different superscript letters (<sup>a-b/A-B</sup>) in the same column are significantly different ( $P < 0.05$ ). SED represents standard error of the difference between two means for comparisons across inputs and N fertilizer treatments.

Treatments	Geochemical properties						
	Soil pH	HWEC [mg kg <sup>-1</sup> ]	HWEN [mg kg <sup>-1</sup> ]	TC [%]	N <sub>t</sub> [%]	NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]
CON	4.9 <sup>b</sup>	485 <sup>b</sup>	46 <sup>a</sup>	2.24 <sup>b</sup>	0.21 <sup>b</sup>	23 <sup>a</sup>	9 <sup>a</sup>
CC	5.0 <sup>b</sup>	504 <sup>b</sup>	51 <sup>a</sup>	2.63 <sup>b</sup>	0.24 <sup>a</sup>	20 <sup>ab</sup>	16 <sup>a</sup>
ZM	5.2 <sup>a</sup>	606 <sup>ab</sup>	54 <sup>a</sup>	2.75 <sup>ab</sup>	0.25 <sup>a</sup>	20 <sup>ab</sup>	10 <sup>a</sup>
TD	5.2 <sup>a</sup>	630 <sup>a</sup>	56 <sup>a</sup>	2.81 <sup>a</sup>	0.26 <sup>a</sup>	17 <sup>b</sup>	15 <sup>a</sup>
SED	± 0.1	± 31	± 5		± 0.01		
0 N	5.3 <sup>A</sup>	549 <sup>A</sup>	40 <sup>B</sup>	2.64 <sup>A</sup>	0.24 <sup>A</sup>	12 <sup>B</sup>	6 <sup>B</sup>
120 N	4.8 <sup>B</sup>	563 <sup>A</sup>	64 <sup>A</sup>	2.55 <sup>A</sup>	0.23 <sup>A</sup>	35 <sup>A</sup>	20 <sup>A</sup>
SED	± 0.1	± 15	± 4		± 0.004		
Source of variation and statistical significance							
OI	**	***	n.s	***	***	n.s	ns
N	***	n.s	***	*	n.s	***	***
OI x N	n.s	***	n.s	n.s	n.s	n.s	ns

Parameters without SED values show the treatments (medians) after back transformation of the least square means (lsmeans) from analyses.

OI = organic inputs; N = Mineral fertilizer.

Soil property definition: HWEC/N = Hot water extractable carbon/nitrogen, TC = Total carbon, N<sub>t</sub> = Total nitrogen, NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrate. Treatment codes: CON = control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*, 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>. Significance levels: n.s:  $P > 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

**Table 2.6:** Regression analysis ( $R^2$ ) showing relationships between abundance and community composition of prokaryotic genes and geochemical data.

Geochemical property	Prokaryotic group							
	Total bacteria		Total archaea		Ammonia-oxidizing bacteria (AOB)		Ammonia-oxidizing archaea (AOA)	
	qPCR	TRFLP	qPCR	TRFLP	qPCR	TRFLP	qPCR	TRFLP
Soil pH	0.802***	n.s	0.597***	0.512***	n.s	n.s	0.747***	0.333**
HWEC	0.573***	n.s	0.619***	n.s	0.517**	0.175*	0.599***	n.s
HWEN	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
TC	0.713***	n.s	0.694***	n.s	0.456*	0.252*	0.743***	n.s
$N_t$	0.607***	0.202*	0.635***	n.s	0.412*	0.283*	0.645***	0.173*
$NH_4^+$	0.472**	n.s	n.s	0.479***	n.s	n.s	0.515**	n.s
$NO_3^-$	0.360*	n.s	n.s	0.333**	n.s	n.s	n.s	n.s

Geochemical property definition: HWEC/N = Hot water extractable carbon/nitrogen, TC = Total carbon,  $N_t$  = Total nitrogen,  $NH_4^+$  = Ammonium,  $NO_3^-$  = Nitrate.

Significance levels: n.s:  $P > 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

## 2.4 Discussion

### 2.4.1 Organic input quality effects on microbial communities

Low quality (*Z. mays*, ZM) and intermediate quality (*C. calothyrsus*, CC) as opposed to high quality (*T. diversifolia*, TD) organic inputs decreased the abundance of bacterial *amoA* genes (AOB). In support of this finding, CC revealed lower TC and HWEC values than TD. Accordingly, over 45 % increase in AOB abundance was attributed to TC and HWEC independently. This corroborated the vital role of organic C in regulation of AOB abundance. This finding was in line with the significant organic input quality influence on the community composition of AOB. In this respect, TD promoted AOB abundance compared to ZM which matched a significant community composition separation. This difference was explained by contrasting biochemical qualities of organic inputs, where both input types (TD, ZM) had similar lignin and polyphenol proportions, but differed greatly in C/N ratio (Gentile et al., 2011a, 2011b) availing high and easily accessible  $\text{NH}_3^-$  to AOB compared to ZM. Support for this was further given by AOB community composition similarities between TD and CC which both had contrasting lignin and polyphenol proportions, but similar C/N ratios. This fact also revealed that presence of polyphenols, leading to input type specific formation of polyphenol-protein complexes (Chivenge et al., 2011; Gentile et al., 2011a; Mutabaruka et al., 2007), did not pose a regulating effect on AOB community composition; although they reduced its abundance due to reduced C and N accessibility. Similarly, Wessén et al. (2010) reported a distinct AOB community composition between straw with a lower C/N ratio (high ammonia availability) compared to peat treatments in the Ultuna long-term experiment in Sweden.

Contrastingly, abundance of archaeal *amoA* genes (AOA) was promoted by both ZM and TD inputs in comparison to CC inputs. This finding was linked to increased soil pH in TD and ZM treatments as opposed to CC, emphasizing the sensitivity of AOA to pH changes in comparison to AOB as further discussed in the next section of this paper. AOA abundance was supported by a weak but significant community composition separation between TD versus CC and ZM. These findings revealed that the difference in C/N ratios between low and high quality organic inputs (Gentile et al., 2011a, 2011b), which controls the availability of organic N, did not pose a regulating effect on AOA abundance, although it altered its community composition. Similar effects of different C/N ratios on AOA community composition were reported by Wessén et al. (2010).

AOA are capable of exploring alternative sources of energy and nutrients especially in N limited soils (Levičnik-Höfferle et al., 2012) as was observed under ZM treatment. The variations in abundance and composition of AOB and AOA in response to organic input quality suggested their niche differentiation in soils with organic N limitation. These findings contrasted others, where formation of polyphenol-protein complexes induced positive shifts of other microbial groups such as proteolytic bacterial and fungal decomposer communities in sandy soils (Kamolmanit et al., 2013; Mutabaruka et al., 2007; Rasche et al., 2014). This uncertainty raises the need for prospective work to verify if effects of polyphenol-protein complexes are specific to particular microbial groups and soil types. Overall, AOA abundance dominated that of AOB in our study. This finding was supported by strong relationships between AOA abundance and soil chemical properties (i.e., HWEC, TC and N<sub>t</sub>) compared to that of AOB. It could therefore be deduced that AOA are more adapted to acquisition of organic nutrients compared to AOB particularly when the soils are moisture limiting, considering that we conducted soil sampling during a dry spell. This scenario was linked to substrates limitation associated with water limitation (Stark and Firestone, 1995) meaning that AOA are water stress tolerant compared to AOB. In support of this, Gleeson et al. (2010) demonstrated sensitivity of AOB abundance to water availability whereas AOA remained unaffected. Therefore, the response of AOB and AOA to organic input quality may also have been shaped by environmental factors like soil moisture contributing to their niche differentiation in soils (Erguder et al., 2009; Gleeson et al., 2010; Wessén et al., 2010).

Similar to the ammonia-oxidizing communities, the abundance of total bacterial 16S rRNA genes was significantly lower in CC compared to TD, whereas total archaeal abundance was significantly lower in both CC and ZM in comparison to TD. Contrastingly, community composition of the same genes remained unchanged. These findings were supported by geochemical properties (i.e., HWEC, TC) showing significant responses to biochemical quality of studied organic inputs, and were further supported by positive correlations between geochemical properties and gene abundance. The observed organic input effects were mainly explained by high polyphenols and lignin contents in CC as opposed to ZM and TD. Resulting polyphenol-protein complexes in CC shield organic N (i.e., proteins), but also C from microbial access (Hagerman, 2012; Schmidt et al., 2013; Verkaik et al., 2006). A previous incubation study with soil from the same field experiment revealed that organic input quality efficiently controlled short-term soil C dynamics, although the anticipated mid-term (three year field trial) effect on the soil organic matter pool was not detectable (Chivenge et al. 2011; Gentile et al., 2011a, 2011b). On the other hand, Nziguheba et al. (2005) did also not observe any effect of

input quality on soil C and N dynamics after five seasons of incorporation of similar organic inputs differing in their biochemical quality. Moreover, Rasche et al. (2014) reported an absence of organic input quality effects on bacterial 16S rRNA gene abundance and community composition in the same field experiment after 9 years. Consequently, we argue that organic input quality regulated the abundance of particular groups of the soil microbial community, but was at the current state of the field experiment not the primary driving actor in altering the community composition, although short-term changes may occur directly after input application (Chivenge et al., 2011; Gentile et al., 2011a).

We suggest that the observed stability of the total microbial community composition irrespective of organic input quality was most probably related to the clayey structure of the soil under the long-term field experiment providing a strong background signal of soil organic carbon (SOC) (Bossuyt et al., 2001; Fonte et al., 2009; Gentile et al., 2011a, 2011b). This suggested that the soil type per se but not organic input quality might have regulated to a large extent the composition of the total microbial community (Rasche et al., 2014). This assumption was supported by Neumann et al. (2013) and Sessitsch et al. (2001) who confirmed that particle size fractions were more responsible for distinct alterations of bacterial community composition than the use of organic amendments. The dominance of clay in our soil could have thus masked the hypothesized alterations of total microbial community composition in response to different organic inputs. However, this speculative assumption clearly raises the need for prospective research if distinct soil types with their individual soil textures and aggregation processes and hence SOC stabilization are more responsible for the determination of the composition of microbial communities including ammonia-oxidizers over other factors such as the use of contrasting organic soil amendments (Rasche et al., 2014). Additionally, it will be necessary to enhance the currently limited understanding on the potential of biochemically contrasting organic inputs and accompanied microbial decomposition processes to shape the physical properties (e.g., aggregation) of soils (Kunlanit et al., 2014; Puttaso et al., 2013).

#### **2.4.2 Mineral N fertilizer depresses microbial abundance except AOB**

Use of mineral N in combination with organic inputs was marked by a significant reduction in soil pH (4.8) compared to treatments which did not receive mineral N (pH = 5.3). This was associated with a substantial depression of the abundance of AOA as well as total bacteria and archaea. The latter two revealed also a significant alteration in their community composition. Similar trends were observed in the Ultuna long-term field experiment (Hallin et al., 2009).

Our findings were attributed to the indirect effects of long-term application of  $\text{NH}_4^+$  fertilizers through soil pH reduction (Geisseler and Scow, 2014). The acidification effect due to  $\text{NH}_4^+$  fertilizer might have per se inhibited microbial growth, but may have also promoted nitrifying activities through provision of  $\text{NH}_4^+$  substrate explaining high  $\text{NO}_3^-$  in the plots receiving mineral N fertilizer. This direct effect was also supported by lack of significant differences in  $\text{NO}_3^-$  concentrations between the studied organic treatments. Though AOA dominated this strongly acidic soil compared to AOB, a finding in line with Prosser and Nicol (2012), stability of AOB demonstrated its obviously higher resilience to reduction in soil pH compared to AOA (Wessén et al., 2010).

Overall, use of mineral N fertilizer in this study altered AOB but not AOA community composition according to CAP and PERMANOVA analyses. In line with our finding, Wu et al. (2011) found distinct shifts in community composition of AOB, but not for AOA in fertilized soils ( $180 \text{ kg urea N ha}^{-1} \text{ yr}^{-1}$  combined with rice straw over no input controls) in a 22 year old field experiment in China. Similarly, it was recently shown that AOA community composition was not stimulated by inorganic  $\text{NH}_3^-$  amendments (Stopnišek et al., 2010; Verhamme et al., 2011). In our study, the variation in abundance and community composition of AOB and AOA in response to mineral fertilizer suggested their niche specialization despite their functional redundancy. In another laboratory microcosm experiment, AOB community composition responded rapidly to  $\text{NH}_4^+$  amendments within a few weeks but their AOA counterpart remained unaffected (Avrahami et al., 2003). Similarly, Jia and Conrad (2009) and Wang et al. (2009) demonstrated changes in AOB but not AOA composition with use of  $\text{NH}_4^+$  fertilizer. This suggested that AOB mainly utilized  $\text{NH}_3^-$  from fertilizer application, while AOA most probably utilized  $\text{NH}_3^-$  as N source from mineralization of organic inputs and soil organic matter. These assumptions were justified by negative correlations (data not shown) between  $\text{NH}_4^+$  and AOA indicating that application of  $\text{NH}_4^+$  did not promote AOA (Schleper, 2010; Valentine, 2007). These results corroborated that AOB had a competitive advantage to immobilize N supplied with N fertilizer, hence promoting their abundance although the promotion did not supersede that of sole use of organic inputs. Additionally, mineral fertilizer application was shown to reduce soil aggregate stability through accelerated aggregate turnover potentially reducing SOC stabilization (Bossuyt et al., 2001; Fonte et al., 2009). Moreover, oxygen limitation due to disruption of microbial protection sites associated with reduced aggregate stability could also have led to the reduction of microbial abundance. The two speculations need however further corroborating research.

## 2.5 Conclusions and recommendations

Contrasting biochemical quality of organic inputs (CC, ZM, TD) regulated the abundance of ammonia-oxidizers and total communities. Overall, the high quality organic input (TD) increased while the intermediate quality organic input (CC) reduced the abundance of assayed genes. However, influence of low quality organic input (ZM) on abundance was specific to the different genes. On the other hand, effects of organic input types on community composition were only of minor extent compared to those on abundance. It was thus concluded that the duration of the SOM field experiment of ten years might not have been sufficient to induce distinctions in the composition of assayed total prokaryotic communities. This assumption was supported by Kamaa et al. (2011) and Shen et al. (2010). Their findings on long-term field experiments revealed similar compositions of total bacterial communities in farm yard and composted pig manure treated soils compared to unfertilized controls. Hence, a prolonged duration of the SOM field experiment is strongly required to determine the actual time point when organic input quality effects on composition of the total soil microbial decomposer communities as studied by 16S rRNA genes can be verified.

This study focused on the assessment of the functional potential of soil ammonia-oxidizing communities as was based on DNA-based quantification of *amoA* gene abundance. To further understand the dynamics of ammonia-oxidizers as regulated by the biochemically contrasting organic inputs, we recommend rRNA-based studies along with nucleic acid stable isotope probing (España et al., 2011) to demarcate those community members which are actually activated by application of individual organic inputs. This strategy should also include phylogenetic characterizations of microbial communities (Junier et al., 2010).

It is worth noting that this study was established on one occasion soil sampling to elaborate the long-term effects of tested treatments. We recommend prospective research with repeated measurements during critical growth stages of the test crop (Hai et al., 2009) to consider seasonal dynamics of microbial decomposer communities to separate temporal responses to freshly applied organic inputs (direct effects) versus those to altered soil organic matter quality induced by different organic and inorganic inputs in the long-term (secondary effects) (Kunlanit et al., 2014). This will provide the required baseline to better understand the presumed, but not detected interacting effects of organic and inorganic inputs on soil decomposer microbial communities. Such studies need to include, apart from physico-chemical soil properties, also other factors such as agro-ecologies as well as rainfall and temperature patterns which may influence short-term alterations of targeted soil microbial decomposer communities.



### **Chapter 3 Dynamics of bacterial and archaeal *amoA* gene abundance after additions of organic inputs combined with mineral nitrogen to an agricultural soil<sup>2</sup>**

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## Abstract

Dynamics of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) abundance was assayed in a tropical Humic Nitisol during two cropping seasons of a long-term field experiment situated in the central highlands of Kenya. Since 2002, soils were treated yearly with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) of *Tithonia diversifolia* (TD; C/N ratio: 13, lignin: 8.9 %; polyphenols: 1.7 %), *Calliandra calothyrsus* (CC; 13; 13; 9.4) and *Zea mays* (ZM; 59; 5.4; 1.2) combined with and without 120 kg CaNH<sub>4</sub>NO<sub>3</sub> ha<sup>-1</sup> season<sup>-1</sup>. In 2012 and 2013, soils (0-15 cm) were sampled at young growth (EC30) and flowering (EC60) stages of maize and subjected to DNA-based *amoA* gene quantification. ZM and TD increased AOB abundance by 9 % and 19 %, respectively, compared to CC, while AOA remained unaffected. This was ascribed to high organic N in TD and lower lignin and polyphenol contents in ZM than in CC. In CC, formation of polyphenol-protein complexes limited microbial access to N. Sole use of mineral N or its combination with organic inputs decreased AOA abundance by 35 % but not AOB as a consequence of pH reduction by mineral N. Overall, AOB was more responsive than AOA to input quality that became most pronounced under optimal soil moisture conditions found in 2013 and at EC60 in 2012. We recommend prolonged study periods, considering also rRNA-based analyses to explore the dynamics of active nitrifying communities as a consequence of interrelations of contrasting organic inputs, crop growth stages and seasonality in agricultural soils.

**Keywords:** Ammonia-oxidizing bacteria and archaea; Gene abundance; Organic input quality; Mineral nitrogen fertilizer; Crop growth stages; Seasonality.

## 3.1 Introduction

Ammonia (NH<sub>3</sub>) oxidation is the first and the most rate-limiting step of nitrification in the terrestrial nitrogen (N) cycle (Martens-Habbena et al. 2009). It is catalyzed by bacteria (AOB) and archaea (AOA) through synthesis of the *amoA* gene encoding the  $\alpha$ -subunit of the enzyme ammonia monooxygenase (Prosser and Nicol 2008; Zhang et al. 2013). In agricultural soils, NH<sub>3</sub> as critical energy source for nitrifying prokaryotes is mainly supplied by mineral N fertilizers and organic inputs (e.g., crop residues, green manures). The amount and particularly the biochemical quality (e.g., content of N, cellulose, lignin, polyphenols) of organic inputs is a central regulator of decomposition and mineralization rates, hence N release (Palm et al. 2011b; España et al. 2011; Rasche and Cadisch 2013; Kunlanit et al. 2014). Combined effects

of organic inputs of contrasting biochemical quality and mineral N fertilizer on temporal variation of AOB and AOA abundance under field conditions are however not adequately understood (Hai et al. 2009; Wessén et al. 2010).

In reference to the source and quantity of  $\text{NH}_4^+$  (Di et al. 2010), AOA generally dominate their AOB counterparts in low ammonium ( $\text{NH}_4^+$ ) conditions or when  $\text{NH}_4^+$  was derived through mineralization of organic N (Schauss et al. 2009; Zhang et al. 2010; Levičnik-Höfferle et al. 2012). On the other hand, AOB have been shown to dominate under high  $\text{NH}_4^+$  conditions, especially when supplied through mineral fertilizers (Schleper et al. 2005; Jia and Conrad 2009; Di et al. 2009). Hai et al. (2009) confirmed a promoted abundance of AOB by urea-derived  $\text{NH}_4^+$ , while AOA abundance was primarily stimulated by organic inputs derived from sorghum straw and cattle manure. These results clearly corroborate the acknowledged niche partitioning between AOB and AOA in reference to the source and concentration of  $\text{NH}_4^+$  in soils (Martens-Habbena 2009; Schleper 2010; Prosser and Nicol 2012).

The C/N ratio of organic inputs has been used as a common indicator explaining AOB and AOA abundance dynamics (Millar and Baggs 2004; Wessén et al. 2010; Strauss et al. 2014). It is, however, not yet fully understood to which extent other biochemical quality attributes like polyphenols and lignin as constituents of organic input derived C limit the N availability through formation of polyphenol-protein bonds (Palm et al. 2001a; Millar and Baggs 2004; Schmidt et al. 2013) and how this controls the abundance of AOB and AOA. Polyphenol-induced N limitation induced a critical stress response through an increase in abundance of proteolytic bacteria in sandy agricultural soils (Rasche et al. 2014). On the other hand, abundance of AOB and AOA did not respond to exposure to purified polyphenols during short-term incubation periods (Schmidt et al. 2013). This lack of response of AOB and AOA abundance was probably the consequence of the absence of complexation of artificially introduced polyphenols with proteins in soils (Schmidt et al. 2013). This situation is, however, likely to change with the exposure of AOB and AOA to freshly incorporated protein rich organic inputs differing in their potential to bind proteins by polyphenols and lignin in soils. This hypothesis needs further verification, especially when organic inputs are combined with mineral N fertilizers to compensate organic input induced N limitation (Palm et al. 2001a; Partey et al. 2013).

To gain in depth understanding of the suggested effects of either organic or mineral inputs on nitrifying communities in soils, abiotic factors need to be considered. Water stress through reduced precipitation decreased AOB and AOA abundance in soils under young growth (EC30) to the flowering (EC60) stage of sorghum (Hai et al. 2009). Rasche et al. (2011) reported

increased AOB abundance with increased precipitation, while that of AOA increased with decreasing temperatures in a temperate beech forest. Notably, Rasche et al. (2011) in contrast to others (e.g., Leininger et al. 2006; Wessén et al. 2010), focused on the interrelated effects of seasonality and resource availability on nitrifying soil communities in a two year study, rather than deriving conclusions only on a single study year. Such extended study periods need to be also considered for tropical environments, where unreliable climatic conditions (e.g., erratic precipitation patterns) may interfere with target research questions on e.g. organic and mineral input dependent soil microbial C and N dynamics. In this respect, repeated measurements at distinct crop growth stages during individual cropping seasons may allow a sound interpretation of dynamics of functionally relevant microbial groups (e.g., AOB, AOA) under the control of specific organic and mineral fertilization schemes.

The primary objective of this 2-year study was to assess the dynamics of AOB and AOA abundance (DNA-based *amoA* gene quantification) under the influence of biochemically contrasting organic inputs with and without mineral N fertilizer in a tropical agricultural soil. To evaluate the presumed fluctuations of AOB and AOA during the initial decomposition phase of both study seasons, we conducted soil samplings at EC30 and EC60 stages of maize used as test crop. Firstly, we hypothesized higher AOB and AOA abundance under high quality organic inputs at EC30 due to the faster organic N release as opposed to intermediate quality inputs with high polyphenol and lignin contents and low quality inputs with low N contents. We also presumed a reduction of AOB and AOA abundance by high quality inputs at EC60 due to negligible remnants of available N following faster decomposition rates at EC30. A contrasting effect was presumed for intermediate and low quality inputs as traced back to the gradual decomposition and mineralization. Secondly, we hypothesized an increase of AOB but not AOA abundance in soils treated with a combination of intermediate or low quality inputs with mineral N fertilizer compared to their counterparts without fertilizer N. This was justified by the presumed compensation effect of mineral N fertilizer under N limiting conditions induced by intermediate and low quality inputs.

## **3.2 Materials and methods**

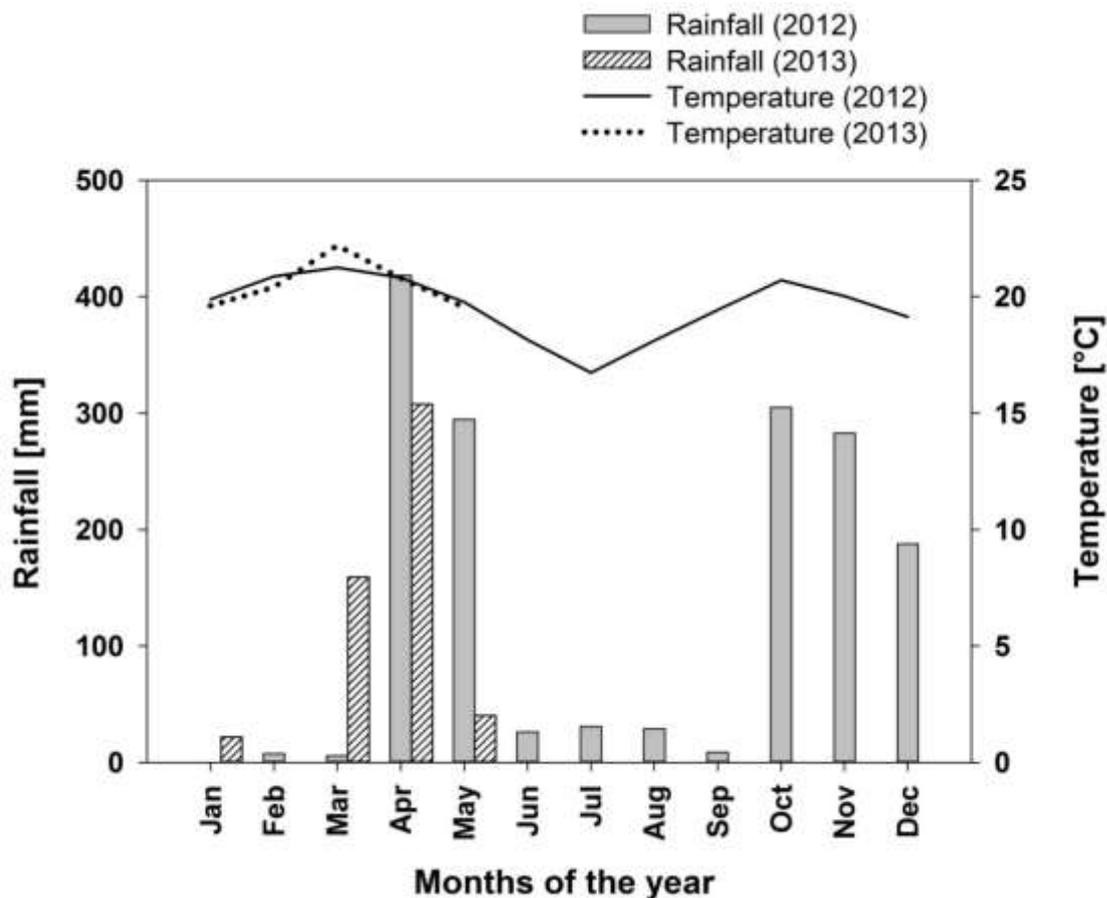
### **3.2.1 Site description and field experiment design**

The experimental site was located in Embu (0°30' S, 37°27' E; 1380 m above sea level) in the central highlands of Kenya (130 km northeast of Nairobi). The soil is defined as a Humic Nitisol (FAO 2006; Jones et al. 2013) dominated by kaolinite minerals derived from basic

volcanic rocks. The texture of the topsoil layer (0-15 cm) was characterized by 17 % sand, 18 % silt and 65 % clay and contained 29.4 g kg<sup>-1</sup> organic C, 2.7 g kg<sup>-1</sup> total N and had a pH (H<sub>2</sub>O) of 5.81 at the start of the field experiment in 2002 (Chivenge et al. 2009; Gentile et al. 2011a). The site has an annual mean temperature of 20°C and a mean annual rainfall of 1200 mm. Rainfall is bimodal with long season rains received from mid-March to June and short season rains from mid-October to December (Fig. 3.1). Comparing the long rain seasons, i.e., mid-March to mid-June, during which the soil samplings were conducted, year 2013 was drier (507 mm rainfall) than 2012 (720 mm).

The soil organic matter (SOM) field experiment was established in year 2002 to primarily study the effect of continuous annual application of biochemically contrasting organic inputs with and without inorganic mineral N fertilizer on crop performance as well as soil C and N dynamics (Chivenge et al. 2009; Gentile et al. 2011a; Chivenge et al. 2011). Five different organic inputs including *Grivellia robusta* (sawdust) and goats manure, *Tithonia diversifolia*, *Calliandra calothyrsus* and *Zea mays* were considered at two application rates (i.e., 1.2 and 4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) including a control treatment. The experiment is laid out in a split plot design with three replicates per treatment with organic inputs as main plots (size, 12 m x 5 m) and mineral N fertilizer as sub-plots (size, 6 m x 5 m) placed inside the organic input plots (Chivenge et al. 2009; Gentile et al. 2009). A corridor of 50 cm in width separated the plots to avoid interference of treatments. The entire experiment consisted of 66 plots (i.e., the five organic inputs at two application rates plus a control, all with versus without mineral N (i.e., 0, 120 kg N ha<sup>-1</sup> season<sup>-1</sup>) which were replicated three times) (Chivenge et al. 2009). In the presented study which focused only on twenty four plots, we considered the treatments with high quality *Tithonia diversifolia* (C/N ratio: 13, Lignin: 8.9 %; Polyphenols: 1.7 %), intermediate quality *Calliandra calothyrsus* (13; 13 %; 9.4 %) and low quality *Zea mays* (59; 5.4 %; 1.2 %) inputs and a control (Gentile et al. 2011b). At the onset of long rains in each year, organic inputs (leaves, petioles and small branches for *C. calothyrsus* in addition to stems for *T. diversifolia* and *Z. mays* stover) are collected and analyzed for dry matter as well as total C and N content. This data is used to determine the amount of each organic material to be applied in fresh weight condition at a rate of 4 Mg C ha<sup>-1</sup> year<sup>-1</sup>. These included; (80, 81 and 102) kg of fresh weight of TD, CC and ZM respectively sub-plot<sup>-1</sup> year<sup>-1</sup>. Prior to maize sowing, organic inputs were chopped into small pieces, broadcast and manually incorporated at a soil depth of 0-15 cm (Gentile et al. 2011a; Chivenge et al. 2011). In the sub-plots of each plot, mineral N fertilizer was applied as calcium ammonium nitrate (CaNH<sub>4</sub>NO<sub>3</sub>) at a rate of 0 and 120 kg N ha<sup>-1</sup> growing season<sup>-1</sup> (Chivenge et al. 2009; Gentile et al. 2011a; Gentile et al.

2011b). One third of the mineral N fertilizer was applied 3 weeks after sowing of maize (test crop) and the remainder 8 weeks later (Chivenge et al. 2009). Mineral N fertilizer was recommended as a complement for the low and intermediate quality organic inputs to compensate the presumed N limitation (Palm et al. 2001a; Partey et al. 2013). For treatment comparison purposes in the presented study, mineral N fertilizer was also combined with the high quality organic input. All plots received a blanket basal application of 60 kg P ha<sup>-1</sup> season<sup>-1</sup> and 60 kg K ha<sup>-1</sup> season<sup>-1</sup> before sowing.



**Figure 3.1:** Rainfall and temperature distribution at Embu site from year 2012 to 2013.

### 3.2.2 Soil sampling

After incorporation of fresh organic inputs and application of mineral N fertilizer in 2012 and 2013, soil samples were collected twice each season (i.e., EC30: last week of April 2012, mid-April 2013; EC60: first week of June 2012, last week of May 2013) (Milling et al. 2005) from the plots designated for maize cropping. The following 8 treatments were considered: 4 treatments without mineral N fertilizer including the no input control (CON) and 3 organic

input treatments (i.e., *C. calothyrsus* (CC), *Z. mays* (ZM) and *T. diversifolia* (TD)). The other 4 treatments were their respective counterparts receiving mineral N fertilizer. In each plot, 10 soil sub-samples were randomly obtained at a depth of 0-15 cm using a soil auger. These 10 sub-samples were bulked to one composite, representative sample per plot, sieved (2-mm mesh) and stored at 4°C. In total, 96 soil samples were obtained. Prior to analysis, each composite soil sample was split into 3 proportions. A fresh sample was used for immediate extraction of ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). The second proportion was air-dried for analysis of pH, hot water extractable C and N (HWEC/N), total C (TC) and total N (N<sub>t</sub>). The third proportion was freeze-dried and shipped to Germany for microbiological analyses.

### **3.2.3 Gene abundance analysis**

Total soil DNA was isolated from 0.5 g of each freeze dried soil and quantified as described by Muema et al. (2015). Quantified DNA was stored at -20°C until further analyses. For DNA-based quantification of target genes (total bacteria and archaea (16S rRNA gene) as well as ammonia-oxidizing bacteria (bacterial *amoA* gene; AOB) and archaea (archaeal *amoA* gene; AOA), plasmid standards were prepared (Table 3.1), purified (Invisorb Fragment CleanUp kit, Stratec Molecular GmbH, Berlin, Germany) and quantified (Rasche et al. (2011)). The qPCRs (3 analytical replicates per DNA sample) were optimized, analyzed and quality checked according to Muema et al. (2015). Gene copy numbers and reaction efficiencies (total bacteria 103 % ± 1 %, total archaea 80 % ± 11 %, AOB 97 % ± 8 %, AOA 77 % ± 10 %) were calculated using StepOne Software version 2.2.2 (Applied Biosystems) and presented per gram of dry soil.

**Table 3.1:** Description of primer sets, PCR ingredients and amplification details used for quantitative PCR analyses.

Target gene	Primer (reference)	qPCR
All bacteria	Eub338f (Lane, D, 1991) Eub518r (Muyzer et al., 1993)	95 °C 5 min; 40 cycles: 95 °C 30s, 55 °C 35s, 72 °C 45s
All archaea	Ar109f (Lueders and Friedrich, 2000) Ar912r (Lueders and Friedrich, 2000)	95 °C 5 min; 40 cycles: 95 °C 30s, 52 °C 35s, 72 °C 45s, 78 °C 20s
Ammonia-oxidizing bacteria (AOB)	AmoA-1f (Rotthauwe et al., 1997) AmoA-2r (Rotthauwe et al., 1997)	95 °C 5 min; 45 cycles: 95 °C 30s, 57 °C 45s, 72 °C 45s, 78 °C 20s
Ammonia-oxidizing archaea (AOA)	Arch-amoAf (Francis et al., 2005) Arch-amoAr (Francis et al., 2005)	95 °C 5 min; 45 cycles: 95 °C 30s, 53 °C 45s, 72 °C 45s, 78 °C 20s

### 3.2.4 Soil physico-chemical analyses

Soil pH was measured in water suspensions at a solid-to-liquid ratio of 1:2.5. Hot water extractable C (HWEC) and N (HWEN) analyses were determined according to Schulz (2004) as well as Schulz and Körschens (1998). C content of hot water extracts was analyzed using potassium dichromate in sulfuric acid milieu (Multi N/C analyzer, Analytik Jena, Jena, Germany), while HWEN was analyzed by a modified Kjeldahl digestion (Hepburn 1908). Total C (TC) and total N ( $N_t$ ) of soils were quantified by dry combustion (Flash EA 1112 Elemental Analyzer, Thermo Fisher Scientific).  $NH_4^+$  and  $NO_3^-$  were calorimetrically measured (ICRAF 1999). Soil sub-samples of 20 g each were used for moisture content (MC) determination by weight loss after drying (105°C, 24 hours).

### 3.2.5 Statistical analysis

Data on abundance of the four studied genes and soil chemical properties were subjected to analysis of variance (ANOVA) using Statistical Analysis Software program (SAS Institute 2014). For statistical analysis of the gene abundance data, a generalized linear mixed model with a negative binomial distribution error and a log link function was used. The model was fitted by restricted log pseudo-likelihood with expansion about the solution for the random effects in the SAS GLIMMIX procedure. For the geochemical data, linear mixed models were fitted in the SAS MIXED procedure. The data were checked for normality and

homoscedasticity on model residuals using quantile-quantile (Q-Q) plots, histograms and studentized residual plots. Different transformations were tried and log base 10 selected as for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in an attempt to satisfy the assumption underlying the ANOVA model (Piepho 2009). A full factorial split plot design model was specified with the effects of main factors “Year” (years 2012 and 2013), “Growth stage” of maize (EC30, EC60) which was nested within the season, “Organic input” (TD, CC, ZM, CON), “Mineral N fertilizer” (0 and 120 kg N ha<sup>-1</sup>) and their interactions as fixed effects on the response variables (i.e., gene abundance, geochemical properties). We accounted for the repeated measurements in the same plots by fitting an error with a first order autoregressive covariance structure to the data. Statistical significance of all effects was assessed at a significance level of  $P < 0.05$ . Blocks and blocks x organic inputs interactions were considered as random effects and model selected accordingly using the Akaike Information Criterion (AIC). Treatments means/medians were compared using the PDIF option of the LSMEANS as well as the letters (letter display) from the SAS generalized linear mixed models procedure and SAS macro statement from the SAS linear mixed models procedure for the abundance and geochemical data, respectively.

It is worth mentioning that no significant interactions between factors “Organic input” and “Mineral N fertilizer” were determined for abundance of all four genes and soil chemical properties data except for  $\text{NH}_4^+$  (Table 3.2). Therefore, treatment means (i.e., CON, CC, ZM, TD) are shown regardless of the mineral N fertilizer levels (i.e., 0 N, 120 N) and those of mineral N levels regardless of organic input type (Fig. 3.2 and 3.3; Tables 3.3 and 3.4).

Pearson linear correlation analyses were conducted in the SAS COR procedure to relate the abundance of the target genes (dependent variables) to the soil physico-chemical properties (independent variables).

### **3.3 Results**

#### **3.3.1 Microbial abundance dynamics in response to organic input quality**

Descriptive means (not subjected to ANOVA) of AOB and AOA abundance, which was given as copy numbers of the respective *amoA* genes, are presented as supplementary information (S3.6.1). AOB and AOA abundance revealed significant interactions between factors “Organic input” and “Growth stage” (AOB,  $P < 0.01$ ; AOA,  $P < 0.05$ , Table 3.2). AOB abundance increased in year 2013 ( $1.90 \times 10^8$  gene copies g<sup>-1</sup> dry soil) compared to 2012 ( $1.54 \times 10^6$  gene copies g<sup>-1</sup> dry soil) ( $P < 0.001$ ) (Table 3.3). On the other hand, AOB abundance was lower in EC60 than EC30 during both years ( $P < 0.001$ , Table 3.3). AOB abundance was higher in ZM

than CC, TD and CON at EC60 in 2012 ( $P < 0.05$ , Fig. 3.2a). In year 2013, TD had marginally higher AOB abundance than CC during both growth stages of maize ( $P < 0.05$ , Fig. 3.2b).

In general, AOA abundance was not different between years 2012 and 2013 ( $P > 0.05$ , Table 3.3). Similar to AOB, AOA abundance was lower in EC60 than EC30 during both years ( $P < 0.05$ , Table 3.3). ZM increased AOA abundance compared to CON at EC30 in 2012 ( $P < 0.05$ , Fig. 3.2c), while plots which received ZM and CC at EC30 as well as TD at EC60 in 2013 had higher AOA abundance than CON ( $P < 0.05$ , Fig. 3.2d). Quality of organic inputs did not influence AOA abundance during the study period ( $P > 0.05$ ).

Descriptive means of bacterial and archaeal 16S rRNA genes abundance are presented as supplementary information (S3.6.2). However, no significant interaction effects between all four factors were determined for both 16S rRNA gene abundance ( $P > 0.05$ , Table 3.2). Therefore, only the main effects of organic input quality, “Year” (seasonality) and “Growth stage” on total prokaryotic communities are shown (Fig. 3.3, Table 3.3). Overall, bacterial 16S rRNA gene abundance revealed  $3.78 \times 10^9$  copies  $g^{-1}$  dry soil in 2012, but was low in 2013 amounting in average to  $1.71 \times 10^9$  copies  $g^{-1}$  dry soil ( $P < 0.001$ , Table 3.3). Bacterial 16S rRNA gene abundance remained relatively stable between EC30 and EC60 in 2012, while an increase at EC60 was measured in 2013 ( $P < 0.05$ , Table 3.3). All plots which received organic inputs had higher bacterial 16S rRNA gene abundance than CON ( $P < 0.05$ , Fig. 3.3a), while quality of organic inputs had no effect ( $P > 0.05$ ).

In contrast to total bacteria, total archaeal 16S rRNA gene abundance was low in 2012 ( $1.47 \times 10^8$  copies  $g^{-1}$  dry soil), but increased in 2013 ( $1.71 \times 10^{10}$  copies  $g^{-1}$  dry soil) ( $P < 0.001$ , Table 3.3). Archaeal 16S rRNA gene abundance was high at EC60 in 2012 but low at EC60 in 2013 ( $P < 0.05$ , Table 3.3). Archaeal 16S rRNA gene abundance was lower in CON than ZM and TD treatments ( $P < 0.05$ ), while biochemical quality of organic inputs did not affect archaeal 16S rRNA gene abundance during the study period ( $P > 0.05$ , Fig. 3.3b).

**Table 3.2:** Statistical evaluation of gene abundance and soil chemical properties by full factorial ANOVA.

Source of variation	Bacterial 16S rRNA gene	Archaeal 16S rRNA gene	Bacterial <i>amoA</i> gene (AOB)	Archaeal <i>amoA</i> gene (AOA)	Soil pH	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	TC	N <sub>t</sub>	HWEC	HWEN	MC
Units	[copies g <sup>-1</sup> dry soil]		[mg kg <sup>-1</sup> ]	[mg kg <sup>-1</sup> ]	[%]	[%]	[mg kg <sup>-1</sup> ]	[mg kg <sup>-1</sup> ]	[%]			
Organic inputs (OI)	*	n.s	***	*	*	n.s	n.s	*	**	**	**	**
Mineral fertilizer(N)	*	**	n.s	**	***	**	***	n.s	n.s	n.s	***	**
Year (Y)	***	***	***	n.s	n.s	n.s	***	n.s	***	***	***	***
Growth stages (EC)	**	**	***	***	***	***	***	***	***	***	***	***
OI x N	n.s	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s	n.s	n.s
OI x Y	n.s	n.s	***	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	*
OI x EC	n.s	n.s	***	*	n.s	*	**	n.s	n.s	**	*	*
N x Y	n.s	n.s	n.s	n.s	n.s	*	*	n.s	n.s	n.s	n.s	n.s
N x EC	n.s	n.s	n.s	n.s	n.s	**	***	n.s	n.s	n.s	**	n.s
OI x N x EC	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

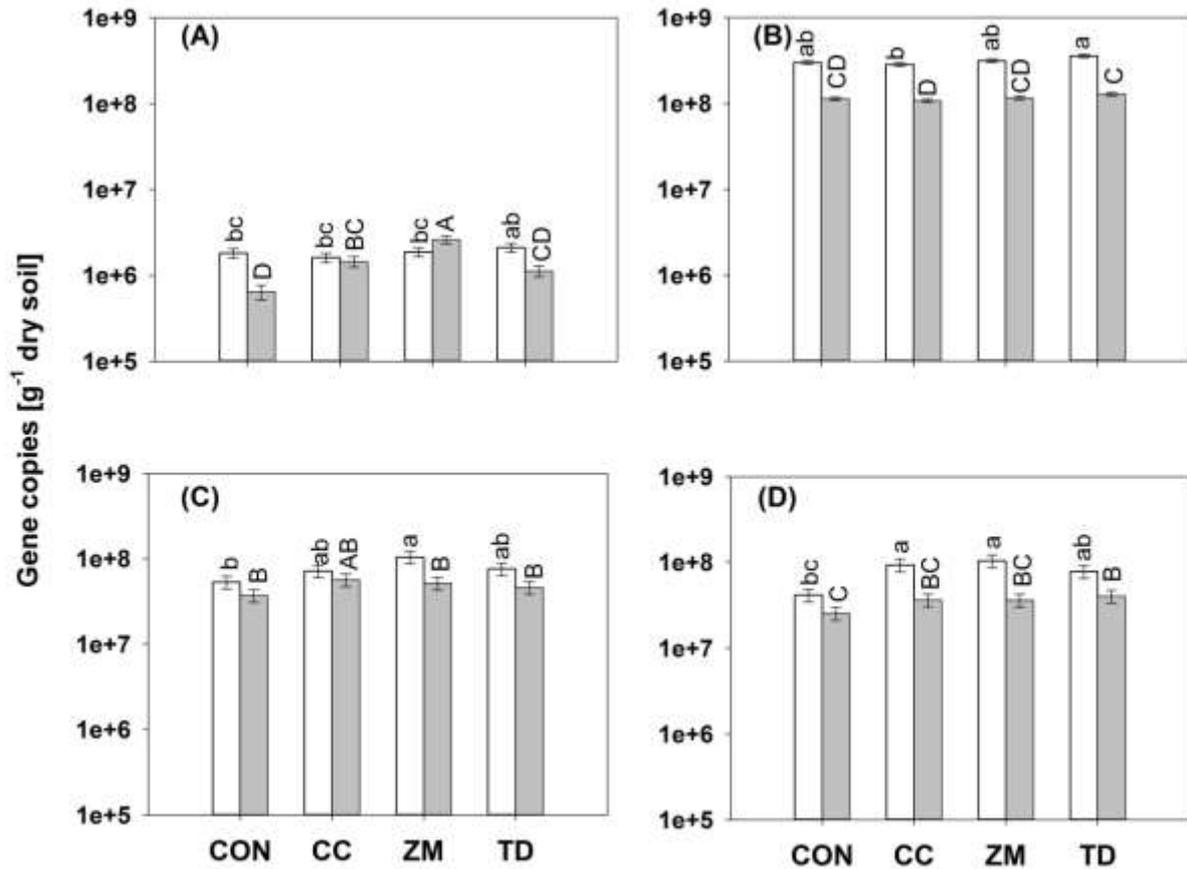
Factors: Organic inputs (TD, CC, ZM, CON); Mineral N fertilizer (0 kg N ha<sup>-1</sup>, 120 kg N ha<sup>-1</sup>); Year/season (years 2012 and 2013); Growth stages of maize (EC30, EC60). Soil property definition: NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrates, TC = Total carbon, N<sub>t</sub> = Total nitrogen, HWEC = Hot water extractable carbon, HWEN = Hot water extractable nitrogen, MC = Soil moisture content.

Significance levels: n.s: P > 0.05; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

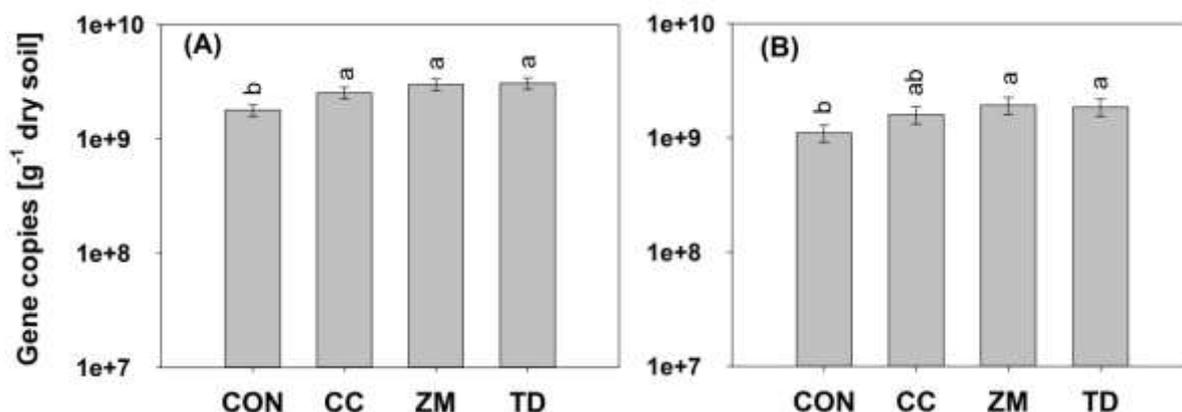
**Table 3.3:** Abundance of the four assayed genes as influenced by crop growth stage, season and mineral N fertilizer. Values are means along with standard errors. Total number of observations (n) = 96.

Effects of Factors	Total bacteria	Total archaea	Bacterial <i>amoA</i> (AOB)	Archaeal <i>amoA</i> (AOA)
EC30 <sub>2012</sub>	3.98 ± 0.32 x 10 <sup>9a</sup>	1.29 ± 0.15 x 10 <sup>8d</sup>	1.85 ± 0.11 x 10 <sup>6c</sup>	7.43 ± 0.63 x 10 <sup>7a</sup>
EC60 <sub>2012</sub>	3.60 ± 0.29 x 10 <sup>9a</sup>	1.67 ± 0.19 x 10 <sup>8c</sup>	1.29 ± 0.09 x 10 <sup>6d</sup>	4.73 ± 0.40 x 10 <sup>7b</sup>
EC30 <sub>2013</sub>	1.44 ± 0.12 x 10 <sup>9c</sup>	1.95 ± 0.23 x 10 <sup>10a</sup>	3.13 ± 0.07 x 10 <sup>8a</sup>	7.50 ± 0.64 x 10 <sup>7a</sup>
EC60 <sub>2013</sub>	2.02 ± 0.16 x 10 <sup>9b</sup>	1.50 ± 0.17 x 10 <sup>10b</sup>	1.16 ± 0.03 x 10 <sup>8b</sup>	3.42 ± 0.29 x 10 <sup>7c</sup>
<b>Year</b>				
Year <sub>2012</sub>	3.78 ± 0.25 x 10 <sup>9a</sup>	1.47 ± 0.15 x 10 <sup>8b</sup>	1.54 ± 0.08 x 10 <sup>6b</sup>	5.93 ± 0.44 x 10 <sup>7a</sup>
Year <sub>2013</sub>	1.71 ± 0.11 x 10 <sup>9b</sup>	1.71 ± 0.17 x 10 <sup>10a</sup>	1.90 ± 0.04 x 10 <sup>8a</sup>	5.06 ± 0.38 x 10 <sup>7a</sup>
<b>Mineral N</b>				
0 N	2.95 ± 0.24 x 10 <sup>9a</sup>	1.96 ± 0.24 x 10 <sup>9a</sup>	1.67 ± 0.07 x 10 <sup>7a</sup>	6.80 ± 0.61 x 10 <sup>7a</sup>
120 N	2.19 ± 0.18 x 10 <sup>9b</sup>	1.26 ± 0.16 x 10 <sup>9b</sup>	1.76 ± 0.07 x 10 <sup>7a</sup>	4.41 ± 0.40 x 10 <sup>7b</sup>

Factors: Growth stages of maize (young growth stage (EC30), flowering stage (EC60)); Year (years 2012 and 2013); Mineral N fertilizer (0 kg N ha<sup>-1</sup>, 120 kg N ha<sup>-1</sup>). For each respective gene, abundance means values are an average of all organic treatments (CC, ZM, TD and CON) values with and without mineral N for specific growth stage and specific year as labeled. Those of specific years under the effect “Year”, are an average of all organic treatments both with and without mineral N regardless of crop growth stage, while those of mineral N are an average of all treatments first, only treatments which did not receive mineral N (0 kg N ha<sup>-1</sup>) regardless of crop growth stage and year/season, and second an average of all treatments which received (120 kg N ha<sup>-1</sup>) only during both years regardless of the crop growth stage and year/season. Different letters (a-d) against the values (means) indicate treatments/groups with significant differences within each factor (P < 0.05).



**Figure 3.2:** Gene copy numbers of the ammonia-oxidizing genes in different treatments (Mean,  $n = 6$ ). Total number of observations = 96. (A) Bacterial *amoA* (AOB) gene in year 2012, (B) AOB in year 2013, (C) Archaeal *amoA* (AOA) in year 2012, and (D) AOA in year 2013 after application of biochemically contrasting organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). White bars = young growth stage (EC30) of maize; Grey bars = flowering stage (EC60). Different letters (a-c/A-D) above bars for the EC30 and EC60, respectively, indicate treatments with significant differences ( $P < 0.05$ ). Treatments codes: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*.



**Figure 3.3:** Gene copy numbers of the total communities in different treatments (Mean,  $n = 24$ ). Total number of observations = 96. (A) total bacteria (Bacterial 16S rRNA gene), (B) total archaea (Archaeal 16S rRNA gene) after application of biochemically contrasting organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). Different letters (a-b) above bars indicate treatments with significant differences ( $P < 0.05$ ). Treatments codes: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*.

### 3.3.2 Effect of mineral N fertilizer on microbial communities

Bacterial and archaeal 16S rRNA as well as AOB and AOA gene abundance showed no interaction between factors “Organic input” and “Mineral N fertilizer” ( $P > 0.05$ , Table 3.2, Fig. 3.2 and 3.3). Therefore, the main effects of mineral N (120 N) as opposed to the zero N rates are shown (Table 3.3). Mineral N or its combination with biochemically contrasting organic inputs reduced 16S rRNA gene abundance (bacteria, 26 %; archaea, 35 %) and also AOA (35 %) compared to their counterparts without mineral N ( $P < 0.05$ ). AOB abundance remained unaffected upon combination of organic inputs with mineral N in comparison to zero mineral N ( $P > 0.05$ ).

### 3.3.3 Effect of input quality and mineral N fertilizer on soil physico-chemical properties

Soil pH, TC and  $N_t$  had no interactions between factors ( $P > 0.05$ , Table 3.2). Therefore, the data of main effects of the factors are presented (Table 3.4). Their descriptive means are however, presented as supplementary information (Table S3.6.1). CC had lower pH than TD ( $P < 0.05$ ). Application of mineral N reduced pH compared to the zero N rates ( $P < 0.01$ , Table 3.4). TC and  $N_t$  values were lower in CON than organic input treatments, but were not influenced by mineral N fertilizer ( $P > 0.05$ , Table 3.4). Since the plots which received 0 or  $120 \text{ kg N ha}^{-1}$  had received organic inputs, it is likely that the organic inputs had strong effect on  $N_t$ , masking the effect of mineral N which generally has a fast turnover, as was measured in

the inorganic and labile forms of N (Sądej, 2008). Stronger effect of organic inputs than mineral N on  $N_t$  is in line with the fact that N in soils is mostly organic N. For  $NH_4^+$ ,  $NO_3^-$ , HWEC/N and MC, interactions between factors were determined ( $P < 0.05$ , Table 3.2). Hence, their individual results are provided (Tables 3.5 and 3.6). Quality of organic inputs did not influence  $NH_4^+$  contents ( $P > 0.05$ , Table 3.5). CON, CC and ZM combined with mineral N increased  $NH_4^+$  at EC60 in 2012 as well as ZM and TD at EC30 in 2013 compared to their counterparts without mineral N ( $P < 0.05$ ). Generally,  $NO_3^-$  contents were lower at EC60 in 2012. This was linked to competition for nitrates from the crop as maize was tasseling. In addition, year 2012 had higher precipitation compared to 2013. Apparently, a heavy rainfall a night before the soil sampling was conducted had been experienced which could have led to leaching of nitrates to level below 0-15 cm we had considered in this study. TD and CC had higher  $NO_3^-$  concentrations than CON and ZM at EC30 in 2013 ( $P < 0.05$ , Table 3.5). Application and combination of mineral N with organic inputs increased  $NO_3^-$  contents at EC60 in both years ( $P < 0.05$ ). Soil moisture content was on average 6 % lower in 2013 than 2012 ( $P < 0.001$ ). In addition, it was lower at EC60 than EC30 in both years ( $P < 0.05$ , Table 3.5). TD at EC30 and ZM at EC60 in 2012 had higher HWEC values than CC ( $P < 0.05$ , Table 3.6). Moreover, sole TD had higher HWEC values than sole ZM at EC30 in 2013 ( $P < 0.05$ ). Mineral N fertilizer did not influence HWEC contents ( $P > 0.05$ ). Organic input quality did not influence HWEN values ( $P > 0.05$ , Table 3.6). HWEN values increased in TD treated plots at EC30, in CC treated plots at EC60 in 2012 as well as in CON and CC treated plots at EC60 in 2013 ( $P < 0.05$ ) upon combination with mineral N fertilizer in comparison to their counterparts without mineral N fertilizer.

**Table 3.4:** Soil chemical properties following amendment of soils with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and mineral N fertilizer. Values are means for soils sampled at young growth (EC30) and flowering (EC60) stages of maize crop during two study seasons.

Factors	Soil chemical properties		
	Soil pH	TC [%]	N <sub>t</sub> [%]
Organic input treatments			
CON	4.74 <sup>c</sup>	2.27 <sup>b</sup>	0.19 <sup>b</sup>
CC	4.86 <sup>bc</sup>	2.64 <sup>a</sup>	0.22 <sup>a</sup>
ZM	5.03 <sup>ab</sup>	2.71 <sup>a</sup>	0.22 <sup>a</sup>
TD	5.13 <sup>a</sup>	2.74 <sup>a</sup>	0.23 <sup>a</sup>
SED	± 0.086	± 0.136	± 0.008
Mineral N treatments			
0 N	5.14 <sup>a</sup>	2.60 <sup>a</sup>	0.22 <sup>a</sup>
120 N	4.74 <sup>b</sup>	2.59 <sup>a</sup>	0.22 <sup>a</sup>
SED	± 0.031	± 0.022	± 0.001
Growth stages			
EC30 <sub>2012</sub>	4.89 <sup>b</sup>	2.69 <sup>a</sup>	0.23 <sup>a</sup>
EC60 <sub>2012</sub>	4.98 <sup>a</sup>	2.52 <sup>c</sup>	0.23 <sup>a</sup>
EC30 <sub>2013</sub>	5.04 <sup>a</sup>	2.59 <sup>b</sup>	0.20 <sup>c</sup>
EC60 <sub>2013</sub>	4.83 <sup>b</sup>	2.57 <sup>bc</sup>	0.22 <sup>b</sup>
SED	± 0.036	± 0.029	± 0.002
Season			
2012	4.93 <sup>a</sup>	2.60 <sup>a</sup>	0.23 <sup>a</sup>
2013	4.94 <sup>a</sup>	2.58 <sup>a</sup>	0.21 <sup>b</sup>
SED	± 0.031	± 0.022	± 0.001

Treatments: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*. 0 N = 0 kg N ha<sup>-1</sup>; 120 N = 120 kg N ha<sup>-1</sup>. Soil property definition: TC = Total carbon, N<sub>t</sub> = Total nitrogen. Different letters (a-c) indicate treatments/groups means (least square means (lsmeans)) with significant differences within each factor (P < 0.05).

**Table 3.5:** Ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and soil moisture (MC) content following amendment of soils with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and mineral N. Values are means/medians for soils sampled at young growth (EC30) and flowering (EC60) stages of maize crop.

		NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]				NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]				MC [%]			
		2012		2013		2012		2013		2012		2013	
		EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60
Treatments													
CON	0 N	5.7 <sup>b</sup>	1.2 <sup>bcd</sup>	2.8 <sup>bc</sup>	4.02 <sup>a</sup>	20.4 <sup>c</sup>	2.7 <sup>b</sup>	1.9 <sup>c</sup>	5.3 <sup>b</sup>	39.3 <sup>bc</sup>	33.6 <sup>de</sup>	38.1 <sup>b</sup>	24.4 <sup>ab</sup>
	120 N	7.1 <sup>ab</sup>	2.6 <sup>a</sup>	2.9 <sup>bc</sup>	4.3 <sup>a</sup>	25.6 <sup>bc</sup>	17.0 <sup>a</sup>	2.6 <sup>bc</sup>	19.2 <sup>a</sup>	38.4 <sup>c</sup>	31.9 <sup>e</sup>	37.9 <sup>b</sup>	24.1 <sup>abc</sup>
CC	0 N	9.5 <sup>a</sup>	1.1 <sup>cde</sup>	2.6 <sup>bc</sup>	4.0 <sup>a</sup>	29.1 <sup>bc</sup>	3.3 <sup>b</sup>	3.9 <sup>ab</sup>	6.0 <sup>b</sup>	40.3 <sup>abc</sup>	35.9 <sup>abc</sup>	40.3 <sup>a</sup>	22.7 <sup>c</sup>
	120 N	8.1 <sup>ab</sup>	1.7 <sup>ab</sup>	3.3 <sup>bc</sup>	3.9 <sup>a</sup>	36.0 <sup>abc</sup>	13.0 <sup>a</sup>	4.6 <sup>a</sup>	14.0 <sup>a</sup>	40.2 <sup>abc</sup>	35.2 <sup>bcd</sup>	39.5 <sup>ab</sup>	22.9 <sup>bc</sup>
ZM	0 N	8.0 <sup>ab</sup>	0.7 <sup>e</sup>	3.6 <sup>bc</sup>	3.6 <sup>a</sup>	34.7 <sup>abc</sup>	3.5 <sup>b</sup>	2.1 <sup>c</sup>	6.0 <sup>b</sup>	41.7 <sup>a</sup>	37.5 <sup>a</sup>	40.8 <sup>a</sup>	25.1 <sup>a</sup>
	120 N	8.6 <sup>ab</sup>	1.7 <sup>abc</sup>	5.2 <sup>a</sup>	3.5 <sup>a</sup>	48.4 <sup>ab</sup>	11.1 <sup>a</sup>	2.4 <sup>bc</sup>	14.0 <sup>a</sup>	40.2 <sup>abc</sup>	36.5 <sup>ab</sup>	40.0 <sup>a</sup>	25.1 <sup>a</sup>
TD	0 N	9.5 <sup>a</sup>	1.0 <sup>de</sup>	3.7 <sup>ab</sup>	3.7 <sup>a</sup>	36.3 <sup>abc</sup>	3.6 <sup>b</sup>	4.3 <sup>a</sup>	6.9 <sup>b</sup>	41.2 <sup>ab</sup>	36.8 <sup>ab</sup>	40.4 <sup>a</sup>	24.8 <sup>a</sup>
	120 N	7.6 <sup>ab</sup>	1.2 <sup>bcd</sup>	2.5 <sup>c</sup>	3.8 <sup>a</sup>	79.6 <sup>a</sup>	12.1 <sup>a</sup>	4.6 <sup>a</sup>	11.7 <sup>a</sup>	40.8 <sup>ab</sup>	34.0 <sup>cde</sup>	39.3 <sup>ab</sup>	24.0 <sup>abc</sup>
SED <sub>inputs</sub>										± 1.06			
SED <sub>nitrogen</sub>										± 1.03			

Treatments codes: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*.

Different letters (a-d) against the values indicate treatments/groups with significant differences within each factor (P < 0.05). Properties without SED values show the treatments (medians) after back transformation of the least square means (lsmeans) from analyses to the original scale.

**Table 3.6:** Hot water extractable carbon and nitrogen (HWEC/N) following amendment of soils with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and mineral N. Values are means for soils sampled at young growth (EC30) and flowering (EC60) stages of maize crop.

		HWEC [mg kg <sup>-1</sup> ]				HWEN [mg kg <sup>-1</sup> ]			
		2012		2013		2012		2013	
		EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60
Treatments									
CON	0 N	350 <sup>b</sup>	224 <sup>d</sup>	432 <sup>d</sup>	402 <sup>c</sup>	25 <sup>d</sup>	19 <sup>b</sup>	14 <sup>d</sup>	13 <sup>c</sup>
	120 N	277 <sup>c</sup>	240 <sup>cd</sup>	447 <sup>dc</sup>	406 <sup>bc</sup>	33 <sup>cd</sup>	26 <sup>ab</sup>	16 <sup>cd</sup>	22 <sup>ab</sup>
CC	0 N	364 <sup>b</sup>	300 <sup>bc</sup>	542 <sup>ab</sup>	478 <sup>ab</sup>	39 <sup>bc</sup>	24 <sup>bc</sup>	27 <sup>ab</sup>	20 <sup>b</sup>
	120 N	363 <sup>b</sup>	303 <sup>bc</sup>	528 <sup>ab</sup>	483 <sup>a</sup>	46 <sup>ab</sup>	31 <sup>a</sup>	27 <sup>ab</sup>	28 <sup>a</sup>
ZM	0 N	403 <sup>ab</sup>	378 <sup>a</sup>	500 <sup>bcd</sup>	549 <sup>a</sup>	35 <sup>dc</sup>	31 <sup>ab</sup>	24 <sup>ab</sup>	24 <sup>ab</sup>
	120 N	378 <sup>b</sup>	332 <sup>ab</sup>	513 <sup>abc</sup>	538 <sup>a</sup>	41 <sup>bc</sup>	30 <sup>a</sup>	22 <sup>bc</sup>	28 <sup>a</sup>
TD	0 N	464 <sup>a</sup>	350 <sup>ab</sup>	579 <sup>a</sup>	526 <sup>a</sup>	41 <sup>bc</sup>	31 <sup>ab</sup>	28 <sup>a</sup>	23 <sup>ab</sup>
	120 N	454 <sup>a</sup>	364 <sup>ab</sup>	530 <sup>ab</sup>	502 <sup>a</sup>	53 <sup>a</sup>	34 <sup>a</sup>	26 <sup>ab</sup>	27 <sup>a</sup>
SED <sub>inputs</sub>				± 33.				± 4.9	
SED <sub>nitrogen</sub>				± 29.				± 4.7	

Treatments codes: CON = Control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.

Different letters (a-d) against the values indicate treatments/groups with significant differences within each factor (P < 0.05).

### 3.3.4 Correlation between soil physico-chemical properties and microbial abundance

AOA abundance was positively correlated with all soil chemical properties, except  $\text{NO}_3^-$  (i.e., soil pH ( $r = 0.549$ ), TC ( $r = 0.526$ ), MC ( $r = 0.520$ ) ( $P < 0.001$ ),  $\text{N}_t$  ( $r = 0.336$ ), HWEN ( $r = 0.299$ ) ( $P < 0.01$ ) as well as  $\text{NH}_4^+$  ( $r = 0.264$ ) and HWEC ( $r = 0.247$ ) ( $P < 0.05$ )). On the other hand, AOB abundance was negatively correlated with HWEN ( $r = -0.454$ ),  $\text{NO}_3^-$  ( $r = -0.451$ ),  $\text{N}_t$  ( $r = -0.424$ ) ( $P < 0.001$ ) and  $\text{NH}_4^+$  ( $r = -0.205$ ;  $P < 0.05$ ), but positively correlated with HWEC ( $r = 0.676$ ;  $P < 0.001$ ). Bacterial 16S rRNA gene abundance was positively correlated with  $\text{N}_t$  ( $r = 0.635$ ), HWEN ( $r = 0.498$ ), soil pH ( $r = 0.485$ ), TC ( $r = 0.483$ ) ( $P < 0.001$ ) as well as  $\text{NO}_3^-$  ( $r = 0.255$ ) and MC ( $r = 0.264$ ) ( $P < 0.05$ ). Archaeal 16S rRNA gene abundance revealed positive correlations with HWEC ( $r = 0.797$ ;  $P < 0.001$ ), soil pH ( $r = 0.271$ ) and TC ( $r = 0.208$ ) ( $P < 0.05$ ), but it was negatively correlated with  $\text{NO}_3^-$  ( $r = -0.451$ ), HWEN ( $r = -0.411$ ) ( $P < 0.001$ ) as well as MC ( $r = -0.255$ ;  $P < 0.05$ ).

## 3.4 Discussion

### 3.4.1 AOB and AOA abundance respond conversely to organic input quality

In agreement with our first hypothesis, we determined a promotion of AOB abundance under the influence of high quality TD inputs compared to intermediate quality CC inputs in 2013. The observed promotion of AOB abundance by TD was attributed to the high and available organic N compared to high polyphenols and lignin contents in CC which induce an organic N stress situation through the formation of polyphenol-protein bonds limiting N access by soil microbial communities (Millar and Baggs 2004; Rasche et al. 2014). This finding was partially supported by negative correlations of AOB abundance with HWEN and  $\text{N}_t$  contents suggesting a dependence of AOB on organic input derived N as energy source as indicated by strong organic input effects on AOB in this study (Millar and Baggs 2004; Musyoki et al. 2015).

On the other hand, the hypothesized delayed N release from intermediate quality inputs (CC) was not confirmed by the available data of AOB abundance during both years. At the same time,  $\text{NH}_4^+$  concentration in the soil remained not influenced by quality of organic inputs. This meant that the mineralized N ( $\text{NH}_4^+$ ) from the high quality inputs was immobilized by microbial communities or taken up by plants outcompeting its sorption in the soil (Chivenge et al. 2009; Muema et al. 2015). It has been shown for CC its high polyphenol and N contents (Gentile et al. 2011b) often lead to a strong sequestration (complexation) of organic N (e.g., proteins) by polyphenols (Millar and Baggs 2004; Mutabaruka et al. 2007; Schmidt et al. 2013). Accordingly, we suggested that such protein-polyphenol-complexes were still existent at EC60

delaying protein degradation (Rasche et al. 2014) as precursor of  $\text{NH}_4^+$  provision. This might have explained the inexistent promotion of AOB abundance. A similar effect of polyphenol-protein complexes was reported by Kamolmanit et al. (2013) who observed that the provision of organic N from intermediate quality inputs of *Tamarindus indica* was postponed to later decomposition stages when polyphenol oxidizing microorganisms (e.g., fungi) were stimulated and started to degrade protein-polyphenol complexes. Therefore, it was suggested that CC inputs require a prolonged period during the growing season to be fully decomposed.

Low quality input (i.e., ZM) in comparison to its intermediate quality counterpart (i.e., CC) induced an increase of AOB abundance from EC30 to EC60. A similar trend was observed for total bacteria. This confirmed our hypothesis on the gradual decomposition of low quality inputs over the season (Partey et al. 2013). Accordingly, the slow release of cellulose derived metabolites was presumably controlled by physico-chemical protection of cellulose by lignin impairing its fast and early decomposition (Talbot et al. 2012; Kunlanit et al. 2014).

Generally, AOB abundance tended to reduce at EC60 regardless of input quality. This was linked to competition for nutrients by the maize crop at EC60 for reproduction compared to EC30 when the crops are mainly elongating (Onwonga et al., 2010). A similar finding was found by Hai et al., 2009 who linked such a reduction of microbial communities at EC60 to soil moisture limitation, a trend we also observed in our study.

Conversely to AOB, AOA abundance remained unaffected by organic input quality. This finding was in agreement with the fact that quality of organic inputs did not influence  $\text{NH}_4^+$  and  $\text{N}_t$  concentrations in the soil. We explained this by the strong soil organic carbon (SOC) background of this clayey soil (Gentile et al. 2011a), offering sufficient, labile and easily degradable C substrates to AOA (Stopnišek et al. 2010). In addition, AOA were shown to acquire a diverse array of resources existent in all tested organic input treatments. These arguments were partially corroborated by positive correlations of assayed soil chemical properties (e.g., TC, HWEC, HWEN,  $\text{N}_t$ ,  $\text{NH}_4^+$ ) with AOA abundance. Although it was reported that AOA have a strong affinity for  $\text{NH}_4^+$  (Valentine 2007; Schleper 2010; Levičnik-Höfferle et al. 2012), they can also explore alternative N sources (e.g., soil organic N pool). AOA were shown also to be unaffected by changes in  $\text{NH}_4^+$  concentrations (Jia and Conrad 2009) and after exposure to organic inputs including biochemically contrasting sorghum straw and cattle manure (Hai et al. 2009).

The effect of organic input quality on AOB and AOA abundance and total prokaryotic communities was partially masked by climatic differences between the two study years and seasons. Although the obtained results in general confirmed our hypotheses, we noticed a

season induced inconsistency which corroborated the common difficulty of deriving conclusive interpretations when analyzing single seasons (Hai et al. 2009; Wessén et al. 2010). Accordingly, rainfall dependent soil moisture alterations exposed distinct effects on abundance of studied genes. While total bacterial abundance decreased in 2013, we observed the opposite for total archaea. This suggested total archaea to be more tolerant to low soil moisture conditions as negative correlations were observed. Reduction in soil moisture content also matched decreased  $N_t$  and HWEN. This was particularly evident in 2013 when limited organic N availability induced a decrease of total bacterial abundance. Contrastingly, total archaea proliferated despite the prevailing low soil moisture or substrate conditions. This supported their hypothesized adaptation potential to chronic energy stress (Valentine 2007).

The low AOB abundance in 2012 corroborated the findings of Muema et al. (2015) obtained from the same soils obtained during a dry spell (March, 2012) before incorporation of organic inputs. In our study, this dry spell before the start of long season rains was more severe in year 2012 than 2013 (Fig. 3.1). Consequently, soil moisture limited the availability of substrates to AOB, which are commonly sensitive to nutrient availability (Levičnik-Höfferle et al. 2012). It was suggested that AOB had not yet fully recovered from the effects of substrate limitation during the dry spell most likely explaining their low abundance during the rainy period in 2012 (Stark and Firestone 1995).

High precipitation in 2012 that was received mainly with proceeding cropping period induced a more positive water balance than year 2013 (data not shown). In addition, an average approximate water filled pore space (WFPS) of 80 % was determined for year 2012 compared to 60 % in 2013 (Gleeson et al. 2010). Gleeson et al. (2010) suggested 65 % to be the optimum WFPS for proliferation and activities of AOB. Furthermore, Avrahami and Bohannan (2007), Szukics et al. (2010) and Gleeson et al. (2010) reported AOB as more sensitive to WFPS alterations than AOA. This meant that the high soil moisture during the cropping season in 2012 limited the oxygen availability for AOB, which were shown to be more sensitive to oxygen concentration than AOA (Morimoto et al. 2011; Zhalnina et al. 2012). This clarified the lack of response of AOB abundance to quality of organic inputs particularly at early maize growth stage in year 2012 as well as their increased abundance in year 2013. Consequently, it could be suggested that influence of precipitation on oxygen concentrations in soil solution (Morimoto et al. 2011; Zhalnina et al. 2012), WFPS and the organic input driven N substrate availability to microbial communities contributed to a niche differentiation between AOB and AOA. The interactions between factors “Year/season” (e.g., soil moisture) and “Organic input quality”, both of which dependently regulated the dynamics of AOB and AOA abundance,

necessitates however, further research to illuminate precisely microbial dynamics with respect to biochemical quality of organic inputs. In this respect, the noticed climatic variation between the two study seasons, which partially masked the hypothesized effects of organic input quality, particularly on archaeal communities (i.e., AOA and total archaea), clearly substantiates the need for a longer and consecutive study period than considered in our study.

### **3.4.2 Effect of mineral N fertilizer on dynamics of soil microbial communities**

Decreased abundance due to application of mineral N fertilizer or its combination with organic inputs as opposed to its zero rates was observed for AOA as well as for total bacteria and archaea. We partially explained these findings with relatively higher pH values observed in the plots without mineral N fertilizer as opposed to those treated with fertilizer N (Hallin et al. 2009). Accordingly, the observed reduction of gene abundances was attributed to the indirect effect of  $\text{NH}_4^+$  fertilizer application reducing soil pH (Hallin et al. 2009; He et al. 2007) and thereby suppressing the proliferation of soil microbial communities including AOA (Liu et al. 2004; Hatzenpichler et al. 2008; Onwonga et al. 2010; Geisseler and Scow 2014). Contrarily, Hallin et al. (2009) reported a promotion of AOA abundance following an increased soil pH with application of calcium nitrate fertilizer as opposed to  $\text{NH}_4^+$  fertilizer. It could thus be postulated that fertilizer type dependent soil pH alterations had contrasting effects on AOA abundance. However, other studies contradicted our observations by reporting the dominance of AOA in acidic soils due to their high affinity for  $\text{NH}_4^+$  compared to AOB (Gubry-Rangin et al., 2010; Martens-Habbena et al., 2009; Nicol et al., 2008). Therefore, an alternative explanation to the reduced AOA abundance in relation to use of inorganic N fertilizer was the chaotropic effect of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  components in the fertilizer (Hallsworth et al. 2003; Cray et al. 2015; de Lima Alves et al. 2015). High  $\text{NH}_4^+$  concentration in soils was reported to inhibit AOA nitrification (Di et al. 2009; Tourna et al. 2010). Specifically under reduced water availability, a chaotropic situation inhibiting microbial growth was thus likely to have occurred (Cray et al. 2013; Stevenson et al. 2014; Stevenson and Hallsworth 2014).

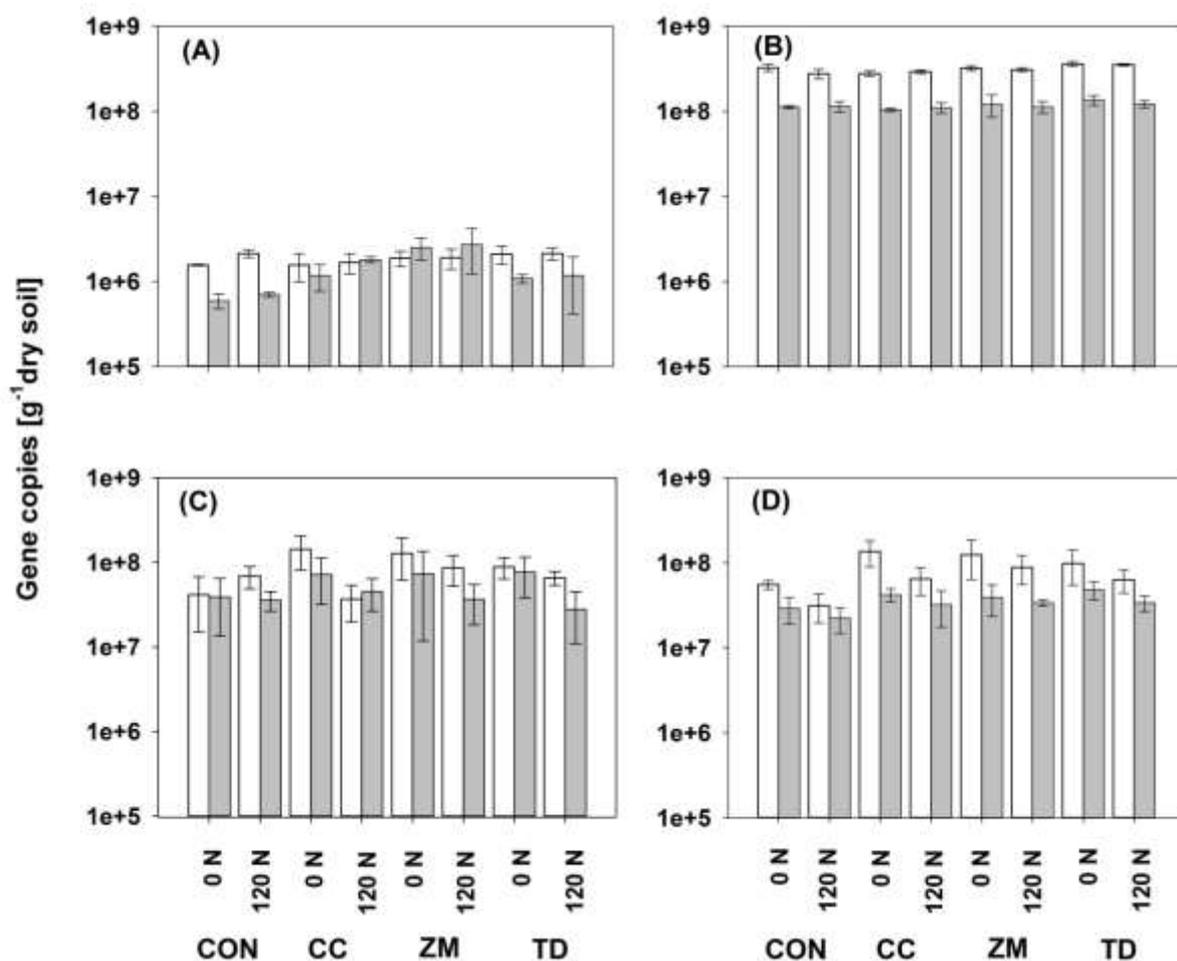
Conversely to AOA, AOB abundance remained unaffected by mineral N addition, and further there was no interaction found between factors “Organic input” and “Mineral N fertilizer”. It is therefore proposed that AOB utilized both organic and inorganic N while AOA might have mainly utilized organic N. Similar findings were provided by Wessén et al. (2010) who did not observe any interaction between organic inputs (e.g., cereal straw, peat) and mineral N applied as calcium nitrate on AOB abundance. This presumed stability of AOB towards  $\text{NH}_4^+$  fertilizer

additions indirectly indicates AOB being less sensitive to N fertilizer induced pH changes than AOA. This finding supports the acknowledged AOB dominated nitrification under mineral fertilizer conditions (Jia and Conrad 2009; Di et al. 2009, Di et al. 2010). This implied that chaotropism effect of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  compounds on AOB was balanced by its adaptation to high concentrations of readily available nutrients (Jia and Conrad 2009; Zhahnina et al. 2012; de Gannes et al. 2014).

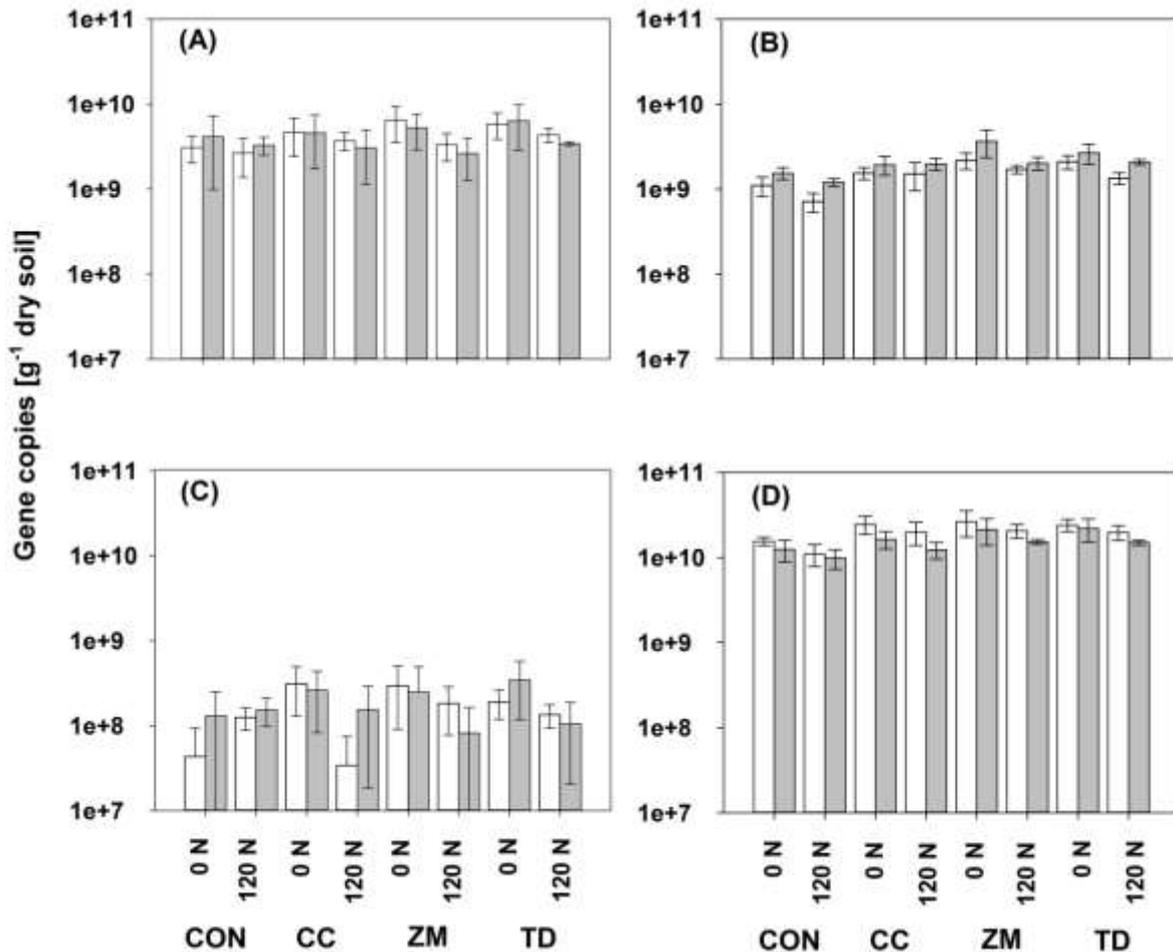
### **3.5 Conclusions**

Our 2-year study in the SOM field experiment allowed us to corroborate the regulating effect of contrasting biochemical quality of organic inputs on soil prokaryotic communities. We observed that particularly AOB revealed a sensitive biochemical quality dependent response, while AOA remained largely unaffected. On the other hand, sole use of mineral N fertilizer or its combination with organic inputs, regardless of their biochemical quality, decreased abundance of AOA, total bacteria and archaea, but not that of AOB. We presumed that this discrepant response was mostly controlled by seasonality dependent soil moisture alternations. Hence, future studies should consider the determination of soil water availability throughout seasons to ascertain the explicit chaotropic effect of inorganic fertilizer components (i.e.,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) on the proliferation and activities of archaeal and bacterial ammonia-oxidizing communities. In this respect, we recommend rRNA-based studies along with nucleic acid stable isotope probing (España et al., 2011) to demarcate those community members which are particularly active at defined time points during a cropping season. This will generate a more conclusive understanding about the resource- and soil climate-dependent niche differentiation between active AOB and AOA communities in agricultural soils.

### 3.6 Supplement



**Supplement S3.6.1:** Gene copy numbers of the ammonia-oxidizing genes in different treatments (descriptive means  $\pm$  standard deviation,  $n=3$ ). Total number of observations = 96. (A) bacterial *amoA* (AOB) gene in year 2012, (B) AOB in year 2013, (C) archaea *amoA* (AOA) in year 2012, and (D) AOA in year 2013 after application of biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and combination with mineral N. White bars = young growth stage (EC30) of maize; Grey bars = Flowering stage (EC60) of maize. Treatments codes: Organic inputs; CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*. Mineral N; 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>.



**Supplement S3.6.2:** Gene copy numbers of the total communities in different treatments (descriptive means  $\pm$  standard deviation,  $n=3$ ). Total number of observations = 96. (A) total bacteria (Bacterial 16S rRNA gene) in year 2012, (B) total bacteria (Bacterial 16S rRNA gene) in year 2013, (C) total archaea (Archaeal 16S rRNA gene) in year 2012, and (D) total archaea (Archaeal 16S rRNA gene) in year 2013 after application of biochemically contrasting organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ) and combination with mineral N. White bars = young growth stage (EC30) of maize; Grey bars = flowering stage (EC60) of maize. Treatments codes: Organic inputs; CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*. Mineral N; 0 N =  $0 \text{ kg N ha}^{-1}$ , 120 N =  $120 \text{ kg N ha}^{-1}$ .

**Table S3.6.1:** Soil pH, total carbon (TC) and total nitrogen (N<sub>t</sub>) content following amendment of soils with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and mineral N. Values are descriptive means ± standard deviations for soils sampled at young growth (EC30) and flowering (EC60) stages of maize crop. Total number of observations = 96.

Treatments		Soil pH				TC [%]				N <sub>t</sub> [%]			
		2012		2013		2012		2013		2012		2013	
		EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60
CON	0 N	4.88 ± 0.17	4.94 ± 0.12	5.03 ± 0.13	4.87 ± 0.12	2.44 ±0.10	2.24 ± 0.17	2.27 ± 0.10	2.25 ± 0.12	0.21 ± 0.004	0.20 ±0.012	0.18 ± 0.009	0.18 ± 0.012
CON	120 N	4.49 ± 0.10	4.55 ± 0.11	4.70 ± 0.09	4.46 ± 0.13	2.31 ± 0.10	2.19 ± 0.08	2.23 ± 0.03	2.23 ± 0.09	0.20 ± 0.011	0.20 ± 0.007	0.17 ± 0.007	0.19 ± 0.003
CC	0 N	4.96 ± 0.11	5.06 ± 0.25	5.09 ± 0.26	4.93 ± 0.30	2.71 ± 0.24	2.56 ± 0.21	2.67 ± 0.31	2.65 ± 0.27	0.24 ± 0.016	0.24 ± 0.015	0.21 ± 0.020	0.23 ± 0.016
CC	120 N	4.70 ± 0.19	4.74 ± 0.18	4.86 ± 0.30	4.55 ± 0.23	2.83 ± 0.14	2.51 ± 0.24	2.62 ± 0.18	2.62 ± 0.26	0.25 ±0.013	0.23 ± 0.020	0.21 ± 0.016	0.23 ± 0.020
ZM	0 N	5.25 ± 0.32	5.38 ± 0.30	5.40 ± 0.26	5.08 ± 0.36	2.82 ± 0.27	2.60 ± 0.34	2.71 ± 0.43	2.75 ± 0.36	0.24 ± 0.015	0.24 ± 0.028	0.21 ± 0.030	0.23 ± 0.024
ZM	120 N	4.75 ± 0.20	4.90 ± 0.16	4.94 ± 0.16	4.57 ± 0.13	2.66 ± 0.30	2.67 ± 0.32	2.79 ± 0.38	2.74 ± 0.18	0.23 ± 0.011	0.23 ± 0.021	0.21 ± 0.022	0.23 ± 0.012
TD	0 N	5.35 ± 0.31	5.40 ± 0.26	5.40 ± 0.11	5.28 ± 0.35	2.88 ± 0.12	2.66 ± 0.21	2.73 ± 0.10	2.71 ± 0.22	0.25 ± 0.005	0.24 ± 0.015	0.21 ± 0.008	0.23 ± 0.020
TD	120 N	4.77 ± 0.16	4.94 ± 0.12	4.96 ± 0.14	4.92 ± 0.08	2.86 ± 0.23	2.73 ± 0.13	2.71 ± 0.19	2.65 ± 0.09	0.25 ± 0.018	0.25 ± 0.012	0.22 ± 0.014	0.23 ± 0.013

Treatments codes: CON = Control, CC = *Calliandra calothyrsus*, ZM = *Zea mays*, TD = *Tithonia diversifolia*, 0 N = 0 kg N ha<sup>-1</sup>, 120 N = 120 kg N ha<sup>-1</sup>.

## **Chapter 4 Soil texture interrelations with organic inputs quality contrastingly influence abundance and composition of ammonia-oxidizing prokaryotes in a tropical arable soil**

### **Abstract**

Interrelations of soil texture and organic inputs quality influence the availability of organic nutrients. This was assumed to distinctly shape the dynamics of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities. We tested two contrasting soils (i.e., clayey Humic Nitisol and sandy Ferric Alisol) of soil organic matter (SOM) long-term trials established in Kenya in 2002. Since 2002, soils were continuously treated ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ) with biochemically contrasting organic inputs including *Tithonia diversifolia* (TD; C/N ratio: 13, lignin: 8.9 %, polyphenols: 1.7 %), *Calliandra calothyrsus* (CC; 13; 13; 9.4) and *Zea mays* stover (ZM; 59; 5.4; 1.2). In 2013, soils (0-15 cm) were sampled at young growth (EC30) and flowering (EC60) stages of maize and subjected to DNA-based community analysis (*amoA* gene quantification and community composition). Generally, soil texture was a major determinant of the abundance and composition of assayed communities (i.e., AOB, AOA, total bacteria and total archaea). In contrast, organic input quality primarily affected bacterial communities. Clayey compared to sandy soil revealed higher AOB and AOA abundance regardless of organic input quality. This was explained by the larger soil organic C background in the clayey soil promoting AOB and AOA proliferation. In the sandy soil, N-rich TD and cellulose-rich ZM promoted AOB abundance compared to CC. This AOB depression under CC was linked to the high contents of polyphenols and lignin in CC inducing a considerable C and N stress, inhibiting AOB's growth during the early stages of decomposition. In the clayey soil, TD versus ZM and CC versus ZM revealed separations of AOB composition. This implied that AOB was able to access N in CC and that N availability was the driving force for AOB alterations provided that C substrates were available. AOA composition revealed differences between TD versus CC and CC versus ZM in the sandy soil. This suggested that AOA alterations are realized mainly when C and N substrates are simultaneously limiting. Overall, the effects of interrelations between soil texture and organic input quality were specific to functionally relevant microbial groups with AOB being more responsive than AOA. As this work was based on functional potential of microbial communities using DNA-based analyses, RNA-based approach is recommended to capture those communities that are actively involved in nitrification process.

**Keywords:** Clayey and Sandy texture; Organic input quality; Ammonia-oxidizing bacteria and archaea; Microbial abundance; community composition.

#### 4.1 Introduction

Tropical agro-ecosystems are generally resource limited justifying the use of organic inputs in complementation of mineral fertilizers to replenish soil nutrients (e.g., nitrogen (N)) for crop growth (Chivenge et al., 2009; Mtambanengwe et al., 2006; Rasche and Cadisch, 2013). Small-holder farmers can rely on a broad array of organic inputs (e.g., crop residues, prunings from shrubs and trees) differing in their biochemical quality. Organic input quality is commonly defined by the contents of N, lignin, polyphenols and also cellulose (Kunlanit et al., 2014; Palm et al., 2001a). In consequence, it was acknowledged that decomposition and mineralization activities of soil microorganisms, which play a key role in providing organically bound nutrients to crops, are critically regulated by organic input quality (Rasche and Cadisch, 2013). In this respect, organic inputs such as *Tithonia diversifolia* (TD; C/N ratio: 13, Lignin: 8.9 %; Polyphenols: 1.7 %) accelerated decomposition availing N early in the cropping season, while other resources such as *Calliandra calothyrsus* (CC; 13; 13; 9.4) showed delayed decomposition patterns (Palm et al., 2001b). The delayed decomposition of CC was associated with the formation of polyphenol protein bonds limiting the accessibility of organic N by microbial decomposers (Millar and Baggs, 2004). Recently, it was shown that such polyphenol-induced N limitation prompted a critical stress response through increase in abundance of proteolytic bacteria in sandy agricultural soils (Rasche et al., 2014).

However, it remains yet to be fully understood to which extent microbial driven nitrification is regulated by organic input quality. This may count for both nitrifying archaea (AOA) and bacteria (AOB) whose community dynamics are generally assessed on the basis of the functional marker *amoA* gene ( $\alpha$ -subunit of the enzyme ammonia-monooxygenase) (Prosser and Nicol, 2008; Zhang et al., 2013). Up to date, existing observations of organic input quality effects on AOA and AOB community dynamics have not displayed clear response patterns. For example, low C/N ratios, hence easily decomposable organic materials (e.g., *Brassica napus*, cereal straw) in comparison to high C/N ratio inputs (e.g., compost, peat) with a gradual decomposition pattern promoted the abundance of AOA in both sandy and clay loam soils (Strauss et al., 2014; Wessén et al. 2010). Conversely, no treatment effect was observed on AOB abundance. Moreover, Hai et al. (2009) found a promotion of AOA and AOB abundance

in sandy soils after treatment with nutrient rich semi-decomposed cattle manure (low C/N ratio), while both groups were decreased by sorghum straw with a high C/N ratio. On the other hand, Wessén et al. (2010) observed a clear community composition differentiation of both, AOB and AOA in a clay loam soil treated with biochemically distinct organic inputs.

These contradictory results clearly imply that the response of nitrifying communities to organic input quality may be also determined by other factors including physico-chemical soil characteristics (Kögel-Knabner et al., 2008). Clayey compared to coarse textured sandy soils show a generally higher accumulation potential of soil organic carbon (SOC) either through physical protection of freshly added organic inputs or via organo-mineral associations (Dieckow et al., 2009; Kögel-Knabner et al., 2008). In consequence, these characteristics of fine-textured clayey soils induced a higher abundance of total bacteria than in sandy soils. This was attributed to the higher surface area of clay particles promoting bacterial growth as well as providing protection from predation (Kögel-Knabner et al., 2008; Neumann et al., 2013; Poll et al., 2003). With respect to nitrifying soil microorganisms, Pereira e Silva et al. (2012) reported a higher abundance of AOB and AOA in clayey than in sandy soils, supporting the mechanisms of clayey soil linked promotion of microbial populations (Kögel-Knabner et al., 2008). Other observations evidenced that AOA in fine textured soils preferentially utilize native SOC (Stopnišek et al., 2010; Zhalnina et al., 2012; Zhang et al., 2010) explaining their increased abundance over sandy soils. In contrast, Wessén et al. (2011) found AOA abundance to be negatively correlated with clay content, which supported its general adaptation to nutrient limiting conditions (Levičnik-Höfferle et al., 2012; Valentine, 2007). In conclusion, it remains speculative if organic inputs of similar biochemical quality induce consistent responses of nitrifying communities in soils with distinct textures.

Our objective was therefore to determine the interrelations between soil texture and organic inputs of contrasting biochemical quality on the abundance and community composition of AOB and AOA in tropical agricultural soils. We hypothesized a higher AOB and AOA abundance in clayey than sandy soils regardless of organic input quality. This appears justified by the strong SOC background of clayey soils as determined by the acknowledged beneficial characteristics of clay minerals in sequestering organic substrates fostering microbial proliferation (Gentile et al., 2011a; Kögel-Knabner et al., 2008). Furthermore, alteration of community composition, i.e. AOB and AOA is proposed in clayey compared to sandy soil due to its high nutrients status promoting community alterations. Consequently, we argue that the general resource limitation in sandy soils is compensated with the addition of high quality organic inputs (low C/N) availing high amounts of organic C and N to AOB and AOA early in

the season compared to intermediate quality inputs due to high polyphenols and lignin contents and low quality inputs (high C/N) limiting faster decomposition. In this respect, we hypothesized a promotion of AOB and AOA abundance and community composition alteration in sandy soils later in the season as a consequence of gradual decomposition of intermediate and low quality inputs availing C and N substrates to the communities.

## **4.2 Materials and methods**

### **4.2.1 Sites description and experimental design**

The study was carried out at the soil organic matter (SOM) long-term field experiments in Embu (0° 30' S, 37° 30' E) and Machanga (0° 47' S, 37° 40' E) which were established in 2002. Embu site (1380 m above sea level (a.s.l)) is located in Embu district in the central highlands of Kenya (130 km northeast of Nairobi). Machanga site (1060 a.s.l) is located in Mbeere district, approximately 200 km northeast of Nairobi. Embu site has an annual mean temperature of 20 °C and a mean annual rainfall of 1200 mm, while Machanga site is characterized by frequent droughts due to erratic and unreliable rains with a mean annual temperature of 26 °C and an average annual rainfall of 900 mm. Rainfall at both sites occurs bimodal with long rains received from mid-March to June and short rains from mid-October to December. The soil at Embu is defined as a Humic Nitisol (FAO, 2006) dominated by kaolinite minerals derived from basic volcanic rocks. The texture of the topsoil layer (0-15 cm) was characterized by 17 % sand, 18 % silt and 65 % clay and contained 29.3 g kg<sup>-1</sup> organic C, 2.8 g kg<sup>-1</sup> total N and a pH of 5.8 (H<sub>2</sub>O) at the start of the experiment (Chivenge et al., 2009; Gentile et al., 2011a). Machanga site is characterized by a sandy soil derived from granitic gneisses and is classified as Ferric Alisols (FAO, 2006). The texture of the top layer (0-15 cm) contained 67 % sand, 11 % silt and 22 % clay with 5.3 g kg<sup>-1</sup> organic C, 0.6 g kg<sup>-1</sup> total N and a pH (H<sub>2</sub>O) of 6.1 at the onset of the experiment (Gentile et al., 2011a). Moreover, Machanga soil was reported to be deficient in major crop nutrients such as N and P (Gentile et al., 2011a), while the Embu soil was considered fertile with greater total carbon and nitrogen (TC, N<sub>t</sub>), as well as higher exchangeable bases measured at the onset of both experiments (Chivenge et al., 2009; Gentile et al., 2011a).

The two SOM long-term field experiments were implemented to primarily study the effect of continuous annual application of biochemically contrasting organic inputs and their combination with mineral N fertilizer on the performance of *Zea mays* as well as soil C and N dynamics (Chivenge et al. 2009, 2011; Gentile et al. 2011a; Rasche et al., 2014). This presented

work however, focused only on organic input treatments excluding the effect of fertilizer N by not considering the plots that received mineral N fertilizer. The experiment was laid out in a randomized complete block design (RCBD) with three replicates with plot sizes of 6 m x 5 m and 6 m x 6 m in the clayey and sandy soil respectively. Further details of the field experiment set-up can be retrieved from Chivenge et al. (2009) and Gentile et al. (2009). Three organic input types were considered in this study: high quality *Tithonia diversifolia* (C/N ratio: 13, lignin: 8.9 %; polyphenols: 1.7 %), intermediate quality *Calliandra calothyrsus* (13; 13 %; 9.4 %) and low quality *Zea mays* (59; 5.4 %; 1.2 %) (Gentile et al., 2011b). At the onset of long rains in each year, these organic inputs (leaves, petioles and small branches for *C. calothyrsus* in addition to stems for *T. diversifolia* and *Z. mays* stover) were collected and analyzed for dry matter and total C and N content. Dry matter and total C data were then used to determine the amount of each organic material to be applied at 4 Mg C ha<sup>-1</sup> a<sup>-1</sup> (Chivenge et al. 2009; Gentile et al. 2011a). Prior to maize (*Zea mays*) sowing, organic inputs were chopped into small pieces, broadcast and hand-incorporated at a soil depth of 0-15 cm (Chivenge et al. 2009; Gentile et al. 2011a, 2011b). All plots received a blanket basal application of 60 kg P ha<sup>-1</sup> season<sup>-1</sup> and 60 kg K ha<sup>-1</sup> season<sup>-1</sup> before sowing.

#### **4.2.2 Soil sampling**

In April 2013, at young maize growth stage (EC30) and early June at flowering stage (EC60) (Milling et al., 2005), soil samples were collected from the no input control (CON), *C. calothyrsus* (CC), *Z. mays* (ZM) and *T. diversifolia* (TD) treatments. In each plot, 10 soil sub-samples were randomly obtained at a depth of 0-15 cm using a soil auger. These 10 sub-samples were bulked to one composite sample per plot, sieved (2-mm mesh) and stored at 4°C. In total, 24 soil samples were obtained per site. Prior to analysis, each composite soil sample was split into three proportions. A fresh sample was used for immediate extraction of ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). The second proportion was air-dried for analysis of pH, hot water extractable C and N (HWEC/N), total C (TC) and total N (N<sub>t</sub>). The third proportion was freeze-dried to be shipped to Germany for microbial community analyses.

#### **4.2.3 Microbiological soil analysis**

##### **4.2.3.1 Soil DNA extraction**

Total soil DNA was isolated from 0.5 g of each freeze dried soil sample using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, Ohio, USA) according to the manufacturer's instructions. Quality of isolated DNA was checked on 1.5 % (w/v) agarose gels. DNA was

quantified photometrically (NanoDrop™ 2000/2000c spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). For each DNA sample, two consistent NanoDrop readings, whose variation was less than 5 %, were considered for subsequent determination of DNA concentration. Quantified DNA was stored at -20 °C for further analysis.

#### **4.2.3.2 Microbial abundance**

For DNA-based quantification of target genes (total bacteria and archaea (16S rRNA gene) as well as ammonia-oxidizing bacteria (bacterial *amoA* gene; AOB) and archaea (archaeal *amoA* gene; AOA)), plasmid standards were prepared (Table 4.1), purified (Invisorb Fragment CleanUp kit, Stratec Molecular GmbH, Berlin, Germany) and quantified according to Rasche et al. (2011). The qPCRs (three analytical replicates per DNA sample) were conducted in a 25 µl reaction cocktail containing 12.5 µl Power SYBR green master mix (Applied Biosystems, Foster City, CA, USA), 0.4 µM of each primer (Table 1), 0.25 µl T4 gene 32 protein (500 µg ml<sup>-1</sup>, MP Biomedicals) and 5 ng DNA template (except for AOB gene (10 ng)) from the prior prepared DNA working stock of 5 ng µl<sup>-1</sup>. The final dilution of DNA extracts was optimized in advance to avoid any reaction inhibition by humic acids co-extracted during DNA isolation (data not shown). Reactions were run on a StepOne Plus Real-time PCR detection system (Applied Biosystems) starting with an initial denaturation step at 95 °C for 10 min, followed by amplification cycles specific for each target gene (Table 4.1). Melting curve analysis of amplicons was conducted to ensure that fluorescence signals originated from specific amplicons and not from primer dimers or other artifacts. Gene copy numbers and reaction efficiencies (total bacteria 102 % ± 1, total archaea 85 % ± 14, AOB 97 % ± 11, and AOA 86 % ± 5) were calculated using StepOne Software version 2.2.2 (Applied Biosystems) and presented per gram of dry soil.

#### **4.2.3.3 Microbial community composition**

For terminal restriction fragment length polymorphism (TRFLP) analysis, bacterial *amoA* (AOB) and archaeal *amoA* (AOA) genes were amplified as previously described (Rasche et al., 2011) (Table 4.1). All forward primers were labeled with 6-carboxyfluorescein at their 5' ends. Amplicons were purified (Invisorb Fragment CleanUp kit) prior to digestion. Two hundred ng of each purified amplicon were digested with a 5 U combination of *AluI* and *RsaI* (New England Biolabs (NEB), Ipswich, USA) and the reactions incubated at 37 °C for 4 hours. Digested products were purified (Sephadex G-50, GE Healthcare Biosciences, Waukesha, WI, USA) (Rasche et al., 2006) and an aliquot of 2 µl was mixed with 17.75 µl HiDi formamide

(Applied Biosystems) and 0.25  $\mu$ l internal 500 ROX<sup>TM</sup> size standard (Applied Biosystems). Labeled terminal-restriction fragments (T-RFs) were denatured at 95 °C for 3 min, chilled on ice and detected on an ABI 3130 automatic DNA sequencer (Applied Biosystems). Peak Scanner<sup>TM</sup> software package (version 1.0, Applied Biosystems) was used to compare relative lengths of T-RFs with the internal size standard and to compile electropherograms into numeric data set, in which fragment length and peak height >50 fluorescence units were used for profile comparison. TRFLP profiles used for statistical analyses were normalized according to Dunbar et al. (2001).

**Table 4.1:** Description of primer sets, PCR ingredients and amplification details used for quantitative PCR (qPCR) and TRFLP analyses.

Target gene	Primer (reference)	qPCR	TRFLP
All bacteria	Eub338f (Lane, 1991) Eub518r (Muyzer et al., 1993)	95 °C 5 min; 40 cycles: 95 °C 30s, 55 °C 35s, 72 °C 45s	
All archaea	Ar109f (Lueders and Friedrich, 2000) Ar912r (Lueders and Friedrich, 2000)	95 °C 5 min; 40 cycles: 95 °C 30s, 52 °C 35s, 72 °C 45s, 78 °C 20s	
Ammonia-oxidizing bacteria (AOB)	AmoA-1f (Rotthauwe et al., 1997) AmoA-2r (Rotthauwe et al., 1997)	95 °C 5 min; 45 cycles: 95 °C 30s, 57 °C 45s, 72 °C 45s, 78 °C 20s	95 °C 5 min; 40 cycles: 94 °C 30s, 53 °C 30s, 72 °C 1 min; 72 °C 10 min
Ammonia-oxidizing archaea (AOA)	Arch-amoAf (Francis et al., 2005) Arch-amoAr (Francis et al., 2005)	95 °C 5 min; 45 cycles: 95 °C 30s, 53 °C 45s, 72 °C 45s, 78 °C 20s	94 °C 5 min; 35 cycles: 94 °C 30s, 53 °C 45s, 72 °C 10 min; 72 °C 1 min

#### 4.2.4 Physico-soil chemical analyses

Soil pH values were measured in water suspensions at a solid-to-liquid ratio of 1-to-2.5. Hot water extractable C (HWEC) and N (HWEN) analyses were determined according to Schulz (2004) as well as Schulz and Körschens (1998). C content of hot water extracts was analyzed using potassium dichromate in sulfuric acid milieu (Multi N/C analyzer, Analytik Jena, Jena, Germany), while HWEN was analyzed by a modified Kjeldahl digestion. Total C (TC) and total N ( $N_t$ ) of soils were quantified by dry combustion (Flash EA 1112 Elemental Analyzer, Thermo Fisher Scientific). Ammonium ( $NH_4^+$ ) and nitrate ( $NO_3^-$ ) were calorimetrically measured with a spectrophotometer on field fresh soils (ICRAF, 1999). Soil subsamples of 20 g each were taken for moisture content (MC) determination by weight loss after drying (105 °C, 24 hours).

#### 4.2.5 Statistical analysis

Analyses of variance (ANOVA) were conducted using Statistical Analysis Software program SAS (SAS Institute, 2015). For the abundance data of the four studied genes, a generalized linear mixed model with a negative binomial distribution error and a log link function was used. The model was fitted by restricted log pseudo-likelihood with expansion about the solution for the random effects in the SAS GLIMMIX procedure. For the geochemical data, linear mixed models were fitted in the SAS MIXED procedure. The data were checked for normality and homoscedasticity on model residuals using quantile-quantile (Q-Q) plots, histograms and studentized residual plots. Different transformations were tried and selected accordingly in an attempt to satisfy the assumption underlying the ANOVA model (Piepho, 2009). A full factorial model was specified with the effects of main factors “Soil type” (Humic Nitisol (Embu), Ferric Alisol (Machanga)), “Organic input” (TD, CC, ZM, CON), “Growth stage” (EC30, EC60), and their interactions as fixed effects on the response variables (i.e., abundance of the four target genes and geochemical properties). We accounted for the repeated measurements in the same plots by fitting an error with a first order autoregressive covariance structure to the data. Statistical significance of all effects was assessed at a significance level of  $P < 0.05$ . Blocks were considered as fixed or random effect and model selected accordingly using the Akaike Information Criterion (AIC). Treatment means/medians were separated using the PDIF option of the LSMEANS as well as the letters (letter display) from the SAS generalized linear mixed models procedure. Descriptive mean values with standard deviations for abundance of all the studied genes are presented as supplementary information (S4.6.1). Pearson linear correlation analyses were conducted in the SAS COR procedure to relate the

abundance (dependent variables) of target genes to soil chemical properties (independent variables).

Main factors and their interaction effects on TRFLP data sets generated for each ammonia-oxidizing gene (i.e., AOB, AOA) were tested using permutation multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Treatment effects were further assayed based on Bray-Curtis similarity coefficients (Clarke, 1993). A similarity matrix was generated for all possible pairs of samples for each target gene (Rasche et al., 2014, 2011; Rees et al., 2005). The similarity matrix was used for analysis of similarity (ANOSIM) to test the hypothesis that composition of studied microbial communities was altered by the three factors. ANOSIM is based on rank similarities between the sample matrix and produces a test statistic called '*R*' (Rasche et al., 2014, 2011; Rees et al., 2005). A 'global' *R* was first calculated in ANOSIM, which evaluated the overall effect of a factor in the data set. This step was followed by a pairwise comparison, whereby the magnitude of *R* indicated the degree of separation between two tested communities. An *R* score of 1 indicated a complete separation, 0 indicated no separation, while a negative *R* score suggests that the greatest differences are within, instead of between groups (Clarke and Gorley, 2001; Rasche et al., 2011; Rees et al., 2005). For graphical visualization of the compositions of ammonia-oxidizing communities as shaped by the studied factors, canonical analysis of principal coordinates (CAP) was performed on resemblance matrix data generated based on Bray-Curtis similarity coefficients (Anderson and Ter Braak, 2003; Anderson and Robinson, 2003; Clarke, 1993). In addition, regression analyses were performed based on Shannon-Weaver indices ( $H'$ , Shannon and Weaver, 1949) of the TRFLP data to related the community composition (predicted variables) to soil chemical properties (predictor variables) using distance-based linear models (DISTLM) (Boj et al., 2011; Legendre and Anderson, 1999; McArdle and Anderson, 2001). DISTLM is a routine for analyzing the relationship between a multivariate data, based on resemblance matrix, and one or more predictor variables (Legendre and Anderson, 1999; McArdle and Anderson, 2001). It partitions variation in a data set (i.e., community composition of target genes, soil chemical data) according to regression models. PERMANOVA, ANOSIM, CAP and DISTLM analyses were conducted using Primer6 for Windows (version 6.1.13, Primer-E Ltd., Plymouth, UK) with (PERMANOVA+ version 1.0.6) add-on for Primer 6 software (Anderson, 2001).

## 4.3 Results

### 4.3.1 Dynamics of abundance of microbial communities

A full factorial analysis of variance (ANOVA) summary of the influence of all factors and their interactions as fixed factors on the abundance of the studied genes is presented in Table 4.2. Soil type exhibited the strongest effect and was significant for all microbial parameters tested and exhibited in most cases a strong interaction with crop growth stage.

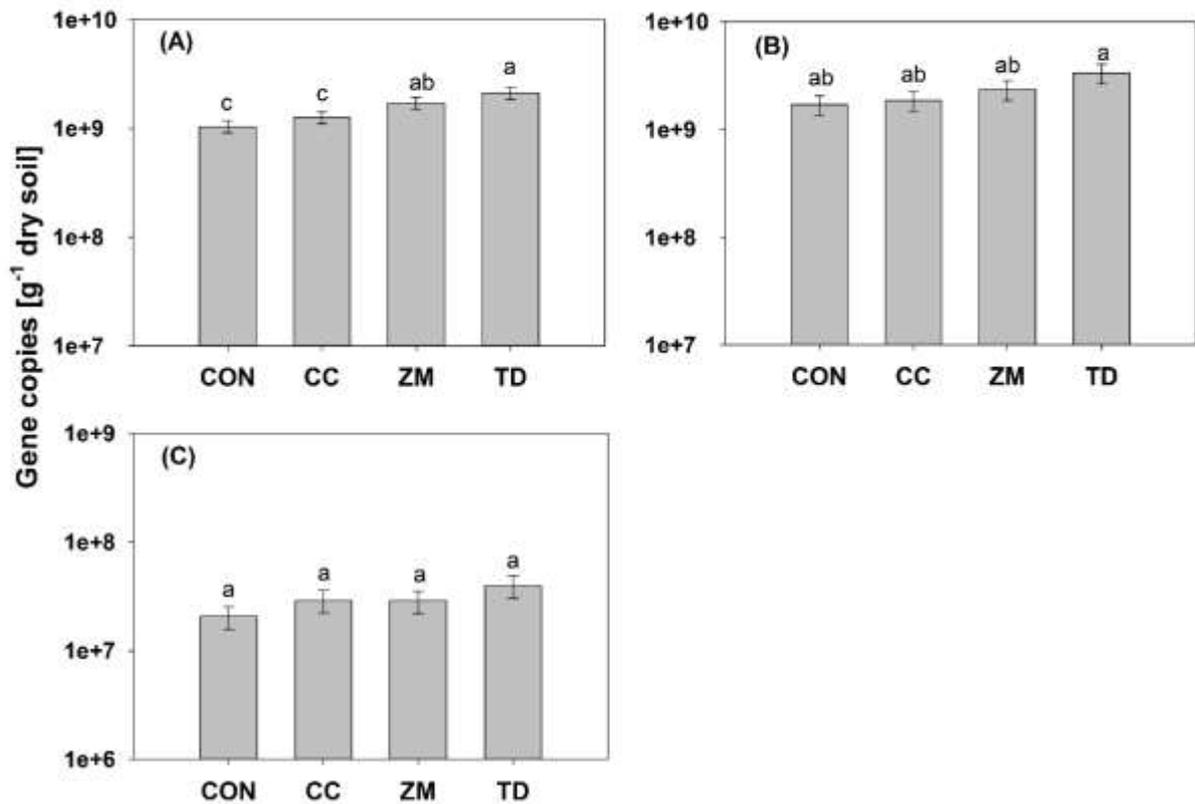
Total bacterial 16S rRNA gene copy numbers were generally higher in the clayey ( $1.97 \times 10^9$  copies  $g^{-1}$  dry soil) than sandy soil ( $1.11 \times 10^9$  copies  $g^{-1}$  dry soil) ( $P < 0.05$ ) (Table 4.3). In both soils, bacterial 16S rRNA gene copy numbers increased at flowering stage (EC60) ( $P < 0.05$ ) (Table 4.3). Total archaeal 16S rRNA gene copy numbers were also higher in the clayey soil ( $1.97 \times 10^{10}$  copies  $g^{-1}$  dry soil) than in the sandy soil ( $2.54 \times 10^8$  copies  $g^{-1}$  dry soil) ( $P < 0.001$ ) (Table 4.3). Archaeal abundance, however, showed a significant interaction with growth stage being lower at EC60 than EC30 in the clayey soil, while a contrasting effect was observed for the sandy soil ( $P < 0.05$ ) (Table 4.3).

In contrast to total bacterial abundance, the sandy soil revealed a higher abundance of AOB ( $2.48 \times 10^8$  copies  $g^{-1}$  dry soil) compared to the clayey soil ( $1.94 \times 10^8$  copies  $g^{-1}$  dry soil) ( $P < 0.05$ ) (Table 4.3). In the clayey soil, AOB abundance was lower in all treatments at EC60 than EC30 ( $P < 0.001$ ), while it increased with advanced maize growth stage in the sandy soil ( $P < 0.05$ ) (Table 4.3). Abundance of AOA was higher in the clayey soil ( $6.16 \times 10^7$  copies  $g^{-1}$  dry soil) compared to the sandy soil ( $1.34 \times 10^7$  copies  $g^{-1}$  dry soil) ( $P < 0.05$ ) (Table 4.3). In the clayey soil, AOA reduced at EC60 in all treatments compared to EC30 ( $P < 0.05$ ), while no significant differences in abundance of AOA was determined between crop growth stages in the sandy soil ( $P > 0.05$ ).

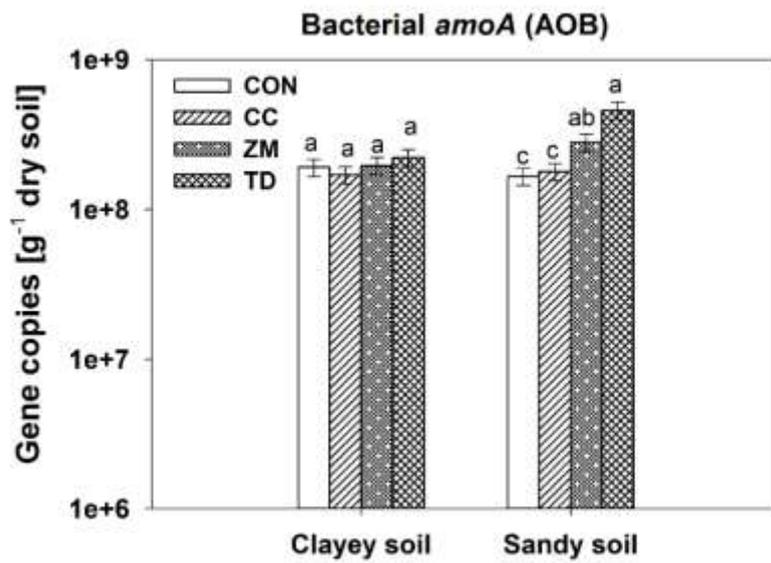
With respect to organic inputs, only total bacterial 16S rRNA gene and AOB abundance were significantly affected by organic input quality. TD showed higher bacterial 16S rRNA gene abundance than CC ( $P < 0.05$ ) (Fig 4.1A).

AOB abundance further revealed a significant interaction between soil type, growth stages and quality of organic inputs ( $P < 0.05$ ). Respective analysis in the sandy soil only revealed that TD and ZM had higher AOB *amoA* abundance than CC ( $P < 0.05$ ) (Fig 4.2).

Both archaeal 16S rRNA gene and AOA *amoA* abundance did not respond to organic input quality independent of soil ( $P > 0.05$ ) (Fig 4.1B and 4.1C).



**Figure 4.1:** Gene copy numbers of total communities and ammonia-oxidizing archaea in different treatments (mean values  $n = 12$ ). (A) total bacteria (Bacterial 16S rRNA gene), (B) total archaea (Archaeal 16S rRNA gene), (C) archaeal *amoA* (AOA) gene after application of different biochemical quality organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). Different letters (a-c), above the bars indicate treatments with significant differences ( $P < 0.05$ ). Treatments: CON = Control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.



**Figure 4.2:** Gene copy numbers of ammonia-oxidizing bacteria (AOB) in clayey and sandy soils in different treatments after application of different biochemical quality organic inputs (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) (mean values  $n = 6$ ). Different letters (a-c), above the bars indicate treatments with significant differences ( $P < 0.05$ ). Treatments: CON = Control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.

**Table 4.2:** Statistical evaluation of gene abundance, community composition and soil chemical properties by full factorial ANOVA and PERMANOVA.

Microbial groups and soil properties	Units	Effects/source of variation						
		S	EC	OI	S x EC	S x OI	EC x OI	S x EC x OI
<b>qPCR</b>								
Bacterial 16S rRNA gene	(copies g <sup>-1</sup> dry soil)	***	***	**	n.s	n.s	n.s	n.s
Archaeal 16S rRNA gene	(copies g <sup>-1</sup> dry soil)	***	***	n.s	***	n.s	n.s	n.s
Bacterial <i>amoA</i> gene (AOB)	(copies g <sup>-1</sup> dry soil)	*	***	**	***	*	n.s	*
Archaeal <i>amoA</i> gene (AOA)	(copies g <sup>-1</sup> dry soil)	***	n.s	n.s	*	n.s	n.s	n.s
<b>TRFLP</b>								
Bacterial <i>amoA</i> gene (AOB)		***	n.s	*	n.s	*	n.s	n.s
Archaeal <i>amoA</i> gene (AOA)		*	n.s	*	n.s	n.s	n.s	n.s
<b>Soil properties</b>								
Soil pH		n.s	***	***	***	*	n.s	n.s
NH <sub>4</sub> <sup>+</sup>	(mg kg <sup>-1</sup> )	*	n.s	n.s	*	n.s	n.s	n.s
NO <sub>3</sub> <sup>-</sup>	(mg kg <sup>-1</sup> )	n.s	n.s	n.s	*	n.s	n.s	n.s
TC	(%)	***	n.s	n.s	n.s	n.s	n.s	n.s
N <sub>t</sub>	(%)	***	**	*	***	*	n.s	*
HWEC	(mg kg <sup>-1</sup> )	***	n.s	**	*	n.s	*	n.s
HWEN	(mg kg <sup>-1</sup> )	***	n.s	***	***	**	n.s	*
MC	(%)	***	***	n.s	***	n.s	n.s	n.s

Abbreviations: S = Soil type, EC = Crop growth stage OI = Organic inputs, NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrate, TC = Total carbon, N<sub>t</sub> = Total nitrogen, HWEC = Hot water extractable carbon, HWEN = Hot water extractable nitrogen, MC = Soil moisture content.

Significance levels: n.s: P > 0.05; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

**Table 4.3:** Abundance of the four assayed microbial communities as influenced by soil type at different growth stages of *Zea mays* during the study period.

Effects	Total bacteria (16S rRNA gene)	Total archaea (16S rRNA gene)	Bacterial <i>amoA</i> gene (AOB)	Archaeal <i>amoA</i> gene (AOA)
Clayey soil	$1.97 \pm 0.17 \times 10^{9a}$	$1.97 \pm 0.29 \times 10^{10a}$	$1.94 \pm 0.13 \times 10^{8b}$	$6.16 \pm 1.02 \times 10^{7a}$
Sandy soil	$1.11 \pm 0.09 \times 10^{9b}$	$2.54 \pm 0.37 \times 10^{8b}$	$2.48 \pm 0.22 \times 10^{8a}$	$1.34 \pm 0.22 \times 10^{7b}$
EC30 <sub>clayey</sub>	$1.67 \pm 0.16 \times 10^{9b}$	$2.21 \pm 0.34 \times 10^{10a}$	$3.19 \pm 0.22 \times 10^{8a}$	$9.74 \pm 2.52 \times 10^{7a}$
EC60 <sub>clayey</sub>	$2.33 \pm 0.23 \times 10^{9a}$	$1.75 \pm 0.27 \times 10^{10b}$	$1.17 \pm 0.08 \times 10^{8c}$	$3.89 \pm 1.01 \times 10^{7b}$
EC30 <sub>sandy</sub>	$9.41 \pm 0.91 \times 10^{8c}$	$1.72 \pm 0.26 \times 10^{8d}$	$2.06 \pm 0.14 \times 10^{8b}$	$9.60 \pm 2.49 \times 10^{6c}$
EC60 <sub>sandy</sub>	$1.30 \pm 0.13 \times 10^{9b}$	$3.75 \pm 0.57 \times 10^{8c}$	$3.00 \pm 0.21 \times 10^{8a}$	$1.87 \pm 0.49 \times 10^{7bc}$

EC30 = Young growth stage of *Zea mays*; EC60 = Flowering stage of *Zea mays*.

Values are means  $\pm$  standard error for soil type effect ( $n = 24$ ) (i.e., means are averages of abundance values of all treatments for both crop growth stages) and mean values of growth stage effect ( $n = 12$ ) are averages of all treatments for respective crop growth stages according to soil type. Different letters (a-d) against the values indicate groups with significant differences within each factor ( $P < 0.05$ ).

### 4.3.2 Dynamics of microbial community composition

PERMANOVA revealed individual effects of soil type and organic input quality on AOA and AOB community composition ( $P < 0.05$ , Table 4.2). For AOB, a significant interaction between soil type and organic input quality was determined ( $P < 0.05$ , Table 4.2). These effects were strengthened by ANOSIM which showed a significant effect of soil type on AOB (Global  $R = 0.505$ ) and AOA (Global  $R = 0.373$ ) community composition ( $P < 0.01$ ) (Table 4.4). Moreover, significant alterations of AOB community composition were observed between the two crop growth stages within each soil type (i.e., clayey soil: Global  $R = 0.239$  ( $P < 0.01$ ), sandy soil: Global  $R = 0.1$  ( $P < 0.05$ )). No significant changes of AOA community composition were found between the crop growth stages within each soil type ( $P > 0.05$ ) (Table 4.4). Organic input quality induced a significant influence on AOB composition (i.e., Global  $R = 0.14$ ) for both soils ( $P < 0.05$ ), while AOA composition was only altered by organic input quality in the sandy soil (Global  $R = 0.291$ ) ( $P < 0.01$ ) (Table 4.4).

To assess the explicit effect of organic input quality on the composition of AOB and AOA communities at specific growth stages, ANOSIM was performed individually for each soil type (Table 4.5). In the clayey soil, AOB revealed a Global  $R = 0.244$  ( $P < 0.05$ ) at EC30 and a Global  $R = 0.639$  ( $P < 0.01$ ) at EC60. In the sandy soil, a Global  $R = 0.225$  ( $P < 0.05$ ) was revealed at EC30, while organic input quality did not reveal a significant Global  $R$  for AOB at EC60 ( $P > 0.05$ ). AOA community composition was only altered by biochemical quality of inputs in the sandy soil at EC60 (Global  $R = 0.259$ ;  $P < 0.05$ ). Pairwise comparisons for AOB composition in the clayey soil showed an  $R = 0.333$  between CC versus ZM at EC30. At EC60, an  $R = 0.704$  was revealed between TD and ZM, while CC versus ZM showed an  $R = 0.815$ . In the sandy soil, AOB revealed an  $R = 0.296$  between TD versus CC (EC30) and an  $R = 0.222$  between CC and ZM (EC30 and EC60) (Table 4.5). AOA community composition distinctions in the sandy soil revealed an  $R = 0.333$  and an  $R = 0.444$  between TD versus CC at EC30 and EC60, respectively, while CC versus ZM revealed an  $R = 0.481$  at EC60. No effects of organic input quality on AOA composition were found in the clayey soil ( $P > 0.05$ ).

**Table 4.4:** Global *R* values for the three main factors “Site,” “Growth stage” and “Input type” obtained from the analysis of similarity (ANOSIM) of TRFLP fingerprints (*n* = 48).

Factors	Bacterial <i>amoA</i> gene (AOB)	Archaeal <i>amoA</i> gene (AOA)
Site type	0.505**	0.373**
Growth stages (EC)		
Clayey soil	0.239**	n.s
Sandy soil	0.1*	n.s
Organic inputs (OI)		
Clayey soil	0.140*	n.s
Sandy soil	0.141*	0.291*

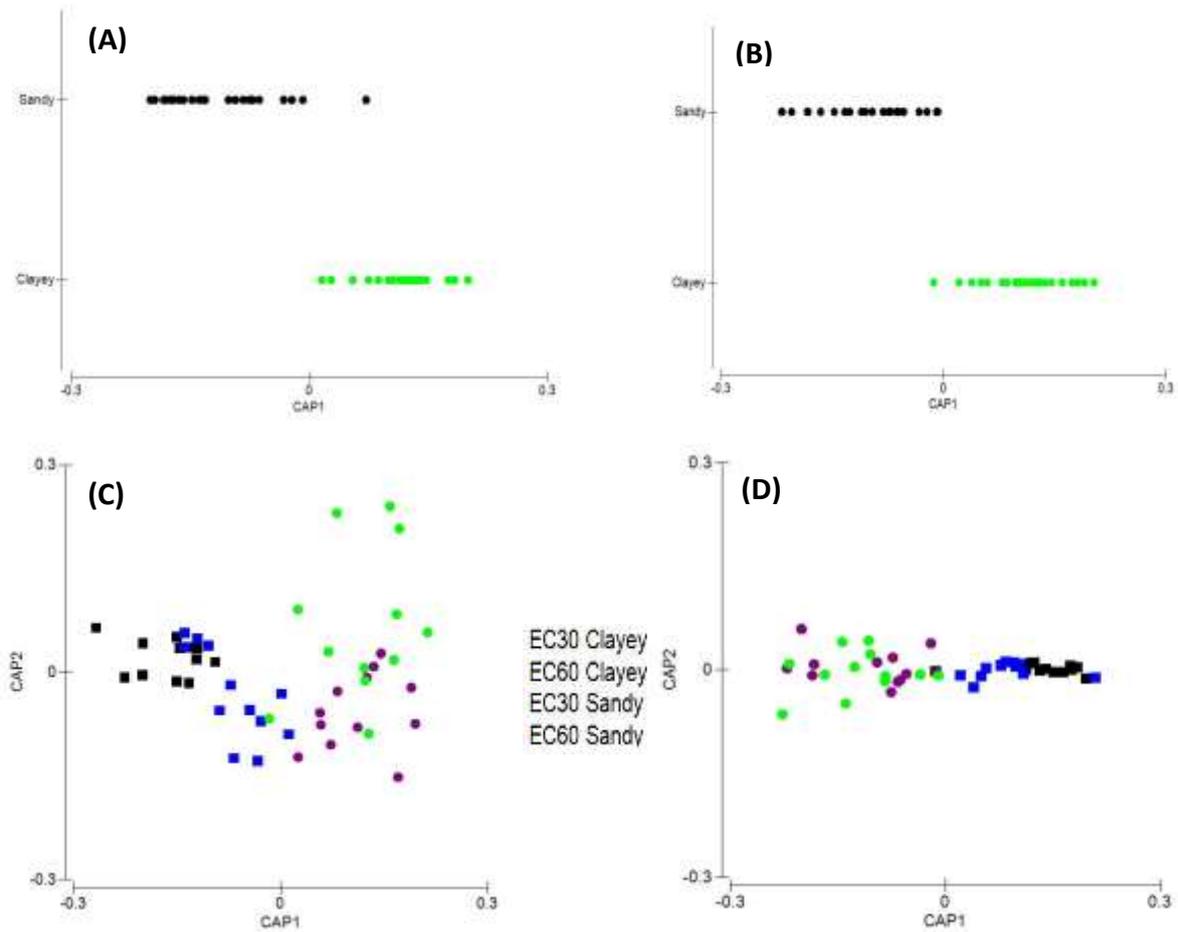
Significance levels: n.s:  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$

**Table 4.5:** Analysis of similarity (ANOSIM) to determine the influence of biochemical quality of organic inputs on composition of total and ammonia-oxidizing prokaryotes in the clayey and sandy soils at young growth stage (EC30) and flowering stage (EC60) of *Zea mays* (*n* = 48).

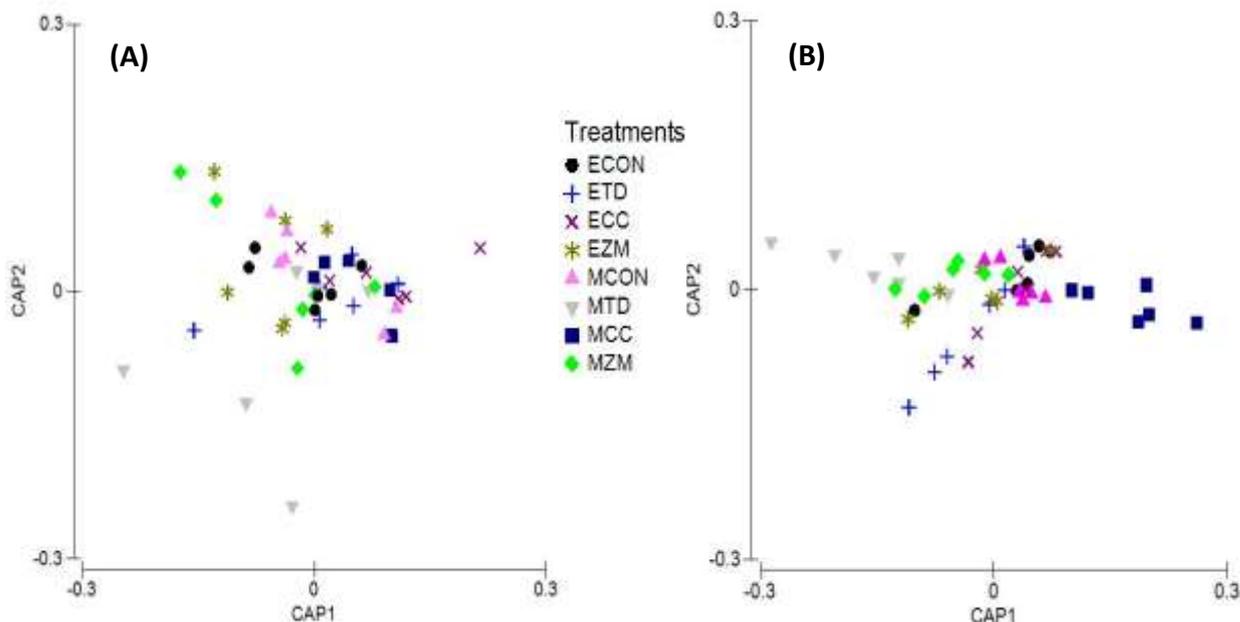
	Bacterial <i>amoA</i> gene (AOB)				Archaeal <i>amoA</i> gene (AOA)			
	Clayey soil		Sandy soil		Clayey soil		Sandy soil	
	EC30	EC60	EC30	EC60	EC30	EC60	EC30	EC60
Global <i>R</i> across treatments	0.244*	0.639**	0.225*	n.s	n.s	n.s	n.s	0.259*
Pairwise test groups	<i>R</i> statistic							
CON vs. TD	0.259	0.185	0.407	0.148	0.333	-0.167	0.259	0.370
CON vs. CC	-0.148	1.000	0.370	-0.037	0.148	0.056	0.222	0.444
CON vs. ZM	0.630	1.000	0.407	0.370	0.519	-0.056	0.019	-0.185
TD vs. CC	-0.074	0.185	0.296	0.148	-0.185	0.019	0.333	0.444
TD vs. ZM	0.185	0.704	-0.148	0.074	0.074	-0.093	-0.296	0.037
CC vs. ZM	0.333	0.815	0.222	0.222	0.000	-0.111	0.148	0.481

Treatment codes: CON = Control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*. Significance levels: n.s:  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ .

Canonical analysis of principal coordinates (CAP) was used to visualize the influence of the studied factors (i.e., soil type, crop growth stage, biochemical quality of organic inputs) on AOA and AOB community composition (Figs. 4.3 and 4.4). CAP ordination revealed distinct effect of soil type and quality of organic inputs but not crop growth stages on composition of AOB and AOA. For instance, ZM separated from CC and TD for AOB composition in the clayey soil (Fig. 4.4C). Further, clear separations between TD versus CC and ZM versus CC were evident for AOA composition in the sandy soil (Fig. 4.4D).



**Figure 4.3:** Canonical analysis of principal coordinates (CAP) for visual presentation of patterns of composition of ammonia-oxidizing communities as shaped by soil texture [(A) and (B)] as well as crop growth stages [(C) and (D)]. Ammonia-oxidizing communities: (A), (C) = ammonia-oxidizing bacteria (AOB); (B), (D) = ammonia-oxidizing archaea (AOA). Crop growth stages: EC30 = young growth stage, EC60= Flowering stage.



**Figure 4.4:** Canonical analysis of principal coordinates (CAP) for visual presentation of patterns of composition of ammonia-oxidizing communities as shaped by the contrasting biochemical organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). (A) = ammonia-oxidizing bacteria (AOB) and (B) = ammonia-oxidizing archaea (AOA). Treatments codes: E = Embu (clayey soil), M = Machanga (sandy soil), CON = Control, TD = *Tithonia diversifolia*, CC = *Calliandra calothyrsus*, ZM = *Zea mays*.

#### 4.3.3 Effects of biochemical quality of inputs on soil chemical properties

Overall, soil pH values were not different between the clayey (pH = 5.13) and sandy (pH = 5.05) soil ( $P > 0.05$ ) (Table 4.6). In more detail, ZM in the clayey soil had a higher pH than CON and CC at EC60 ( $P < 0.05$ ), but there was no organic input quality effect on soil pH at EC30 ( $P > 0.05$ ). In the sandy soil, TD showed higher pH than CON, CC and ZM at both crop growth stages ( $P < 0.01$ ). The latter also showed a higher pH than CC during both growth stages ( $P < 0.05$ ). The clayey soil showed higher  $\text{NH}_4^+$  concentrations than the sandy soil ( $P < 0.05$ ). However,  $\text{NH}_4^+$  concentration was neither influenced by crop growth stages nor by biochemical quality of organic inputs within each soil type ( $P > 0.05$ ).  $\text{NO}_3^-$  was not influenced by soil type as well as biochemical quality of organic inputs ( $P > 0.05$ ). Clayey soil had higher TC values than the sandy soil ( $P < 0.001$ ). On the other hand, crop growth stages and the biochemical quality of organic inputs did not influence TC within each soil type ( $P > 0.05$ ).  $\text{N}_t$  values were higher in the clayey than the sandy soil ( $P < 0.001$ ). In the clayey soil,  $\text{N}_t$  increased at EC60 ( $P < 0.001$ ). All plots which received organic input increased  $\text{N}_t$  compared to CON ( $P < 0.05$ ), but

no difference between biochemical contrasting organic inputs was measured ( $P > 0.05$ ). In the sandy soil, neither the crop growth stages nor organic input quality influenced  $N_t$  ( $P > 0.05$ ). The clayey soil revealed higher HWEC values than the sandy soil ( $P < 0.001$ ). In the clayey soil, TD had higher HWEC than ZM and CC at EC30 and EC60, respectively ( $P < 0.05$ ). At EC60, ZM showed higher HWEC than CC ( $P < 0.05$ ). In the sandy soil, TD had higher HWEC than CC and ZM at EC30 ( $P < 0.05$ ). The clayey soil had higher HWEN than the sandy soil ( $P < 0.001$ ). In the clayey soil, HWEN was low at EC60 compared to EC30 ( $P < 0.05$ ). ZM had high HWEN compared to CC and CON at EC60 ( $P < 0.05$ ). Biochemical quality of organic inputs did not influence HWEN at EC30 ( $P > 0.05$ ). On the other hand, an increase of HWEN at EC60 was measured in the sandy soil ( $P < 0.001$ ). TD revealed high HWEN compared to CON, CC and ZM ( $P < 0.05$ ). Soil moisture content (MC) was high in the clayey soil compared to the sandy soil ( $P < 0.001$ ) which corresponded with the precipitation patterns at the two study sites. Accordingly, MC was high at EC30 compared to EC60 within each site ( $P < 0.001$ ). Overall, biochemical quality of inputs did not influence MC in both soil types ( $P > 0.05$ ).

**Table 4.6:** Chemical properties of Embu (clayey) and Machanga (sandy) soils amended with biochemically contrasting organic inputs (4 Mg C ha<sup>-1</sup>). Values are means for soils sampled at young (EC30) and flowering (EC60) stages of maize crop after incorporation of organic inputs.

Factors	Treatments	Soil properties							
		Soil pH	NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]	TC [%]	N <sub>t</sub> [%]	HWEC [mg kg <sup>-1</sup> ]	HWEN [mg kg <sup>-1</sup> ]	MC [%]
EC30									
Clayey soil	CON	5.03 <sup>cd</sup>	2.8 <sup>a</sup>	1.9 <sup>c</sup>	2.27 <sup>bc</sup>	0.18 <sup>b</sup>	432. <sup>de</sup>	13.8 <sup>d</sup>	38.1 <sup>a</sup>
	CC	5.09 <sup>abcd</sup>	2.8 <sup>a</sup>	3.9 <sup>bc</sup>	2.67 <sup>abc</sup>	0.21 <sup>a</sup>	543 <sup>abc</sup>	26.5 <sup>ab</sup>	40.3 <sup>a</sup>
	ZM	5.40 <sup>a</sup>	3.6 <sup>a</sup>	2.1 <sup>c</sup>	2.70 <sup>abc</sup>	0.21 <sup>a</sup>	500 <sup>bcd</sup>	26.7 <sup>ab</sup>	40.8 <sup>a</sup>
	TD	5.39 <sup>ab</sup>	3.8 <sup>a</sup>	4.5 <sup>bc</sup>	2.73 <sup>ab</sup>	0.21 <sup>a</sup>	579 <sup>a</sup>	28.2 <sup>a</sup>	40.4 <sup>a</sup>
Sandy soil	CON	4.54 <sup>D</sup>	2.9 <sup>AB</sup>	5.9 <sup>AB</sup>	0.32 <sup>A</sup>	0.04 <sup>A</sup>	220 <sup>AB</sup>	3.9 <sup>C</sup>	13.1 <sup>AB</sup>
	CC	4.14 <sup>E</sup>	3.7 <sup>A</sup>	7.2 <sup>A</sup>	0.32 <sup>A</sup>	0.04 <sup>A</sup>	147 <sup>B</sup>	4.1 <sup>C</sup>	10.1 <sup>B</sup>
	ZM	4.64 <sup>D</sup>	2.8 <sup>AB</sup>	6.5 <sup>AB</sup>	0.30 <sup>A</sup>	0.04 <sup>A</sup>	172 <sup>B</sup>	4.9 <sup>C</sup>	12.2 <sup>AB</sup>
	TD	5.18 <sup>A</sup>	3.7 <sup>A</sup>	7.9 <sup>A</sup>	0.41 <sup>A</sup>	0.05 <sup>A</sup>	271 <sup>A</sup>	11.9 <sup>A</sup>	14.1 <sup>A</sup>
	SED	± 0.12	± 0.50	± 0.91		± 0.007	± 24.8		± 1.08
EC60									
Clayey soil	CON	4.87 <sup>d</sup>	4.0 <sup>a</sup>	5.8 <sup>ab</sup>	2.25 <sup>c</sup>	0.18 <sup>b</sup>	402 <sup>e</sup>	13.3 <sup>d</sup>	24.4 <sup>b</sup>
	CC	4.93 <sup>d</sup>	4.0 <sup>a</sup>	6.3 <sup>ab</sup>	2.64 <sup>abc</sup>	0.23 <sup>a</sup>	478 <sup>cd</sup>	19.9 <sup>c</sup>	22.3 <sup>b</sup>
	ZM	5.08 <sup>bcd</sup>	3.6 <sup>a</sup>	6.0 <sup>ab</sup>	2.74 <sup>a</sup>	0.23 <sup>a</sup>	549 <sup>ab</sup>	24.6 <sup>ab</sup>	25.1 <sup>b</sup>
	TD	5.28 <sup>abc</sup>	3.7 <sup>a</sup>	7.0 <sup>a</sup>	2.71 <sup>abc</sup>	0.23 <sup>a</sup>	524 <sup>abc</sup>	22.8 <sup>bc</sup>	24.8 <sup>b</sup>
Sandy soil	CON	5.14 <sup>C</sup>	2.5 <sup>AB</sup>	2.7 <sup>C</sup>	0.32 <sup>A</sup>	0.04 <sup>A</sup>	163 <sup>B</sup>	3.6 <sup>C</sup>	5.2 <sup>C</sup>
	CC	5.06 <sup>C</sup>	2.7 <sup>AB</sup>	4.0 <sup>BC</sup>	0.30 <sup>A</sup>	0.04 <sup>A</sup>	275 <sup>A</sup>	8.2 <sup>B</sup>	2.6 <sup>C</sup>
	ZM	5.63 <sup>B</sup>	2.3 <sup>AB</sup>	4.3 <sup>BC</sup>	0.31 <sup>A</sup>	0.03 <sup>A</sup>	248 <sup>A</sup>	7.5 <sup>B</sup>	3.4 <sup>C</sup>
	TD	6.06 <sup>A</sup>	2.1 <sup>B</sup>	5.5 <sup>AB</sup>	0.42 <sup>A</sup>	0.04 <sup>A</sup>	263 <sup>A</sup>	14.5 <sup>A</sup>	3.6 <sup>C</sup>
	SED	± 0.12	± 0.55	± 0.91		± 0.007	± 25.6		± 1.08
Soil type	Clayey soil	5.13 <sup>A</sup>	3.6 <sup>A</sup>	4.7 <sup>A</sup>	2.58 <sup>A</sup>	0.21 <sup>A</sup>	501 <sup>A</sup>	21.6 <sup>A</sup>	3.6 <sup>A</sup>
	Sandy soil	5.05 <sup>A</sup>	2.9 <sup>B</sup>	5.5 <sup>A</sup>	0.34 <sup>B</sup>	0.04 <sup>B</sup>	220 <sup>B</sup>	6.9 <sup>B</sup>	2.9 <sup>B</sup>
	SED	± 0.07	± 0.31	± 0.50		± 0.005	± 12.7		± 0.31

**Table 4.6:** Continued.

Factors	Soil properties	Soil pH	NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]	TC [%]	N <sub>t</sub> [%]	HWEC [mg kg <sup>-1</sup> ]	HWEN [mg kg <sup>-1</sup> ]	MC [%]
	Treatments								
Growth stages	EC30 <sub>clayey</sub>	5.23 <sup>b</sup>	3.3 <sup>ab</sup>	3.1 <sup>b</sup>	2.59 <sup>a</sup>	0.20 <sup>b</sup>	513 <sup>a</sup>	23.4 <sup>a</sup>	39.9 <sup>a</sup>
	EC60 <sub>clayey</sub>	5.04 <sup>c</sup>	3.8 <sup>a</sup>	6.3 <sup>a</sup>	2.58 <sup>a</sup>	0.22 <sup>a</sup>	489 <sup>a</sup>	19.9 <sup>b</sup>	24.3 <sup>b</sup>
	EC30 <sub>sandy</sub>	4.62 <sup>d</sup>	3.2 <sup>ab</sup>	6.8 <sup>a</sup>	0.34 <sup>b</sup>	0.04 <sup>c</sup>	203 <sup>b</sup>	5.8 <sup>d</sup>	12.4 <sup>c</sup>
	EC60 <sub>sandy</sub>	5.47 <sup>a</sup>	2.5 <sup>b</sup>	4.1 <sup>b</sup>	0.34 <sup>b</sup>	0.04 <sup>c</sup>	234 <sup>b</sup>	8.0 <sup>c</sup>	3.7 <sup>d</sup>
	SED	± 0.08	± 0.25	± 0.46		±0.004	± 12.7		± 0.54

Abbreviations: NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrate, TC = Total carbon, N<sub>t</sub> = Total nitrogen, HWEC = Hot water extractable carbon, HWEN = Hot water extractable nitrogen, MC = Soil moisture content.

Treatment codes: CON = Control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.

Values are means for organic inputs quality effects ( $n = 3$ ); soil type effect ( $n = 24$ ); growth stage effect ( $n = 12$ ).

SED = Standard error of the difference between two means for comparisons across organic inputs, growth stages and soil type.

Different letters (a-d/A-D), against the values indicate treatments with significant differences within factors accordingly ( $P < 0.05$ ).

#### **4.3.4 Linear correlations and regression analyses between soil chemical properties and soil microbial communities**

Total bacterial and archaeal 16S rRNA gene abundances were positively correlated with TC, N<sub>t</sub>, HWEC and HWEN in both soils ( $P < 0.01$ ) (Table 4.7). Archaeal *amoA* (AOA) gene abundance revealed positive correlations with TC, HWEC and HWEN in the clayey soil, while positive correlations between bacterial *amoA* (AOB) gene abundance with TC, N<sub>t</sub> and HWEN were shown in the sandy soil ( $P < 0.05$ ). Total archaeal, AOB and AOA gene abundances revealed positive correlations with MC in clayey soil ( $P < 0.05$ ). Negative correlations were determined for AOB ( $P < 0.001$ ) and AOA ( $P < 0.05$ ) gene abundances with NO<sub>3</sub><sup>-</sup> in the clayey soil. Abundance of all studied genes in both soils, except AOA in sandy soil, revealed positive correlations with soil pH ( $P < 0.05$ ).

Distance-based linear models (DISTLM) analyses between ammonia-oxidizing AOB and AOA community composition and soil chemical properties revealed significant relationships in the clayey, but not in the sandy soil (Table 4.7). Accordingly, variation in AOB community composition was explained by MC, N<sub>t</sub> and NO<sub>3</sub><sup>-</sup> (34 %, 24 % and 18 %, respectively;  $P < 0.05$ ), while the community composition variation of AOA was explained by soil pH (35 %), TC (34 %) and N<sub>t</sub> (29 %) ( $P < 0.01$ ).

**Table 4.7:** Linear correlations ( $r$ ) and regression ( $r^2$ ) analyses between the soil chemical properties, abundance (qPCR) and composition (TRFLP) of microbial communities ( $n = 48$ ).

Soil type	Soil properties	Bacterial 16S rRNA gene (qPCR) ( $r$ )	Archaeal 16S rRNA gene (qPCR) ( $r$ )	Archaeal <i>amoA</i> gene (AOA) (qPCR) ( $r$ )	Archaeal <i>amoA</i> gene (AOA) (TRFLP) ( $r^2$ )	Bacterial <i>amoA</i> gene (AOB) (qPCR) ( $r$ )	Bacterial <i>amoA</i> gene (AOB) (TRFLP) ( $r^2$ )
Clayey	Soil pH	0.52*	0.84***	0.59**	0.35**	0.47*	n.s
	NH <sub>4</sub> <sup>+</sup>	n.s	n.s	n.s	n.s	n.s	n.s
	NO <sub>3</sub> <sup>-</sup>	n.s	n.s	-0.44*	n.s	-0.68***	0.18*
	TC	0.63***	0.83***	0.55**	0.35**	n.s	n.s
	N <sub>t</sub>	0.73***	0.63**	n.s	0.29**	n.s	0.24*
	HWEC	0.65**	0.77***	0.48*	n.s	n.s	n.s
	HWEN	0.56**	0.80***	0.60**	n.s	n.s	n.s
	MC	n.s	0.43*	0.71***	n.s	0.96***	0.34**
Sandy	Soil pH	0.55*	0.55*	n.s	n.s	0.75***	n.s
	NH <sub>4</sub> <sup>+</sup>	n.s	n.s	n.s	n.s	n.s	n.s
	NO <sub>3</sub> <sup>-</sup>	n.s	n.s	n.s	n.s	n.s	n.s
	TC	0.79***	0.72***	n.s	n.s	0.51*	n.s
	N <sub>t</sub>	0.78***	0.67***	n.s	n.s	0.42*	n.s
	HWEC	0.41*	0.49*	n.s	n.s	n.s	n.s
	HWEN	0.66***	0.68***	n.s	n.s	0.64***	n.s
	MC	n.s	n.s	n.s	n.s	n.s	n.s

Abbreviations: NH<sub>4</sub><sup>+</sup> = Ammonium, NO<sub>3</sub><sup>-</sup> = Nitrate, TC = Total carbon, N<sub>t</sub> = Total nitrogen, HWEC = Hot water extractable carbon, HWEN = Hot water extractable nitrogen, MC = Soil moisture content.

Significance levels: n.s:  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

## **4.4 Discussion**

### **4.4.1 Response of microbial gene abundance and community composition to texture**

The abundance of all the studied microbial communities was higher in the clayey than sandy soil including AOB except for flowering stage. In addition, community compositions of AOB and AOA were clearly distinct according to the soil texture. Influence of soil texture on dynamics of soil microbial communities has widely been reported (Girvan et al., 2003; Morimoto et al., 2011; Musyoki et al., 2015; Neumann et al., 2013; Rasche et al., 2014). The distinct influence of texture on the dynamics of microbial communities in this study was attributed to differences in native soil fertility status between Embu (clayey) and Machanga (sandy) soils as was revealed by soil chemical properties. TC, N<sub>t</sub>, HWEC and HWEN values were higher in the clayey than the sandy soil which was also in line with initial soils characterization (Gentile et al., 2011a). Climatic conditions which influence soil moisture content also contributed to higher microbial abundance in the clayey soil. Accordingly, AOB and archaeal communities (total and AOA) abundances were positively correlated with moisture content. However, dynamics of microbial communities were not only confounded to soil texture but specific interaction effects between organic inputs and soil texture on dynamics of AOB and AOA were also evident.

### **4.4.2 Response of AOB to interrelations of soil texture and organic input quality**

Amendment of sandy soil with organic inputs had offset the presumed nutrient deficiency by availing organic nutrient substrates at high levels favored by microbial groups including AOB (Di et al., 2009; Musyoki et al., 2015; Wessén et al., 2011, 2010). Accordingly, the response of AOB abundance to high availability of mineralized organic N was observed for N-rich TD during the entire study period. Contrary, a reduced AOB abundance by intermediate quality inputs (CC) in comparison to high (TD) and low (ZM) quality inputs was also evident. This was explained by high polyphenol and lignin contents in CC binding organic C and N, thus limiting their availability to microbial communities (Millar and Baggs, 2004; Palm et al., 2001b; Schmidt et al., 2013). Promotion of AOB abundance in low and intermediate quality inputs was observed at flowering stage (EC60) in the sandy soil substantiating their gradual decomposition (Partey et al., 2013; Palm et al., 2001b). This effect was, however, more pronounced for the low quality inputs since its related AOB abundance increased matching that of high quality TD. A similar trend was observed

for total bacterial and archaeal abundance in the sandy soil which was further attributed to the high cellulose content in the low quality inputs (ZM) accelerating decomposition (Kunlanit et al., 2014; Samahadthai et al., 2010). In the sandy soil, we noticed a promotion of bacterial abundance in the control treatment (CON) at EC60 presumably induced by promoted excretion of rhizodeposits that might have boosted AOB proliferation (Bürgmann et al., 2005). These assumptions were further supported by positive correlations between AOB abundance with TC,  $N_t$  and HWEN indicating that AOB utilized organic C and  $NH_3$  derived from mineralization of organic substrates (i.e., rhizodeposition, organic inputs). Our results clearly implied that when nutrients are limited in sandy soils, AOB utilize mainly C and N substrates derived from freshly incorporated organic inputs (Musyoki et al., 2015).

In accordance with our expectations, high abundance of AOB regardless of organic input quality was revealed in the clayey soil, particularly at early growth stage (EC30). These findings were attributed to the native resource rich characteristics of this particular clayey soil (Gentile et al., 2011a) and corresponded to those observed by Musyoki et al. (2015) on the same soils. Moreover, the dominance of AOB abundance under non-nutrient limiting conditions particularly in response to high  $NH_4^+$  levels was earlier reported (de Gannes et al., 2014; Jia and Conrad, 2009; Levičnik-Höfferle et al., 2012). In addition, clayey soils protect freshly added organic inputs from immediate access by microbial communities (Dieckow et al., 2009; Krull et al., 2001; Zinn et al., 2005). We assumed that such protection mechanism along with the strong SOC background in the clayey soil which provided sufficient substrates to microorganisms had most likely masked the effect of quality of fresh organic inputs on AOB abundance.

In contrast to the abundance of AOB, protection of organic inputs by the clayey soil induced an input quality dependent separation of AOB community composition between CC versus ZM and TD versus ZM at EC60. This finding supported an argument by Krull et al. (2001) that protection of organic C by a clayey texture only reduces the immediate decomposition rates, with organic resources becoming available for decomposition over time. Such separation was therefore attributed to the biochemical differences, particularly the C/N ratio, between ZM (high C/N) and the low C/N inputs CC and TD (Gentile et al., 2011a; Palm et al., 2001b). This suggested that when C was not a limiting factor, N was the driving force for AOB community composition alterations. Wessén et al. (2010) also reported alterations of AOB composition due to C/N ratio differences between straw and peat inputs in a supposedly non-nutrient limiting clay loam soil.

Hence, it could be deduced that the mechanism by clayey soil to protect organic inputs (availing organic C) gave AOB a clear advantage of evolving to those communities being adapted to degrade organic inputs of distinct biochemical quality.

However, no substantial separation of AOB community composition in response to organic input quality was found in the sandy soil. This was attributed to organic C limitation as a substrate for AOB due to low SOC sequestration levels compared to the clayey soil. In addition, we argued that low organo-mineral associations in the sandy soil along with the limited surface area for adsorption and less protection of AOB against predation were supposedly responsible for the inexistent AOB composition alterations as explained (Dieckow et al., 2009; Kögel-Knabner et al., 2008). This finding was further supported by Poll et al. (2003) and Sessitsch et al. (2001) who reported substantial differences in total bacterial composition in clayey relative to sandy soils. It could therefore be suggested that N is most likely the driving force for observed AOB community alterations when C substrates are not limited.

#### **4.4.3 Soil texture exert stronger effect on AOA than organic input quality**

According to our hypothesis, higher abundance of AOA, regardless of biochemical quality of organic inputs was determined in the clayey compared to the sandy soil. This was primarily attributed to the characteristic high SOC retention of clayey soils providing sufficient C and N substrates for AOA proliferation, which masked the effects of quality of organic inputs (Gentile et al., 2011a; Musyoki et al., 2015; Zhang et al., 2010). These assumptions were corroborated by positive relationships between AOA with TC and  $N_t$  in the clayey soil possibly implying that archaea are adapted to decomposition of native and probably recalcitrant organic matter, in addition to freshly added inputs in clayey soils.

We did not observe any interrelation between the clayey soil and organic input quality on the community composition of AOA. This observation contradicted findings by Wessén et al. (2010) in a clay loam soil of a long-term fertilization field experiment in Ultuna (Sweden). According to Rasche and Cadisch (2013), faster turnover rates of SOC in tropical compared to temperate conditions might have reduced organic matter accumulation to limits whereby, in comparison to AOB, alterations of AOA community composition were not detectable. This implied that AOA was most likely less sensitive to low SOC in the tropics compared to temperate regions.

On the other hand, we determined effects of organic input quality on the community composition of AOA only in the sandy soil. This was particularly evident between TD versus CC as well as CC

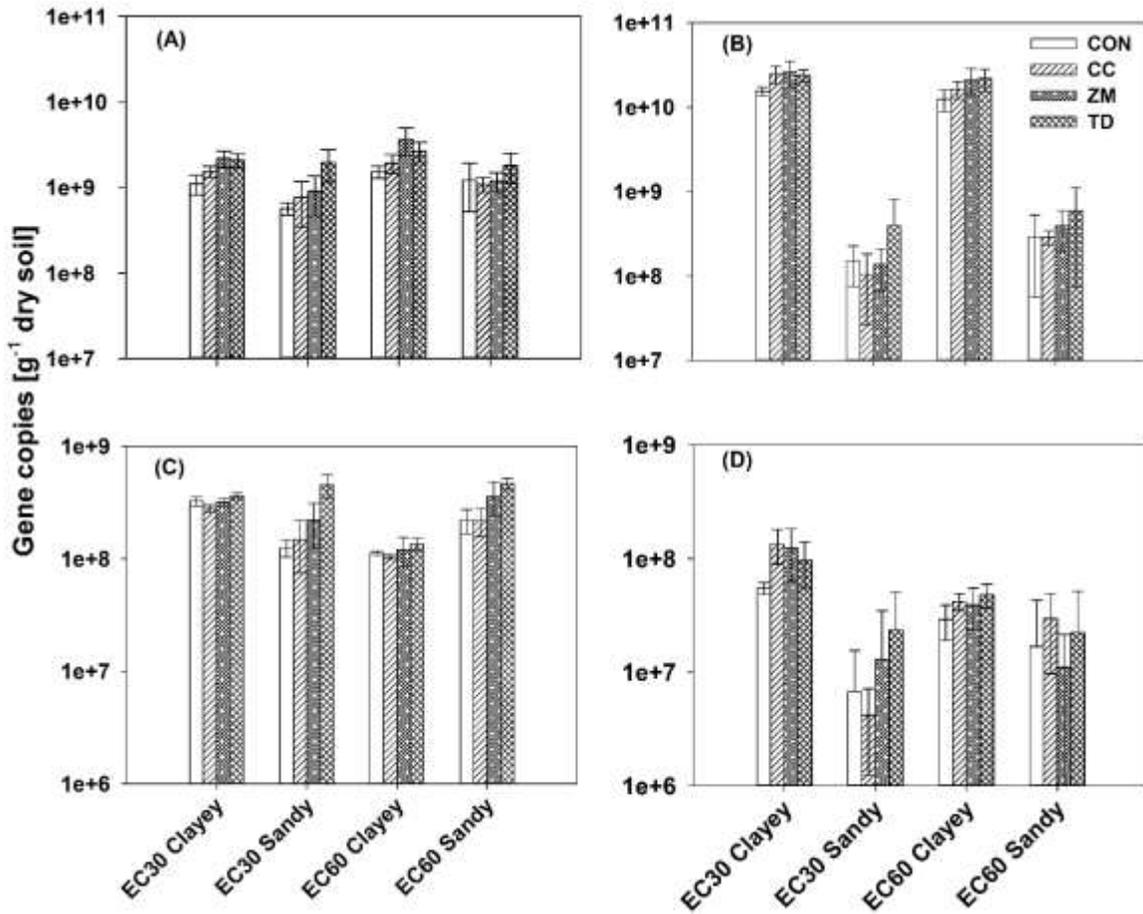
versus ZM at EC60. These alterations were attributed to higher polyphenol contents in CC causing the formation of chemical bonds (i.e., polyphenols-proteins bonds) which limited microbial access to organic N and C as opposed to TD and ZM (Millar and Baggs, 2004; Palm et al., 2001b). The findings suggested that polyphenol and lignin contents are the driving force for AOA composition alterations in soil conditions where C and N substrates are simultaneously limiting.

#### **4.5 Conclusions**

We observed that organic inputs of contrasting biochemical quality induced distinct responses to functional microbial groups of bacteria in specific soil types. We linked this to the background SOC between the two soil types as SOC regulates C substrates to microbes. For example, AOB abundance responded to soil amendments in the sandy soil when C was limiting. Specifically, high (TD) (i.e., low C/N) and low (ZM) (i.e., high C/N) quality inputs increased AOB abundance in comparison to the intermediate (CC) quality inputs suggesting that AOB primarily utilized both available organic N and C from fresh inputs for proliferation. On the other hand, its composition was mainly altered by N availability in the clayey soil when organic C was not limiting. Accordingly, alterations of AOB in the plots which received high N residues such as TD and CC from those which received low N such as ZM implied that N was the driving force to AOB composition dynamics provided that C substrates were available.

Contrastingly, AOA composition was altered by inputs amendment in the sandy soil when C substrate was limiting. Distinct separations of AOA assessed between plots that received TD and CC as well as between CC and ZM, implied that polyphenol and lignin contents are the driving force for AOA composition alterations in soil conditions where C and N substrates are simultaneously limiting. Variations in responses of AOB and AOA to interrelations of soil texture and organic inputs also support the acknowledged concept of niche differentiation between AOB and AOA (Jia and Conrad, 2009; Levičnik-Höfferle et al., 2012; Verhamme et al., 2011) with AOB being more adapted to high resource conditions than AOA which responded to recalcitrant substrates. As this work was based on DNA analysis, RNA-based approach is recommended to capture those communities that are actively involved in nitrification process.

## 4.6 Supplement



**Supplement S4.6.1:** Gene copy numbers of the four assayed genes in clayey and sandy soil types in the different treatments (descriptive means  $\pm$  standard deviation,  $n=3$ ): (A) total bacteria (bacterial 16S rRNA gene), (B) total archaea (archaeal 16S rRNA gene), (C) ammonia-oxidizing bacteria (AOB, bacterial *amoA* gene), and (D) ammonia-oxidizing archaea (AOA, archaeal *amoA* gene) after application of different biochemical quality organic inputs ( $4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). Crop growth stages: EC30 = young growth stage, EC60= Flowering stage. Treatments: CON = control, CC = *Calliandra calothyrsus*, TD = *Tithonia diversifolia*, ZM = *Zea mays*.

## **Chapter 5 General discussion**

The main goal of the presented PhD work was to understand the impact of long and short-term use of organic and inorganic inputs on the population dynamics of soil microbial communities involved in nitrification process in two contrasting environments in the tropics. Knowledge on dynamics of microbial communities is underpinned by their fundamental roles as regulators of SOM turnover, provision of organic nutrients in inorganic forms that are accessible to plants as well as being indicators of soil quality. The latter, although mainly ignored due to lack of tangible and immediate benefits, is a critical tool in assessment of sustainable management of soil resources. Consideration of contrasting soil types reflected the environmental differences existing in the tropics.

Development of molecular tools, and more importantly the affordability of PCR-based approaches like qPCR and TRFLP techniques laid the foundation to undertake this study. Although these approaches were limited to use of 16S rRNA genes rather than the whole genome DNA (**chapter one**), they made it possible to understand and draw important conclusions with regards to the dynamics of soil microbial communities and their interactions with the environment. For example using the qPCR (DNA-based functional potential abundance) and TRFLP (diversity), it was possible to prove, and therefore contribute to science that biochemical quality of organic resources combined with synthetic fertilizers and their interactions with abiotic factors like precipitation and both chemical and physical properties of soils influence the populations of microbial communities in distinctive ways. Such basic information is relevant to understand the function of the ecosystem of concern and could form basis for further analyses using more expensive but whole community analyses approaches. Integration of soil and input types with climate as well as soil chemical properties are therefore worth considering when deliberating on viable means to solve the issue of soil degradation if sustainable food production particularly in the developing countries has to be achieved.

These factors were studied and presented in various chapters in this thesis with specific objectives and obvious findings intended for publication. Thus, all the chapters contribute to a common objective of understanding how in the long and short-term, the biochemical quality of organic inputs and combination with synthetic fertilizer shape the dynamics of soil microbial communities.

The major findings of this thesis are contextualized and future work directions provided in the following sections.

### **5.1 Is continuous application of organic resources to boost SOM a solution to soil degradation in the tropics?**

This question was resolved through comparison of the results of initial soil characterization and those generated in year 2013. Table 5.1 therefore presents an overview of changes of total carbon (TC), total nitrogen ( $N_t$ ) and soil pH over 11 years since the establishment of the SOM trials in clayey and sandy soil types in Kenya in 2002. Continuous cultivation without and with application of organic residues resulted in degradation of soil resource in both soil types. In comparison to start of the experiment, TC was reduced by 40 % in the absolute control plots and by 35 % in the organic residues treated plots, whereas a 33 % reduction in  $N_t$  was determined for both absolute control (CON) and organic inputs (OR) treated plots in Machanga site (sandy soil) (Table 5.1). At Embu site (clayey soil), TC was reduced by 23 % and 8 %, whereas  $N_t$  reduced by 35 % and 21 % in CON and OR treated plots respectively. The higher rate of C loss in the sandy soil was explained by its low protection of freshly added organic matter from microbial activities to meet their C substrate demand (Puttaso et al., 2011). In addition, increased mineralization rates most likely occurred to provide nutrients for plants uptake since the soil is nutrient limited (Chivenge et al., 2009; Gentile et al., 2011a; Mtambanengwe et al., 2006). On the other hand, the low rate of C loss in the clayey soil was apparent due to its native high SOC background (Gentile et al., 2011a) coupled with strong protection of recently added organic residues. In addition, C retention in clayey compared to sandy soil was associated with formation of clay sized organo-mineral associations promoted by addition of organic residues (Dieckow et al., 2009; Kögel-Knabner et al., 2008; Jones and Singh, 2014). Similar observations were evident for  $N_t$  and soil pH, with soil potential N being very limiting in Machanga site. In summary, TC has remained moderate at Embu site but deteriorated from low to very low at Machanga site for the 11 years considered in this study (Tekalign, 1991).  $N_t$  has significantly reduced from high to moderate at Embu site with a worsening scenario at Machanga site which has reached a limiting state (Hazelton and Murphy, 2007; Tekalign, 1999). However, soil texture for Embu and Machanga soils has remained unaffected after 11 years of continuous application of biochemically different quality organic residues (**Chapter four**). Overall, soil degradation rate is soil type specific. Continuous application

of organic residues alone particularly in the tropics seems therefore, not a solution to soil degradation in general. Nevertheless, repeated application of residues provides a substantial buffer to soil degradation as shown by lower deterioration percentages of soil physico-chemical properties in soils treated with organic residues compared to the absolute control. This therefore justifies the integration of organic and inorganic soil inputs (Vanlauwe et al., 2010).

**Table 5.1:** Changes in soil physico-chemical properties after 11 years of repeated applications of biochemically different quality organic residues (4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) in comparison to the start of the SOM experiments in year 2002. Values are means of respective number of observations as indicated per column.

Site	Soil properties	Year 2002 (n =3)	Year 2013 (CON) (n = 6)	Year 2013 (OR) (n = 18)	[%] reduction (CON)	[%] reduction (OR)
Machanga	TC [g kg <sup>-1</sup> ]	5.3	3.2	3.4	40	35
Machanga	N <sub>t</sub> [g kg <sup>-1</sup> ]	0.6	0.4	0.4	33	33
Machanga	Soil pH	6.13	4.84	5.12	21	16
Embu	TC [g kg <sup>-1</sup> ]	29.3	22.6	27.0	23	8
Embu	N <sub>t</sub> [g kg <sup>-1</sup> ]	2.8	1.8	2.2	35	21
Embu	Soil pH [water]	5.81	4.95	5.20	14	10

Soil chemical parameters: TC = Total carbon, N<sub>t</sub> = Total nitrogen. Treatment codes: CON = absolute control; OR = Organic residues regardless of their quality since there were no residue quality effects on TC and N<sub>t</sub> values in year 2013 (**Chapter four**). Data for year 2002 was sourced from Gentile et al. (2011a).

## **5.2 Implications of quality of organic resources and mineral N fertilizer on dynamics of microbial communities in a clayey soil**

A substantial understanding of the long and short-term effects of quality of organic resources and mineral N fertilizer on the dynamics of soil microbial communities has been achieved by this study (**chapters two and three**). Although a prolongation of the experimental period was recommended to clearly capture the effects of interrelations of soil moisture content as dictated by precipitation patterns with quality of organic inputs (**chapter three**), and also to achieve conclusive findings particularly on the composition of total communities (**chapter two**), clear effects of quality of

organic resources on population dynamics of microbial communities were demonstrated. As revealed and discussed in **chapter two** and **three**, the biochemical quality of organic resources (CC, ZM, TD) regulated the abundance and composition of total and ammonia-oxidizing communities (Muema et al., 2015). In the mid to long-term continuous application of organic inputs, high quality inputs (TD) promoted the abundance of microbial communities, whereas intermediate quality inputs (CC) decreased the abundance, while low quality inputs portrayed specific effects on abundance to distinct microbial groups (**chapter two**, Fig. 2.2). These findings were explained by the contrasting proportions of organic N, lignin as well as polyphenol contents in the materials (Millar and Baggs, 2004; Palm et al., 2001b). Regulation of abundance and composition of microbial communities by the biochemical quality of organic resources have been reported also elsewhere (Chèneby et al., 2010; Hai et al., 2009; Kamolmanit et al., 2013; Rasche et al., 2014; Wessén et al., 2010). Alterations of the composition of microbial communities by the biochemical quality of organic resources occurred to a minor extent (**chapter two**, Fig. 2.4). This justifies the recommendation to prolong the experiment for more conclusive answers. Similar findings were observed in the short-term, (i.e., after fresh application of organic inputs) except that AOA abundance remained unaffected (**chapter three**, Fig. 3.2). Sole use or combination of mineral N fertilizer with organic inputs regardless of their quality revealed a decrease in abundance of microbial communities except for the AOB abundance (**chapter two**, Fig. 2.2; **chapter three**, Table 3.3). This decrease in abundance of microbial communities was attributed to the indirect reduction of soil pH by the mineral N fertilizer suppressing microbial proliferation. It implied that AOB are less sensitive to pH changes. Similar findings were also reported by Wessén et al. (2010). Another possible explanation was the chaotropic nature of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  components of the inorganic fertilizer reducing water activity in soils hence limiting microbial growth (**Chapter three**). Chaotropicity refers to the ability of solutes to induce stress to microbial communities by destabilization of macromolecular systems as a consequent of reduced water activity (de Lima Alves et al., 2015; Hallsworth et al., 2003). However, since this study did not focus on water activity measurements (de Lima Alves et al., 2015; Stevenson and Hallsworth, 2014), this potential effect was proposed for consideration in future related studies. In summary, quality of SOM, whether accumulated over mid to long-term or freshly added into clayey soil, influences the abundance and composition of ammonia-oxidizing communities.

### **5.3 Implications of interrelations of soil texture and quality of organic residues on abundance and composition of microbial communities**

Consideration of two distinct soil types (clayey and sandy) in the tropics provided an understanding of the interrelations between soil texture and quality of organic residues and their impact on microbial communities (**chapter four**). The linkage between soil microorganisms, soil particles and SOM, particularly the freshly incorporated organic residues, is soil aggregation, and has consequences on soil productivity (Chivenge et al., 2009; Fonte et al., 2009; Gentile et al., 2011a; Lucas, 2013). Soil microorganisms promote soil aggregation while decomposing the added organic residues (Six et al., 2004; Tisdall, 1991). Studies conducted in the same experiment by Gentile et al. (2011a) showed promotion of soil aggregation by addition of organic residues but was not related to their quality in both soil textures. Consequently, it was proposed that the strong soil structure associated with clayey soil would protect the freshly incorporated organic inputs hindering their immediate accessibility by microbial communities thereby masking the effects of input quality on microbial communities. On the other hand, the coarse structure of sandy soils would provide less protection of organic inputs rendering them accessible to microbial communities and hence result in fast decomposition. Accordingly, distinct responses of different microbial groups to interrelations of soil texture and organic inputs quality were revealed. For example, AOB abundance was influenced by quality of organic inputs in the sandy soil but remained unaffected in the clayey soil (**chapter four**, Fig. 4.1C). An opposite scenario was true for its composition (**chapter four**, Fig. 4.2C and 2D). Total bacterial abundance was influenced by inputs quality in both soil types (**chapter four**, Fig. 4.2A). On the other hand, archaeal communities (total and AOA) genes abundance were not affected by quality of inputs (**chapter four**, Fig. 4.1B and 1D). However, a clear soil texture distinction was observed (**chapter four**, Table 4.3). Moreover, AOA composition was altered by input quality in the sandy soil (**chapter four**, Fig.4.2C and 2D). In addition, crop growth stages influenced microbial dynamics probably due to interactions with the crop or soil moisture changes (Onwoga et al., 2010). This was more pronounced in the clayey soil with reduction of communities at flowering stage associated with the competition for nutrients and moisture with the crop. However, an increase of AOB abundance was observed at flowering stage of maize in the sandy soil which was associated with a delayed decomposition of low and intermediate quality residues as well as carbon substrates provision to microbial communities through root exudations (Bürgmann et al., 2005). Overall, soil texture

residues interrelated effects were specific to microbial groups with AOB being more sensitive than AOA. This supported the concept of niche differentiation between AOB and AOA which reduces their competition for substrates (Jia and Conrad, 2009; Levičnik-Höfferle et al., 2012; Verhamme et al., 2011).

#### **5.4 Microbial dynamics versus crop performance as shaped by quality of residues and mineral N fertilizer**

From the findings in **Chapter two, three and four**, distinct effects of quality of organic residues on the dynamics of microbial communities have been reported. High quality organic resources promoted the abundance of microbial communities but the intermediate quality resources decreased the abundance of communities. Low quality *Zea mays* (ZM) inputs on the other hand demonstrated contrasting effects on microbial communities depending on microbial group and crop growth stage. For example, in a clayey soil the abundance of AOB and total archaea was decreased by ZM, while that of AOA and total bacteria was promoted (**chapter two**, Fig. 2.2). In the sandy soil at young plants growth stage, the abundance of AOB and total bacteria was decreased but promoted at flowering stage by ZM (**chapter four**, Fig. 4.1C and S4.6.1). In addition, mineral N regardless of type of organic resources decreased the abundance of microbial communities except that of AOB (**chapter two**, Fig. 2.2 and **chapter three**, Table 3.3). Additionally, clear specific effects of quality of organic resources and mineral N on the composition of AOB and AOA were reported (**chapter two**, Figs. 2.3, 2.4 and **chapter four**, Fig. 2), which emphasized the role of C/N ratio, polyphenol contents as well as mineral fertilizer in regulation of dynamics of microbial communities.

On the other hand, in both clayey and sandy soils, high (TD) and intermediate (CC) quality organic resources did not reveal significant treatment differences on maize grain yields. However, their grain yields were significantly higher compared to low quality inputs (ZM) over ten seasons (Chivenge et al., 2009). In addition, combination of low quality inputs with mineral N fertilizer compared to sole use of inputs improved grain yields specifically in the nutrient limited sandy soil. Similar findings on maize grain yields were observed in a sandy soil in Zimbabwe (Mtambanengwe et al., 2006).

From these two scenarios, high quality inputs (TD) followed a similar principle of promotion of abundance of microbial communities and increase of crop yields. This implied that their high and

easily accessible organic N content was sufficient to support microbial activities and plants uptake simultaneously. Intermediate quality inputs on the other hand decreased microbial communities but increased crop yields. The improvement of crop yields was attributed to high initial N in CC (Chivenge et al., 2009; Vanlauwe et al., 2005) coupled with gradual release of mineralized N throughout the growing season ensuring constant N supply which coincided with plants nutrients demand boosting the yields. Nevertheless, its high polyphenol and lignin contents probably caused the decline of microbial communities by favoring only those which could degrade these recalcitrant materials which was supported by the microbial composition data (**Chapter two and four**). Low quality inputs were characterized by contrasting effects on the abundance and composition of microbial communities but a clear decrease of crop grain yields as a consequence of their high C/N leading to N immobilization. This scenario agrees with suggestions that some microbial groups like AOA have alternative nutrient sources (Blainey et al., 2011; Chen et al., 2008; Stopnišek et al., 2010) unlike plants which heavily rely on readily available soil mineral nutrients. Combination of organic inputs with mineral N fertilizer reduced the abundance of AOA as well as total communities and altered the composition of AOB including total communities. Although this study did not measure and link soil nitrification potential to community dynamics of AOB and AOA communities, reduced abundance of AOA might not have necessarily resulted to reduced nitrification. This was because AOB abundance remained unchanged and might therefore have been responsible for nitrification. On the other hand, if reduction in AOA abundance reduced nitrification rate, it would have been an added advantage to the crops as  $\text{NH}_4^+$  would be retained in the soil for plants uptake (Subbarao et al., 2013). This latter argument would therefore support the general greater maize yields observed upon combination of organic resources and mineral N fertilizer than sole application of individual resource (Chivenge et al., 2009). However, negative interaction effects, i.e. yields as a result of combination of organic resources and mineral N were not large enough to surpass the yield increase brought about by the sum of the separate addition of the two resources, were observed (Chivenge et al., 2009). These were attributed to high levels of N both from organic inputs and high rate of fertilizer masking the possible positive interaction effects (Chivenge et al., 2009). However, at the sandy soil field trial, low quality inputs combined with mineral N revealed positive interaction effects compared to their sole use due to the fertilizer compensation effect on N limitation. Overall, it was deduced that high quality inputs followed a similar principle in influencing the dynamics of microbial communities and crop

performance while intermediate and low quality inputs followed different principles, contrastingly influencing the dynamics of microbial communities and crop performance.

## **5.5 Conclusions**

From the research findings and implications of this PhD research firstly, quality of SOM whether accumulated over mid to long-term or freshly added into clayey soil had effects on the abundance and composition of ammonia-oxidizing communities. In relation to the size of microbial communities, high quality organic resources promoted their abundance whereas the intermediate quality residues led to their decrease. On the other hand, low quality residues either promoted or decreased the abundance of microbial communities dependent on microbial group and the crop growth stage. The contrasting quality attributes of organic residues induced separation of composition of communities too. In this regard, microbial groups particularly the ammonia-oxidizing communities differentiated to those communities that were sensitive to high N content, those that could degrade recalcitrant resources from those that could tolerate N limiting conditions. Sole use of mineral N fertilizer or its combination with organic residues decreased the abundance of microbial communities apart from the AOB indicating its lower sensitivity to pH changes created by mineral fertilizer. Secondly, the effects of interrelations of soil texture and quality of organic resources were specific to microbial groups with AOB being more sensitive than AOA. In this respect, sandy soils revealed effects of organic inputs quality on the abundance of AOB and altered the community composition of AOA. On the other hand, clayey soil induced a considerable alteration of the community composition of AOB in response to biochemical quality of organic inputs. These findings supported the concept of niche differentiation between AOB and AOA which reduces their competition for substrates (Jia and Conrad, 2009; Levičnik-Höfferle et al., 2012; Verhamme et al., 2011).

The findings of dynamics of microbial communities were compared to those of crop yields with regard to use of organic residues of different biochemical qualities in the same soils. It was deduced that high quality inputs followed a similar principle in modifying the abundance of microbial communities and crop yields. Intermediate and low quality inputs followed different principles, contrastingly influencing the abundance of microbial communities and crop performance. Finally, it was also found out that soil degradation was specific to soil type and that continuous application

of organic residues particularly in the tropics alone (at least at the tested rate) is not a general solution to soil degradation. Nevertheless, repeated application of residues provided a substantial buffer to soil degradation which should be embraced for sustainable management of soil resource.

## **5.6 Future directions**

Although this study provides substantial insights on influences of agricultural practices on microbial ecology linked to soil N cycle in SSA, it was mainly limited to the nitrification process. In order to have a more complete understanding of N dynamics in the soil ecosystem, focus on other processes which include nitrogen fixation, mineralization, ammonification as well as denitrification within the N-cycle is highly recommended (Hai et al., 2009; Zhang et al., 2013).

DNA-based analyses were essential to understand the influence of different quality of organic resources and mineral N fertilizer on dynamics of microbial communities. However, use of RNA-based analyses are recommended to show those particular communities that are actively involved in specific soil processes like nitrification (España et al., 2011).

Further understanding of N dynamics at a process or activity level can be achieved by carrying out enzymatic analysis (Kamolmanit et al., 2013). Linkages of community diversity and abundance data with nitrification and other potential functions would improve the understanding of the soil ecosystem functions (Wessén et al., 2010).

The role of microbial communities on global climate change is not completely understood. Therefore, the above proposed linkages of specific microbial community data with potential functions in consideration of abiotic factors like precipitation, temperatures among others could improve the understanding of how microorganisms affect climate change and possible mitigation measures. In addition, data on microbial abundance and composition could be incorporated in climate models to reduce uncertainties and improve estimation and predictions of future effects of climate change (Allison et al., 2013; Singh et al., 2010; Todd-Brown et al., 2012).

In order to specifically understand interrelations of soil texture and quality of organic inputs on soil microbial dynamics, consideration of controlled environments in future is recommended to avoid the influence of environmental factors like precipitation which cannot be eliminated in the field experiments.

Only one rate of application of mineral N fertilizer ( $120 \text{ kg N ha}^{-1} \text{ season}^{-1}$ ) was considered for combination with different organic residues. This led to a decrease in abundance of microbial communities in addition to negative interactions on maize performance specifically with high and intermediate quality residues (Muema et al., 2015; Chivenge et al., 2009). Future studies should consider testing different, but lower application rates of mineral fertilizer N. This would prevent negative potential effects of excess N especially with high and intermediate quality inputs, like contamination of the environment such as water bodies which is ecology unfriendly (Galloway et al., 2008; Peter et al., 1997; Subbarao et al., 2013).

Scaling up of findings is critical for the farmers. Therefore, future trials should be set up in farmers' fields so that they can have a sense of ownership of the research findings and interpretation generated. Such an approach would convince farmers to prioritize use of organic resources as soil inputs and perhaps be encouraged to grow some of the shrubs by their farm sides as hedge crops.

ISFM technology particularly the importance of organic compared to inorganic fertilizers has not yet been well understood or appreciated by the policy makers. Therefore, organic inputs are not well integrated in decision making systems at a national level. It would be important to emphasize on their importance at the grass root levels of governance.

## Summary

Tropical agro-ecosystems are limited in nutrient resources as a consequence of i) being composed of highly weathered soils, ii) low native soil organic matter (SOM) content due to conversion of natural forests to arable lands and iii) continuous cropping without replenishing soil nutrients. Recovery of SOM by use of organic residues is faced with other competing uses like animal fodder. Moreover, existing SOM is further reduced by increased turnover rates due to favorable climatic conditions in the tropics. Incorporation of residues is therefore a justified means to restore SOM and to provide crop nutrients through microbial mediated activities like nitrification. Nitrification is a central step of the nitrogen (N) cycle, whereby ammonia is converted into nitrite and then to nitrate by bacteria and archaea through production of the *amoA* gene encoding the  $\alpha$ -subunit of the enzyme ammonia monooxygenase. In order to better understand the impact of organic residues of contrasting biochemical quality (i.e., high quality *Tithonia diversifolia* (TD; C/N ratio: 13, lignin: 8.9 %, polyphenols: 1.7 %), intermediate quality *Calliandra calothyrsus* (CC; 13, 13, 9.4) and low quality *Zea mays* (ZM; 59, 5.4, 1.2)) on nutrient provision, effects of residue quality on dynamics of relevant decomposer microbial communities were studied. In addition, mineral N fertilizer was used to compensate for mineral N limitations especially in case of low and intermediate quality residues. Since N is one of the most limiting crop nutrients in the tropics, this study therefore focused on ammonia-oxidizing prokaryotes, using DNA-based quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (TRFLP) techniques. In addition, soil physicochemical properties were measured and linked to the dynamics of microbial communities. The study hypothesized that soil type due to differences in structure and nutrient background, as well as seasonality, which influences soil moisture, would shape the response of the studied communities to biochemical quality of residues.

In the first part of this research project (**chapter two**), the long-term response of abundance and composition of total and ammonia-oxidizing prokaryotes to biochemically contrasting residues and their combination with mineral N fertilizer in a clayey soil was determined. From the results, the abundance of all studied genes was promoted by high quality inputs of TD due to their high amounts of readily available N. However, they were reduced by intermediate quality inputs (CC) which was linked to its high polyphenol and lignin contents. This fact induced N limitation via the formation of polyphenol-protein bonds protecting organic N from microbial decomposition. TD and CC also showed a separation with regards to AOA composition. Low quality maize inputs

(ZM) due to organic N limitation compared to TD decreased the abundance of AOB and total archaeal genes and revealed a separation of AOA and AOB compositions. However, the promotion of AOA abundance by ZM was attributed to its high affinity for ammonia under low N conditions. Residues, regardless of quality, upon combination with mineral N decreased the abundance of communities except AOB and altered their composition apart from AOA. This was attributed to the indirect reduction of soil pH by the fertilizer with AOB abundance being less sensitive to pH changes compared to AOA. The findings suggested residue type and mineral N dependent effects on dynamics of AOB and AOA.

In the second part (**chapter three**), building on the results of first study in the clayey soil, seasonality effects particularly the precipitation were considered. To achieve this, data from two consecutive long rains seasons in years 2012 and 2013 was integrated. Two distinct growth stages (young and flowering) of *Zea mays* were also considered. The results revealed temporal responses of abundance of AOB but not AOA genes to biochemical quality of residues. Generally, a similar trend of organic input effect on AOB abundance as in **chapter two** was revealed, except that ZM increased AOB abundance at flowering stage of maize. This was a confirmation of delayed decomposition of ZM throughout the season as a result of N limitation. Use of N fertilizer or its combination with organic inputs revealed similar results as explained in **chapter two**. This emphasized the lower sensitivity of AOB abundance to pH changes compared to AOA. In addition, precipitation, regardless of residue quality, distinctly influenced all studied genes apart from AOA. Accordingly, AOB and total archaeal abundance increased while that of total bacteria reduced in year 2013 which was drier compared to 2012. It was thus suggested that optimal water filled pore space, which favored proliferation of AOB, was achieved in year 2013. Total archaea were adapted to reduced precipitation while higher precipitation in year 2012 favored the proliferation of total bacteria. Overall, the results suggested that the effects of interrelations of residue quality and seasonality, which also regulate soil moisture, contributed to niche differentiation between AOB and AOA.

In the third component (**chapter four**), a sandy soil texture was considered in addition to clayey soil, which was the focus of the previous two studies. The two contrasting soils showed specific interrelations with SOM with regards to their soil structure as supported by their differences in background soil organic carbon (SOC). It was thus hypothesized that such differences will lead to

distinct effects on dynamics of microbial communities. In clayey soils, apart from high SOC, clay particles form organo-mineral associations, which provide a large surface area for proliferation and protection of microorganisms from predation, favored a higher abundance of AOB and AOA regardless of residue type in this study. On the other hand, sandy soils having a coarse structure providing less protection of freshly added residues from microbial access. Interrelations of sandy texture and quality of organic residues promoted AOB abundance and altered AOA composition. Accordingly, TD (high organic N) and ZM (high organic C) compared to CC with high polyphenol and lignin contents promoted the abundance of AOB and altered AOA composition. Interrelations of the clayey soil with organic residues altered AOB composition. Accordingly, AOB was separated between TD versus ZM and CC versus ZM. These findings implied that AOB was able to access N from CC and that N was the driving force for AOB alterations when C was not limited (clayey soil). On the other hand, AOA composition alteration was only possible when C and N substrates were simultaneously limiting, as in the case of the studied sandy soil. Therefore, soil texture and residues interrelations effects are microbial group specific with AOB being more sensitive compared to AOA.

Overall, the results of this PhD research revealed specific responses of dynamics of AOB and AOA to quality of organic residues and their combinations with mineral N fertilizer. They also revealed effects of interrelations between quality of residues and soil texture as well as seasonality particularly precipitation on dynamics of microbial communities. Future investigation of active microbial communities with the use of RNA-based approaches need to be considered to further improve our understanding of quality of SOM on soil nutrient dynamics.

## Zusammenfassung

Tropische Agrarökosysteme haben limitierte Nährstoffressourcen als Folge von i) stark verwitterten Böden, ii) einem niedrigem Gehalt an natürlicher organischer Bodensubstanz (OBS) aufgrund der Konversion von natürlichen Wäldern zu landwirtschaftlicher Nutzfläche und iii) kontinuierlichem Ackerbau ohne ergänzende Nährstoffzufuhr. Die Wiederherstellung der OBS durch Einarbeitung von organischen Ernterückständen steht in Konkurrenz zu anderen Nutzungen wie etwa Tierfutter. Aufgrund der günstigen klimatischen Bedingungen in den Tropen wird darüber hinaus die bestehende OBS durch erhöhte Umsatzraten weiter reduziert. Daher ist für den Wiederaufbau der OBS und von Pflanzennährstoffen, die durch mikrobielle Aktivitäten wie Nitrifikation bereitgestellt werden, die Einarbeitung von Ernterückständen eine zwingende Notwendigkeit. Nitrifizierung ist ein zentraler Schritt im N-Kreislauf, wobei das mineralisierte Ammonium zuerst in Nitrit und danach in Nitrat von Bakterien und Archaeen durch das *amoA* Gen, welches die  $\alpha$ -Untereinheit des Enzyms Ammoniummonooxygenase (AMO) codiert, umgewandelt wird. Um die Auswirkungen von Ernterückständen von kontrastierender biochemischer Qualität (d.h., hohe Qualität *Tithonia diversifolia* (TD; C / N-Verhältnis: 13, Lignin: 8.9%; Polyphenole: 1.7%), mittlere Qualität *Calliandra calothyrsus* (CC; 13, 13,4) und niedrige Qualität *Zea mays* (ZM; 59, 5.4, 1.2) auf die Nährstoffverfügbarkeit besser verstehen zu können, wurden die Auswirkungen der Rückstandsqualität auf die Dynamik der relevanten mikrobiellen Zersetzergemeinschaften untersucht. Zusätzlich wurde mineralischer N-Dünger appliziert, um den mineralische N-Mangel zu kompensieren, der vor allem bei Ernterückständen niedriger und mittlerer Qualität auftritt. Da N einer der am meist limitierenden Pflanzennährstoffe in den Tropen ist, konzentriert sich die Studie auf Ammonium-oxidierende Prokaryoten, unter Verwendung von DNA-basierter quantitativer PCR (qPCR) sowie des terminalen Restriktionsfragmentlängenpolymorphismus (TRFLP). Darüber hinaus wurden die physikalisch-chemischen Eigenschaften des Bodens gemessen und mit der Dynamik der mikrobiellen Gemeinschaften verknüpft. Es wurde die Hypothese aufgestellt, dass der Bodentyp, aufgrund von Unterschieden in Struktur und Nährstoffgehalt als auch die Saisonalität, welche die Bodenfeuchte beeinflusst, die Reaktion der untersuchten mikrobiellen Gesellschaften auf die biochemische Qualität der Ernterückstände bestimmen würde.

Im ersten Teil der Forschungsstudie (Kapitel 2) wurde die langfristige Reaktion der Abundanz und Zusammensetzung aller Ammonium-oxidierenden Prokaryoten auf biochemisch kontrastierendes organisches Material und deren Kombination mit mineralischem N Dünger in einem tonigen Boden untersucht. Die Ergebnisse zeigten, dass durch die Zugabe qualitativ hochwertiger organischer Einträge (TD), aufgrund des leicht verfügbaren N, die Abundanz aller untersuchten Gene stimuliert wurde. Bei Zugabe von organischem Material mittlerer Qualität (CC) wurden sie jedoch reduziert, was auf den hohen Polyphenol- und Ligninanteil des zugeführten Materials zurückgeführt werden kann. Durch die Bildung von Polyphenol-Protein-Verbindungen, welche organischen N vor mikrobieller Zersetzung schützen, wird somit ein N-Mangel herbeigeführt. Weiterhin wurde eine unterschiedliche Zusammensetzung der AOA Gemeinschaft bei TD und CC beobachtet. Die Nutzung von Ernterückständen geringer Qualität (ZM) verringerte die Abundanz der AOB und der Gesamtarchaeenpopulation im Vergleich zur Behandlung TD wobei sich außerdem eine unterschiedliche Zusammensetzung der AOA und AOB Gemeinschaften zwischen den beiden Behandlungen ZM und TD zeigte. Die Förderung der AOA Abundanz in Behandlung ZM kann der hohen Affinität der AOA zu Ammonium bei niedrigen N Verhältnissen zugeschrieben werden. Alle Ernterückstände verringerten bei Kombination mit mineralischen N, unabhängig von der Qualität, die Abundanz der untersuchten mikrobiellen Gemeinschaften mit Ausnahme von AOB und veränderten deren Zusammensetzung mit Ausnahme von AOA. Dies wurde auf die indirekte Reduktion des Boden pH-Werts durch den mineralischen Dünger zurückgeführt, basierend auf der Tatsache dass AOB weniger sensibel auf Veränderungen des pH Werts reagiert als AOA. Die Ergebnisse zeigen, dass die Qualität des Ernterückstands und mineralischer N die Dynamik von AOA und AOB beeinflussen.

Aufbauend auf den Ergebnissen der ersten Studie im Lehm Boden, wurden im zweiten Teil (Kapitel 3) die saisonalen Effekte, besonders die Niederschläge, berücksichtigt. Um dies zu erreichen wurden Daten aus zwei aufeinanderfolgenden, langen Regenzeiten der Jahre 2012 und 2013 integriert. Zwei unterschiedliche Wachstumsstadien (junges Stadium und Blütezeit) bei *Zea mays* wurden ebenfalls berücksichtigt. Die Ergebnisse zeigten, dass die biochemische Qualität der Ernterückstände die Abundanz von AOB, aber nicht von AOA Genen temporär beeinflusste. Generell wurde ein ähnlicher Trend des Effekts von organischem Eintrag auf die AOB Abundanz wie in Kapitel 2 deutlich, mit dem Unterschied, dass ZM die AOB Abundanz während der Blütezeit von Mais erhöht hat. Dies war eine Bestätigung für die verzögerte Zersetzung von ZM

während der Saison aufgrund der N Limitierung. Die Verwendung von N-Dünger oder dessen Kombination mit organischen Einträgen ergab ähnliche Ergebnisse wie in Kapitel 2 beschrieben. Dies betonte die geringe Sensitivität der AOB Abundanz zu pH Wert Änderungen im Vergleich zu den AOA. Zusätzlich beeinflusste der Niederschlag alle untersuchten Gene mit Ausnahme der AOA, ohne Bezug zur Qualität der Ernterückstände. Im Jahr 2013 konnte, im Vergleich zum Jahr 2012, eine Stimulation der AOB und Gesamtarchaeenpopulation festgestellt werden, während die Gesamtbakterienpopulation zurück ging. Dies wurde mit den geringeren Niederschlägen in 2013 erklärt. Es wurde deshalb angenommen, dass das Optimum der wassergefüllten Poren (WFPS), welches die Ausbreitung der AOB begünstigte, im Jahr 2013 erreicht wurde. Die Gesamtarchaeenpopulation war an geringere Niederschläge angepasst, wobei höhere Niederschläge im Jahr 2012 die Ausbreitung der Gesamtbakterienpopulation begünstigte. Insgesamt zeigten die Ergebnisse, dass die Wechselbeziehung von Ernterückstandsqualität und Saisonalität, die auch die Bodenfeuchte reguliert, zu einer Nischendifferenzierung zwischen AOB und AOA beitrug.

Im dritten Teil dieser Forschungsarbeit (Kapitel 4) wurde zusätzlich zum tonigen Boden, der in den vorangegangenen Studien untersucht wurde, ein sandiger Boden miteinbezogen. Die beiden kontrastierenden Böden zeigten spezifische Wechselbeziehungen mit der OBS hinsichtlich ihrer Bodenstruktur, begünstigt durch ihren unterschiedlichen Gehalt an organischem Bodenkohlenstoff (SOC). Es wurde daher die Hypothese aufgestellt, dass diese Unterschiede eine deutliche Auswirkung auf die Dynamik der mikrobiellen Gemeinschaften haben. In lehmigen Böden bilden Tonpartikel organisch-mineralische Verbindungen mit großen Oberflächen, die die Vermehrung von Mikroorganismen und deren Schutz vor Prädatoren begünstigen. Diese Tatsache resultierte in dieser Studie in einer höheren Abundanz der AOA und AOB unabhängig vom Ernterückstandstyp. Andererseits haben sandige Böden eine grobe Struktur und bieten für frisch hinzugefügtes organisches Material einen geringeren Schutz vor mikrobiellem Zugriff. Wechselwirkungen zwischen der sandigen Bodentextur und der Qualität der organischen Rückstände förderten die AOB Abundanz und veränderten die AOA Zusammensetzung. Dementsprechend förderten TD (hoher organischer N-Gehalt) und ZM (hoher organischer C-Gehalt), im Vergleich zu CC mit hohem Polyphenol- und Ligningehalt, die Abundanz von AOB und veränderten die AOA Zusammensetzung. Wechselwirkungen des lehmigen Bodens mit organischen Ernteresten veränderten die AOB Zusammensetzung. Dementsprechend zeigte sich eine Veränderung der

AOB Zusammensetzung zwischen den Varianten TD und ZM und zwischen CC und ZM. Diese Ergebnisse implizierten, dass AOB auf N von CC zugreifen konnte und dass N die treibende Kraft der veränderten AOB Zusammensetzung unter nicht limitierenden C Bedingungen (Lehmboden) war. Im Gegensatz hierzu war die Veränderung der AOA Zusammensetzung nur möglich, wenn C und N Substrate gleichzeitig limitierend waren, wie im Fall des untersuchten Sandbodens. Daher sind Wechselwirkungen zwischen Bodentextur- und Ernterückstandseffekten spezifisch für die mikrobielle Gruppe, wobei AOB sensitiver als AOA reagiert.

Insgesamt offenbaren die Ergebnisse dieser Doktorarbeit spezifische Reaktionen der Dynamik von AOB und AOA auf die Qualität von organischen Ernterückständen und deren Kombinationen mit mineralischen N-Dünger. Sie zeigt auch Auswirkungen der Wechselbeziehungen zwischen der Qualität der Ernterückständen und Bodenbeschaffenheit als auch der Saisonabhängigkeit, insbesondere Niederschlag, auf die Dynamik mikrobieller Gemeinschaften. Zukünftige Untersuchungen der aktiven mikrobiellen Gemeinschaften durch Verwendung von RNA-basierten Ansätzen müssen in Betracht gezogen werden, um unser Verständnis für den Einfluss der OBS-Qualität auf die Bodennährstoffdynamik weiter zu verbessern.

## References

- African Development Bank. Building today a better Africa tomorrow. <http://www.afdb.org/en/topics-and-sectors/sectors/agriculture-agro-industries/african-agriculture/>
- Ågren, G.I., Wetterstedt, J.Å.M., Billberger, M.F.K., 2012. Nutrient limitation on terrestrial plant growth – modeling the interaction between nitrogen and phosphorus. *New Phytologist* 194, 953–960.
- Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 94, 714–725.
- Anderson, M., Ter Braak, C.T., 2003. Permutation tests for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation* 73, 85–113.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.
- Anderson, M.J., Robinson, J., 2003. Generalized discriminant analysis based on distances. *Austrian and New Zealand Journal of Statistics* 45, 301–318.
- Angel, R., Soares, M.I.M., Ungar, E.D., Gillor, O., 2010. Biogeography of soil archaea and bacteria along a steep precipitation gradient. *The ISME Journal* 4, 553–563.
- Arp, D.J., Sayavedra-Soto, L.A., Hommes, N.G., 2002. Molecular biology and biochemistry of ammonia oxidation by *Nitrosomonas europaea*. *Archives of Microbiology* 178, 250–255.
- Avrahami, S., Bohannan, B.J.M., 2007. Response of *Nitrosospira* sp. strain AF-like ammonia oxidizers to changes in temperature, soil moisture content, and fertilizer concentration. *Applied and Environmental Microbiology* 73, 1166–1173.
- Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal* 5, 908–917.
- Bationo, A., Waswa, B., Abdou, A., Bado, B.V., Bonzi, M., Iwuafor, E., Kibunja, C., Kihara, J., Mucheru, M., Mugendi, D., Mugwe, J., Mwale, C., Okeyo, J., Olle, A., Roing, K., Sedogo, M., 2012. Overview of long-term experiments in Africa, in: Bationo, A., Waswa, B., Kihara, J., Adolwa, I., Vanlauwe, B., Saidou, K. (Eds.), *Lessons learned from long-term*

- soil fertility management experiments in Africa. Springer Netherlands, Dordrecht, pp. 1–26.
- Belnap, J., 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. *Ecological Studies*. 150, 241–261.
- Blainey, P.C., Mosier, A.C., Potanina, A., Francis, C.A., Quake, S.R., 2011. Genome of a low-salinity ammonia-oxidizing archaeon determined by single-cell and metagenomic analysis. *PLoS ONE* 6, 71–97.
- Boj, E., Fortiana, J., Esteve, A., Claramunt, M.M., Costa, T., 2011. Actuarial applications of distance-based generalized linear models. *ASTIN* pp 1–12.
- Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology* 16, 195–208.
- Bru, D., Ramette, A., Saby, N.P.A., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D., Philippot, L., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *The ISME Journal* 5, 532–542.
- Bürgmann, H., Meier, S., Bunge, M., Widmer, F., Zeyer, J., 2005. Effects of model root exudates on structure and activity of a soil diazotroph community. *Environmental Microbiology* 7, 1711–1724.
- Bustin, S.A., 2005. Real-time reverse transcription PCR. *Encycl. Diagn. Genomics Proteomics* 1131–1135.
- Cadisich, G., Imhof, H., Urquiaga, S., Boddey, R.M., Giller, K.E., 1996. Carbon ( $^{13}\text{C}$ ) and nitrogen mineralization potential of particulate light organic matter after rainforest clearing. *Soil Biology and Biochemistry*. 28, 1555–1567.
- Che, J., Zhao, X.Q., Zhou, X., Jia, Z.J., Shen, R.F., 2015. High pH-enhanced soil nitrification was associated with ammonia-oxidizing bacteria rather than archaea in acidic soils. *Applied Soil Ecology* 85, 21–29.
- Chen, X.-P., Zhu, Y.-G., Xia, Y., Shen, J.-P., He, J.-Z., 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environmental Microbiology* 10, 1978–1987.
- Chèneby, D. Bru, D. Pascault, N. Maron, P., A. Ranjard, L. and Philippot, L., 2010. Role of plant residues in determining temporal patterns of the activity, size, and structure of nitrate reducer communities in soil. *Applied and Environmental Microbiology* 76, 7136–7143.

- Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. *Soil Biology and Biochemistry* 43, 657–666.
- Chivenge, P., Vanlauwe, B., Gentile, R., Wangechi, H., Mugendi, D., van Kessel, C., Six, J., 2009. Organic and mineral input management to enhance crop productivity in central Kenya. *Agronomy Journal* 101, 1266–1275.
- Clarke, K. R., Gorley, R.N., 2001. *Primer5: User Manual/Tutorial*. PRIMER-E, Plymouth, UK.
- Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*. 18, 117–143.
- Cray, J.A., Russell, J.T., Timson, D.J., Singhal, R.S., Hallsworth, J.E., 2013. A universal measure of chaotropicity and kosmotropicity: A universal measure of chao- and kosmotropicity. *Environmental Microbiology* 15, 287–296.
- Cray, J.A., Stevenson, A., Ball, P., Bankar, S.B., Eleutherio, E.C.A., Ezeji, T.C., Singhal, R.S., Thevelein, J.M., Timson, D.J., Hallsworth, J.E., 2015. Chaotropicity: a key factor in product tolerance of biofuel-producing microorganisms. *Current Opinion in Biotechnology* 33, 228–259.
- De Boer, W., and Kowalchuk, G. A., 2001. Nitrification in acid soils: microorganisms and mechanisms. *Soil Biology and Biochemistry* 33, 853–866.
- De Gannes, V., Eudoxie, G., Hickey, W.J., 2014. Impacts of edaphic factors on communities of ammonia-oxidizing archaea, ammonia-oxidizing bacteria and nitrification in tropical soils. *PLoS ONE* 9, 1–14.
- De Lima Alves, F., Stevenson A., Baxter, E., Gillion, J.L.M., Hejazi, F., Hayes, S., Morrison, I.E.G., Prior, B.A., McGenity, T.J., Rangel, D.E.N., Magan, N., Timmis, K.N., Hallsworth, J.E., 2015. Concomitant osmotic and chaotropicity-induced stresses in *Aspergillus wentii*: compatible solutes determine the biotic window. *Current Genetics* 61, 457–477.
- Deutzmann, J.S., Wörner, S., Schink, B., 2011. Activity and diversity of methanotrophic bacteria at methane seeps in eastern lake Constance sediments. *Applied and Environmental Microbiology* 77, 2573–2581.
- Di, H.J., Cameron, K.C., Shen, J.-P., Winefield, C.S., O’Callaghan, M., Bowatte, S., He, J.-Z., 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology* 72, 386–394.

- Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience* 2, 621–624.
- Dieckow, J., Bayer, C., Conceição, P.C., Zanatta, J.A., Martin-Neto, L., Milori, D.B.M., Salton, J.C., Macedo, M.M., Mielniczuk, J., Hernani, L.C., 2009. Land use, tillage, texture and organic matter stock and composition in tropical and subtropical Brazilian soils. *European Journal of Soil Science* 60, 240–249.
- Dunbar, J., Ticknor, L.O., Kuske, C.R., 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. *Applied and Environmental Microbiology* 67, 190–197.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M., Böttger, E.C., 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research* 17, 7843–7853.
- Enwall, K., Hallin, S., 2009. Comparison of TRFLP and DGGE techniques to assess denitrifier community composition in soil. *Letters in Applied Microbiology* 48, 145–148.
- Erguder, T.H., Boon, N., Wittebolle, L., Marzorati, M., Verstraete, W., 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiology Reviews* 33, 855–869.
- España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G.D., Cadisch, G., 2011. Identification of active bacteria involved in decomposition of complex maize and soybean residues in a tropical vertisol using <sup>15</sup>N-DNA stable isotope probing. *Pedobiologia* 54, 187–193.
- FAO, 2006. World Reference Base for Soil Resources, FAO, Rome, Italy.
- FAO, 2008. World reference base for soil resources, FAO, Rome, Italy.
- FAO, 2012. Current world fertilizer trends and outlook to 2016, FAO, Rome, Italy.
- Feinstein, L.M., Sul, W.J., Blackwood, C.B., 2009. Assessment of Bias Associated with Incomplete Extraction of Microbial DNA from Soil. *Applied and Environmental Microbiology* 75, 5428–5433.
- Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? *Soil Biology and Biochemistry* 35, 837–843.

- Fonte, S.J., Yeboah, E., Ofori, P., Quansah, G.W., Vanlauwe, B., Six, J., 2009. Fertilizer and residue quality effects on organic matter stabilization in soil aggregates. *Soil Science Society of America Journal* 73, 961–966.
- Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 102, 14683–14688.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892.
- Garbeva, P., van Veen, J.A., van Elsas, J.D., 2004. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* 42, 243–270.
- Geisseler, D., Scow, K.M., 2014. Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biology and Biochemistry* 75, 54–63.
- Gelsomino, A., Keijzer-Wolters, A.C., Cacco, G., van Elsas, J.D., 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *Journal of Microbiological Methods* 38, 1–15.
- Gentile, R., Vanlauwe, B., Kavoo, A., Chivenge, P., Six, J., 2011a. Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. *Nutrient Cycling in Agroecosystems* 88, 121–131.
- Gentile, R., Vanlauwe, B., Six, J., 2011b. Litter quality impacts short-but not long-term soil carbon dynamics in soil aggregate fractions. *Ecological Applications* 21, 695–703.
- Gentile, R., Vanlauwe, B., van Kessel, C., Six, J., 2009. Managing N availability and losses by combining fertilizer-N with different quality residues in Kenya. *Agriculture Ecosystems and Environments* 131, 308–314.
- Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology* 69, 1800–1809.

- Gleeson, D.B., Müller, C., Banerjee, S., Ma, W., Siciliano, S.D., Murphy, D.V., 2010. Response of ammonia-oxidizing archaea and bacteria to changing water filled pore space. *Soil Biology and Biochemistry* 42, 1888–1891.
- Gubry-Rangin, C., Nicol, G. W., Prosser, J. I., 2010. Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiology Ecology* 74, 566–574.
- Hagerman, A.E., 2012. Fifty years of polyphenol–protein complexes, in: Cheynier, V., Sarni-Manchado, P., Quideau, S. (Eds.), *Recent advances in polyphenol research*. Wiley-Blackwell, pp. 71–97.
- Hai, B., Diallo, N.H., Sall, S., Haesler, F., Schauss, K., Bonzi, M., Assigbetse, K., Chotte, J.-L., Munch, J.C., Schloter, M., 2009. Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. *Applied and Environmental Microbiology* 75, 4993–5000.
- Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME Journal* 3, 597–605.
- Hallsworth, J.E., Heim, S., Timmis, K.N., 2003. Chaotropic solutes cause water stress in *Pseudomonas putida*. *Environmental Microbiology* 5, 1270–1280.
- Hatzenpichler, R., Lebedeva, E.V., Spieck, E., Stoecker, K., Richter, A., Daims, H., Wagner, M., 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proceedings of the National Academy of Sciences* 105, 2134–2139.
- Hazelton, P., Murphy, B., 2007. *Interpreting soil test results: What do all the numbers mean?*, Second edition. Csiro Publishing, Collingwood VIC, Australia.
- He, J., Shen, J., Zhang, L., Zhu, Y., Zheng, Y., Xu, M., Di, H., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology* 9, 2364–2374.
- Heal, O.W., Anderson, J.M., Swift, M.J., 1997. Plant litter quality and decomposition: an historical overview. In: Cadisch, G., Giller, K.E. (eds.), *Driven by nature: Plant litter quality and decomposition*. CAB International, Wallingford, pp. 3–30.
- Henao, J., Baanante, C., 2006. *Agricultural Production and soil nutrient mining in Africa: Implications for resource conservation and policy development*. Muscle Shoals, Alabama,

- U.S.A. An International Center for Soil Fertility and Agricultural Development.  
[www.ifdc.org](http://www.ifdc.org)
- Hepburn, J. S., 1908. The modifications of the Kjeldahl method for the quantitative determination of nitrogen. *Franklin Institute* 166: 81-89.
- Höfferle, S., Nicol, G. W., Pal, L., Hacin, J., Prosser, J. I., Mandic-Mulec, I., 2010. Ammonium supply rate influences archaeal and bacterial ammonia oxidizers in a wetland soil vertical profile. *FEMS Microbiology Ecology* 74, 302–315.
- Hofmockel, S. K., Fierer, N., Benjamin, P. C. and Robert B. J., 2010. Amino acid abundance and proteolytic potential in North. American soils ecosystem ecology. *Oecologia* 10, 1601–1609.
- Hugenholtz, P., 2002. Exploring prokaryotic diversity in the genomic era. *Genome Biology* 3, 1–3.
- Hyman, M.R., Arp, D.J., 1992.  $^{14}\text{C}_2\text{H}_2$ - and  $^{14}\text{CO}_2$ -labeling studies of the de novo synthesis of polypeptides by *Nitrosomonas europaea* during recovery from acetylene and light inactivation of ammonia monooxygenase. *Journal of Biological Chemistry* 267, 1534–1545.
- ICRAF, 1999. Laboratory methods for soil and plant analysis. International Centre for Research in Agroforestry, Nairobi, Kenya.
- Isobe, K., Koba, K., Suwa, Y., Ikutani, J., Fang, Y., Yoh, M., Mo, J., Otsuka, S., Senoo, K., 2012. High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition. *FEMS Microbiology Ecology* 80, 193–203.
- Jia, Z., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology* 11, 1658–1671.
- Jones A, Breuning-Madsen H, Deckers J, Dewitte O, Gallali T, Le Roux, P., Michéli, E., Montanarella, L., Spaargaren, O., Thiombiano, L., Van Ranst, E., Yemefack, M., Zougmore, R., 2013. Soil Atlas of Africa.  
[http://eusoiils.jrc.ec.europa.eu/library/maps/africa\\_atlas/](http://eusoiils.jrc.ec.europa.eu/library/maps/africa_atlas/)
- Jones, E., Singh, B., 2014. Organo-mineral interactions in contrasting soils under natural vegetation. *Frontiers in Environmental Science* 2, 1–15.
- Junier, P., Molina, V., Dorador, C., Hadas, O., Kim, O.S., Junier, T., Witzel, K.P., Imhoff, J.F., 2010. Phylogenetic and functional marker genes to study ammonia-oxidizing

- microorganisms (AOM) in the environment. *Applied Microbiology and Biotechnology* 85, 425–440.
- Kaewpradit, W., Toomsan, B., Vityakon, P., Limpinuntana, V., Saenjan, P., Jogloy, S., Patanothai, A., Cadisch, G., 2008. Regulating mineral N release and greenhouse gas emissions by mixing groundnut residues and rice straw under field conditions. *European Journal of Soil Science* 59, 640–652.
- Kamaa, M.M., Mburu, H.N., Blanchart, E., Chibole, L., Chotte, J.L., Kibunja, C.N., Lesueur, D., 2012. Effects of organic and inorganic fertilization on soil bacterial and fungal microbial diversity in the Kabete long-term trial, Kenya. *Biology and Fertility of Soils* 4, 315–321.
- Kamolmanit, B., Vityakon, P., Kaewpradit, W., Cadisch, G., Rasche, F., 2013. Soil fungal communities and enzyme activities in a sandy, highly weathered tropical soil treated with biochemically contrasting organic inputs. *Biology and Fertility of Soils* 49, 905–917.
- Kanerva, S., Kitunen, V., Kiikkilä, O., Loponen, J., Smolander, A., 2006. Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles. *Soil Biology and Biochemistry* 38, 1364–1374.
- Ke, X., Lu, Y., 2012. Adaptation of ammonia-oxidizing microbes to environment shift of paddy field soil. *FEMS Microbiology Ecology* 80, 87–97.
- Keil, D., Meyer, A., Berner, D., Poll, C., Schützenmeister, A., Piepho, H.-P., Vlasenko, A., Philippot, L., Schlöter, M., Kandeler, E., Marhan, S., 2011. Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. *FEMS Microbiology Ecology* 77, 95–106.
- Kibunja, C.N., 2007. Impact of long-term application of organic and inorganic nutrient sources in maize-bean rotation to soil nitrogen dynamics and soil microbial populations and activities. PhD thesis, University of Nairobi, Kenya.
- Kirschbaum, M. U., 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology and Biochemistry* 27, 753–760.
- Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* 171, 61–82.

- Kononova, M.M., (1966). *Soil Organic Matter*. Pergamon Press, New York, p.544.
- Konstantinidis, K.T., Ramette, A., Tiedje, J.M., 2006. The bacterial species definition in the genomic era. *Philosophical Transactions of the Royal Society B: Biological Science* 361, 1929–1940.
- Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems - a review. *Plant Soil* 256, 41–66.
- Krull, E., Baldock, J., Skjemstad, J., others, 2001. Soil texture effects on decomposition and soil carbon storage, in: *Net Ecosystem Exchange CRC Workshop Proceedings*. pp. 103–110.
- Kunlanit, B., Vityakon, P., Puttaso, A., Cadisch, G., Rasche, F., 2014. Mechanisms controlling soil organic carbon composition pertaining to microbial decomposition of biochemically contrasting organic residues: Evidence from midDRIFTS peak area analysis. *Soil Biology and Biochemistry* 76, 100–108.
- Lane, D, 1991. 16S/23S rRNA sequencing, In: Stackebrandt A Goodfello M (eds) *Nucleic acid techniques systematics*, 1st ed. Wiley, West Sussex, UK pp 115-257, Chichester ; New York.
- Legendre, P., Anderson, J.M., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69, 1–24.
- Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806–809.
- Levičnik-Höfferle, Š., Nicol, G.W., Ausec, L., Mandić-Mulec, I., Prosser, J.I., 2012. Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen. *FEMS Microbiology Ecology* 80, 114–123.
- Liu, D.L., Helyar, K.R., Conyers, M.K., Fisher, R., Poile, G.J., 2004. Response of wheat, triticale and barley to lime application in semi-arid soils. *Field Crops Research* 90, 287–301.
- Liu, W.T., Marsh, T.L., Cheng, H., Forney, L.J., 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology* 63, 4516–4522.
- Lucas, S.T., (2013) *Managing soil microbial communities with organic amendments to promote soil aggregate formation and plant health*. Thesis and dissertations--plant and soil sciences. [http://uknowledge.uky.edu/pss\\_etds/24](http://uknowledge.uky.edu/pss_etds/24).

- Lueders, T., Friedrich, M., 2000. Archaeal population dynamics during sequential reduction processes in rice field soil. *Applied and Environmental Microbiology* 66, 2732–2742.
- Magdalena, F. Stefania, J., 2011. Agricultural utilization of dairy sewage sludge: Its effect on enzymatic activity and microorganisms of the soil environment. *African Journal of Microbiology Research* 5, 1755–1762.
- Magill, A.H., Aber, J.D., 2000. Variation in soil net mineralization rates with dissolved organic carbon additions. *Soil Biology and Biochemistry* 32, 597–601.
- Mando, A., Bonzi, M., Wopereis, M.C.S., Lompo, F., Stroosnijder, L., 2005. Long-term effects of mineral and organic fertilization on soil organic matter fractions and sorghum yield under Sudano-Sahelian conditions. *Soil Use and Management* 21, 396–401.
- Mapfumo, P., Mtambanengwe, F., Vanlauwe, B., 2007. Organic matter quality and management effects on enrichment of soil organic matter fractions in contrasting soils in Zimbabwe. *Plant Soil* 296, 137–150.
- Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 461, 976–979.
- Martens-Habbena, W., Stahl, D.A., 2011. Nitrogen metabolism and kinetics of ammonia-oxidizing archaea. *Methods in Enzymology* 496, 465–487.
- Mcardle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology* 83, 290–297.
- Millar, N., Baggs, E.M., 2004. Chemical composition, or quality, of agroforestry residues influences N<sub>2</sub>O emissions after their addition to soil. *Soil Biology and Biochemistry* 36, 935–943.
- Milling, A., Smalla, K., Maidl, F.X., Schloter, M., Munch, J.C., 2005. Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. *Plant Soil* 266, 23–39.
- Mills, D.K., Entry, J.A., Gillevet, P.M., Mathee, K., 2007. Assessing microbial community diversity using amplicon length heterogeneity polymerase chain reaction. *Soil Science Society of America Journal* 71, 572–578.
- Morimoto, S., Hayatsu, M., Hoshino, Y.T, Nagaoka, K., Yamazaki, M., Karasawa, T., Takenaka, M., Akiyama, H., 2011. Quantitative analyses of ammonia-oxidizing archaea (AOA) and

- ammonia-oxidizing bacteria (AOB) in fields with different soil types. *Microbes and Environments* 26, 248–253.
- Mrkonjic Fuka, M., Engel, M., Gattinger, A., Bausenwein, U., Sommer, M., Munch, J. C. and Schloter, M. . 2008. Factors influencing variability of proteolytic genes and activities in arable soils. *Soil Biology and Biochemistry* 40,1646–1653.
- Mrkonjic Fuka, M., Engel, M., Hagn, A., Munch, J.C., Sommer, M., Scholoter, M., 2009. Changes of diversity pattern of proteolytic bacteria over time and space in an agricultural soil. *Microbial Ecology* 57, 391–401.
- Mtambanengwe, F., Mapfumo, P., Vanlauwe, B., 2006. Comparative short-term effects of different quality organic resources on maize productivity under two different environments in Zimbabwe. *Nutrient Cycling in Agroecosystems*. 76, 271–284.
- Muema, E.K., Cadisch G., Röhl, C., Vanlauwe, B., Rasche, F., 2015. Response of ammonia-oxidizing bacteria and archaea to biochemical quality of organic inputs combined with mineral nitrogen fertilizer in an arable soil. *Applied Soil Ecology* 95, 128–139.
- Musyoki, M.K., Cadisch, G., Enowashu, E., Zimmermann, J., Muema, E., Beed, F., Rasche, F., 2015. Promoting effect of *Fusarium oxysporum* [f.sp. *strigae*] on abundance of nitrifying prokaryotes in a maize rhizosphere across soil types. *Biological Control* 83, 37–45.
- Mutabaruka, R., Hairiah, K., Cadisch, G., 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol–protein complexes in soils from different land-use histories. *Soil Biology and Biochemistry* 39, 1479–1492.
- Muyzer, G., 1999. Genetic fingerprinting of microbial communities-present status and future perspectives, in: *Proceedings of the 8th International Symposium on Microbial Ecology*. pp. 1–10.
- Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59, 695–700.
- Myers, R.J.K., Palm, C.A., Cuevas, E., Gunatilleke, I.U.N., Brossard. M., 1994. The synchronization of nutrient mineralization and plant nutrient demand. In: Wooster, P.L., Swift, M.J. (eds.), *The biological management of tropical soil fertility*. John Wiley & Sons, Chichester, UK.

- Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54, 655–670.
- Neumann, D., Heuer, A., Hemkemeyer, M., Martens, R., Tebbe, C.C., 2013. Response of microbial communities to long-term fertilization depends on their microhabitat. *FEMS Microbiology Ecology* 86, 71–84.
- Nicol, G. W., Leininger, S., Schleper, C., Prosser, J. I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia-oxidizing archaea and bacteria. *Environmental Microbiology* 10, 2966–2978.
- Nicol, G.W., Schleper, C., 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology* 14, 207–212.
- Norton, J. M., Stark, J. M., 2011. Regulation and measurement of nitrification in terrestrial systems. *Methods in Enzymology*. 486, 343–368.
- Nziguheba, G., Merckx, R., Palm, C.A., 2005. Carbon and nitrogen dynamics in a phosphorus-deficient soil amended with organic residues and fertilizers in western Kenya. *Biology and Fertility of Soils* 41, 240–248.
- Okur, N., Altindişl, A., Cengel, M., Gocmez, S., Kayikcioglu, H.H., 2009. Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems. *Turkey Journal of Agriculture and Forestry* 33, 413–423.
- Onwonga, R.N., Lelei, J.J., Mochoge, B.B., 2010. Mineral nitrogen and microbial biomass dynamics under different acid soil management practices for maize production. *Journal of Agricultural Science* 2, 16–30.
- Palm, C.A., Gachengo, C.N., Delve, R.J., Cadisch, G., Giller, K.E., 2001a. Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. *Agriculture, Ecosystems and Environment* 83, 27–42.
- Palm, C.A., Giller, K.E., Mafongoya, P.L., Swift, M.J., 2001b. Management of organic matter in the tropics: translating theory into practice. *Nutrient Cycling in Agroecosystems* 61, 63–75.
- Palm, C.A., Myers, R. J. K., Nandwa, S., 1997. Organic-inorganic nutrient interactions in soil fertility replenishment. In: Buresh, R.J., Sanchez, A., Calhoun, F.G. (eds.), *Replenishing soil fertility in Africa*. ASA-SSSA Special publication 51, 193–218.

- Pandey, J., Ganesan, K., Jain, R.K., 2007. Variations in T-RFLP profiles with differing chemistries of fluorescent dyes used for labeling the PCR primers. *Journal of Microbiological Methods* 68, 633–638.
- Partey, S.T., Preziosi, R.F., Robson, G.D., 2013. Maize residue interaction with high quality organic materials: Effects on decomposition and nutrient release dynamics. *Agricultural Research* 2, 58–67.
- Pereira e Silva, M.C., Poly, F., Guillaumaud, N., van Elsas, J.D., Falcão Salles, J., 2012. Fluctuations in ammonia-oxidizing communities across agricultural soils are driven by soil structure and pH. *Terrestrial Microbiology* 3, 1–22.
- Peter, M.V., John, D., Robert, W.H., Gene, E.L., Pamela, A., David, W.S., William, H.S., David, G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological applications* 7, 737–750.
- Petersen, D.G., Blazewicz, S.J., Firestone, M., Herman, D.J., Turetsky, M., Waldrop, M., 2000. Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environmental Microbiology* 14, 993–1008.
- Piepho, H.P., 2009. Data transformation in statistical analysis of field trials with changing treatment variance. *Agronomy Journal* 101, 865–869.
- Poll, C., Thiede, A., Wermbter, N., Sessitsch, A., Kandeler, E., 2003. Micro-scale distribution of microorganisms and microbial enzymeactivities in a soil with long-term organic amendment. *European Journal of Soil Science* 54, 715–724.
- Population reference bureau, 2015. <http://www.prb.org/Publications/Articles/2015/sahel-demographics.aspx>
- Prosser, J.I., Nicol, G.W., 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology* 10, 2931–2941.
- Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. *Trends in Microbiology* 20, 523–531.
- Puttaso, A., Vityakon, P., Rasche, F., Saenjan, P., Treloges, V., Cadisch, G., 2013. Does organic residue quality influence carbon retention in a tropical sandy soil? *Soil Science Society of America Journal* 77, 1001.

- Puttaso, A., Vityakon, P., Saenjan, P., Trelo-ges, V., Cadisch, G., 2011. Relationship between residue quality, decomposition patterns, and soil organic matter accumulation in a tropical sandy soil after 13 years. *Nutrient Cycling in Agroecosystems* 89, 159–174.
- Rasche, F., Cadisch, G., 2013. The molecular microbial perspective of organic matter turnover and nutrient cycling in tropical agroecosystems - What do we know? *Biology and Fertility of Soils* 49, 251–262.
- Rasche, F., Hödl, V., Poll, C., Kandeler, E., Gerzabek, M.H., Van Elsas, J.D., Sessitsch, A., 2006a. Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. *FEMS Microbiology Ecology* 56, 219–235.
- Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., Sessitsch, A., 2011. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *The ISME Journal* 5, 389–402.
- Rasche, F., Musyoki, M.K., Röhl, C., Muema, E.K., Vanlauwe, B., Cadisch, G., 2014. Lasting influence of biochemically contrasting organic inputs on abundance and community structure of total and proteolytic bacteria in tropical soils. *Soil Biology and Biochemistry* 74, 204–213.
- Rasche, F., Trondl, R., Naglreiter, C., Reichenauer, T.G., Sessitsch, A., 2006b. Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum annum* L.). *Canadian Journal of Microbiology* 52, 1036–1045.
- Rastogi, G., Sani, R.K., 2011. Molecular techniques to assess microbial community structure, function, and dynamics in the environment, in: Ahmad, I., Ahmad, F., Pichtel, J. (Eds.), *Microbes and Microbial Technology*. Springer New York, New York, NY, pp. 29–57.
- Rees, G.N., Baldwin, D.S., Watson, G.O., Perryman, S., Nielsen, D.L., 2005. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. *Antonie Van Leeuwenhoek* 86, 339–347.
- Rothauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology* 63, 4704–4712.

- Sądej, W., Przekwas, K., 2008. Fluctuations of nitrogen levels in soil profile under conditions of a long-term fertilization experiment. *Plant Soil Environ* 54: 197–203.
- Sakala, W., G. Cadisch, and K.E., Giller., 2000. Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N mineralization. *Soil Biology and Biochemistry* 32, 679–688.
- Sakurai, M., Suzuki, K., Onodera, M. Shinano, T., Osaki, M., 2007. Analysis of bacterial communities in soil by PCR–DGGE targeting protease genes. *Soil Biology and Biochemistry* 39, 2777–2784.
- Samahadthai, P., Vityakon, P., Saenjan, P., 2010. Effects of different quality plant residues on soil carbon accumulation and aggregate formation in a tropical sandy soil in Northeast Thailand as revealed by a 10-year field experiment. *Land Degradation and Development* 21, 463–473.
- Sanchez, P.A., Jama, B. A., 2002. Soil fertility replenishment takes off in east and southern Africa. In: Vanlauwe, B., Diels, J., Sanginga, N., Merckx, R. (eds.), *Integrated plant nutrient management in Sub-Saharan Africa: from concept to practice*. CAB International, Wallingford, Oxon.
- Sardans, J. and Penuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biology and Biochemistry* 37, 455–461.
- SAS Institute, 2014. SAS system for windows, Version 9.3. SAS Institute Inc. Cary, NC, USA.
- Schauss, K., Focks, A., Leininger, S., Kotzerke, A., Heuer, H., Thiele-Bruhn, S., Sharma, S., Wilke, B.-M., Matthies, M., Smalla, K., Munch, J.C., Amelung, W., Kaupenjohann, M., Schloter, M., Schleper, C., 2009. Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. *Environmental Microbiology* 11, 446–456.
- Schematic representation of the flow of nitrogen through the environment. [https://en.wikipedia.org/wiki/Nitrogen\\_cycle](https://en.wikipedia.org/wiki/Nitrogen_cycle)
- Schleper, C., 2010. Ammonia oxidation: different niches for bacteria and archaea? *The ISME Journal* 4, 1092–1094.
- Schleper, C., Jurgens, G., Jonuscheit, M., 2005. Genomic studies of uncultivated archaea. *Nature Reviews Microbiology* 3, 479–488.

- Schmidt, M.A., Kreinberg, A.J., Gonzalez, J.M., Halvorson, J.J., French, E., Bollmann, A., Hagerman, A.E., 2013. Soil microbial communities respond differently to three chemically defined polyphenols. *Plant Physiology and Biochemistry* 72, 190–197.
- Schulz, E., 2004. Influence of site conditions and management on different soil organic matter (som) pools. *Archives of Agronomy and Soil Science* 50, 33–47.
- Schulz, E., Körschens, M., 1998. Characterization of the decomposable part of soil organic matter (som) and transformation processes by hot water extraction. *Eurasian Soil Science* 31, 809–813.
- Schütte, U.M.E., Abdo, Z., Bent, S.J., Shyu, C., Williams, C.J., Pierson, J.D., Forney, L.J., 2008. Advances in the use of terminal restriction fragment length polymorphism (TRFLP) analysis of 16S rRNA genes to characterize microbial communities. *Applied Microbiology and Biotechnology* 80, 365–380.
- Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H., Kandeler, E., 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Applied and Environmental Microbiology* 67, 4215–4224.
- Shannon, C.E., Weaver, W., 1949. *The mathematical theory of communication*. Urbana IL: University of Illinois Press.
- Shen, J., Zhang, L., Zhu, Y., Zhang, J., He, J., 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology* 10, 1601–1611.
- Shen, J.P., Zhang, L.M., Guo, J.F., Ray, J.L., He, J.Z., 2010. Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Applied Soil Ecology* 46, 119–124.
- Shepherd, D.K., Cheryl, A.P., Gachengo, N.C., Vanlauwe, B., 2003. Rapid characterization of organic resource quality for soil and livestock management in tropical agroecosystems using Near-Infrared Spectroscopy. *Agronomy Journal* 95, 1314–1322.
- Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology* 8, 779–790.
- Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Res., Advances in Soil Structure Research* 79, 7–31.

- Six, J., Frey, D.S., Thiet, K.R., and Batten, M.K., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70, 555–569.
- Smalla, K., Oros-Sichler, M., Milling, A., Heuer, H., Baumgarte, S., Becker, R., Neuber, G., Kropf, S., Ulrich, A., Tebbe, C.C., 2007. Bacterial diversity of soils assessed by DGGE, TRFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbiological Methods* 69, 470–479.
- Smith, C.J., Osborn, A.M., 2009. Advantages and limitations of quantitative PCR (QPCR)-based approaches in microbial ecology. *FEMS Microbiology Ecology* 67, 6–20.
- Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* 61, 218–221.
- Stevenson, A., Cray, J.A., Williams, J.P., Santos, R., Sahay, R., Neuenkirchen, N., McClure, C.D., Grant, I.R., Houghton, J.D.R., Quinn, J.P., Timson, D.J., Patil, S.V., Singhal, R.S., Anton, J., Dijksterhuis, J., Hocking, A.D., Lieven, B., Rangel, D.E.N., Voytek, M.A., Gunde-Cimerman, N., Oren, A., Timmis, K.N., McGenity, T.J., Hallsworth, J.E., 2015. Is there a common water-activity limit for the three domains of life? *The ISME Journal* 9, 1333–1351.
- Stevenson, A., Hallsworth, J.E., 2014. Water and temperature relations of soil Actinobacteria: Water and temperature relations of Actinobacteria. *Environmental Microbiology Reports* 6, 744–755.
- Stopnišek, N., Gubry-Rangin, C., Höfferle, Š., Nicol, G.W., Mandič-Mulec, I., Prosser, J.I., 2010. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Applied and Environmental Microbiology* 76, 7626–7634.
- Strauss, S.L., Reardon, C.L., Mazzola, M., 2014. The response of ammonia-oxidizer activity and community structure to fertilizer amendment of orchard soils. *Soil Biology and Biochemistry* 68, 410–418.
- Subbarao, G.V., Sahrawat, K.L., Nakahara, K., Rao, I.M., Ishitani, M., Hash, C.T., Kishii, M., Bonnett, D.G., Berry, W.L., Lata, J.C., 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). *Annals of Botany* 112, 297–316.

- Szukics, U., Abell, G.C.J., Hödl, V., Mitter, B., Sessitsch, A., Hackl, E., Zechmeister-Boltenstern, S., 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiology Ecology* 72, 395–406.
- Szukics, U., Hackl, E., Zechmeister-Boltenstern, S., Sessitsch, A., 2012. Rapid and dissimilar response of ammonia-oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils. *Microbiological Research* 167, 103–109.
- Talbot, J., M., Finzi, A.C., 2008. Differential effects of sugar maple, red oak, and hemlock tannins on carbon and nitrogen cycling in temperate forest soils. *Oecologia*. 155, 583–592.
- Talbot, J.M., Yelle, D.J., Nowick, J., Treseder, K.K., 2012. Litter decay rates are determined by lignin chemistry. *Biogeochemistry* 108, 279–295.
- Tekalign, T., Haque, I., Aduayi, E.A., 1991. Soil, plant, water, fertilizer, animal manure and compost analysis manual. Plant science division working document 13, ILCA, Addis Ababa, Ethiopia.
- Tian, G., Brussaard, L., Kang, B.T., 1995. An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the sub-humid tropics. *Applied Soil Ecology* 2, 25–32.
- Tisdall, J.M. 1991. Fungal hyphae and structural stability of soil. *Australian Journal of Soil Research* 29, 729–743.
- Todd-Brown, K.E.O., Hopkins, F.M., Kivlin, S.N., Talbot, J.M., Allison, S.D., 2012. A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry* 109, 19–33.
- Tourna, M., Freitag, T. E., Prosser, J. I., 2010. Stable isotope probing analysis of interactions between ammonia oxidizers. *Applied and Environmental Microbiology* 76, 2468–2477.
- Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A., Schleper, C., 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proceedings of the National Academy of Sciences* 108, 8420–8425.
- Treusch, A. H., Leininger, S., Kletzin, A., Schuster, S. C., Klenk, H. P., Schleper, C., 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environmental Microbiology* 7, 1985–1995.

- Valentine, D.L., 2007. Adaptations to energy stress dictate the ecology and evolution of the archaea. *Nature Reviews Microbiology* 5, 316–323.
- Vanlauwe, B., Aihou, K., Aman, S., Iwuafor, E.N.O., Tossah, B.K., Diels, J., Sanginga, N., Lyasse, O., Merckx, R., Deckers, J., 2001b. Maize yield as affected by organic inputs and urea in the West African moist savanna. *Agronomy Journal* 93, 1191–1199.
- Vanlauwe, B., Bationo, A., Chianu, J., Giller, K.E., Merckx, R., Mkwunye, U., Ohiokpehai, O., Pypers, P., Tabo, R., Shepherd, K.D., Smaling, E.M.A., Woomer, P.L., Sanginga, N., 2010. Integrated soil fertility management: Operational definition and consequences for implementation and dissemination. *Outlook in Agriculture* 39, 17–24.
- Vanlauwe, B., Gachengo, C., Shepherd, K., Barrios, E., Cadisch, G., Palm, C.A., 2005. Laboratory validation of a resource quality-based conceptual framework for organic matter management. *Soil Science Society of America Journal*, 69, 1135–1145.
- Vanlauwe, B., Wendt, J., Diels, J., 2001a. Combined application of organic matter and fertilizer. In: Tian, G., Ishida, F., Keatinge, J.D.H. (eds.), *Sustaining soil fertility in west Africa*. Soil Science Society of America and American Society of Agronomy, Madison, pp. 247–279.
- Verhamme, D.T., Prosser, J.I., Nicol, G.W., 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal* 5, 1067–1071.
- Verkaik, E., Jongkind, A.G., Berendse, F., 2006. Short-term and long-term effects of tannins on nitrogen mineralisation and litter decomposition in kauri (*Agathis australis* (D. Don) Lindl.) forests. *Plant Soil* 287, 337–345.
- Wang, Y., Ke, X., Wu, L., Lu, Y., 2009. Community composition of ammonia-oxidizing bacteria and archaea in rice field soil as affected by nitrogen fertilization. *Systematic and Applied Microbiology* 32, 27–36.
- Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. *Soil Biology and Biochemistry* 28, 1549–1554.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173, 697–703.
- Wessén, E., Nyberg, K., Jansson, J.K., Hallin, S., 2010. Responses of bacterial and archaeal ammonia-oxidizers to soil organic and fertilizer amendments under long-term management. *Applied Soil Ecology* 45, 193–200.

- Wessén, E., Söderström, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., Thomsen, F., Klemmedtson, L., Philippot, L., Hallin, S., 2011. Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. *The ISME Journal* 5, 1213–1225.
- World Bank, 2012. *Global Food Policy Report*. Washington, DC, USA. <http://www.ifpri.org/gfpr/2012/employment-agriculture>
- World Bank, 2012. *World population prospects*. United Nations, New York, USA.
- Wu, Y., Lu, L., Wang, B., Lin, X., Zhu, J., Cai, Z., Yan, X., Jia, Z., 2011. Long-term field fertilization significantly alters community structure of ammonia-oxidizing bacteria rather than archaea in a paddy soil. *Soil Science Society of America Journal* 75, 1431–1439.
- Xu, M., Schnorr, J., Keibler, B., Simon, H.M., 2012. Comparative analysis of 16S rRNA and amoA genes from archaea selected with organic and inorganic amendments in enrichment culture. *Applied and Environmental Microbiology* 78, 2137–2146.
- Yao, H., Gao, Y., Nicol, G. W., Campbell, C. D., Prosser, J. I., Zhang, L., Han, W., Singh, B. K., 2011. Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. *Applied and Environmental Microbiology* 77, 4618–4625.
- Zhalnina, K., Dörr de Quadros, P., A. O. Camargo, F., Triplett, E.W., 2012. Drivers of archaeal ammonia-oxidizing communities in soil. *Terrestrial Microbiology* 3, 1–9.
- Zhang, L., Hu, H., Shen, J., He, J., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *The ISME Journal* 6, 1032–1045.
- Zhang, L.-M., Offre, P.R., He, J.-Z., Verhamme, D.T., Nicol, G.W., Prosser, J.I., 2010. Autotrophic ammonia oxidation by soil thaumarchaea. *Proceedings of the National Academy of Sciences* 107, 17240–17245.
- Zhang, X., Liu, W., Schloter, M., Zhang, G., Chen, Q., Huang, J., Li, L., Elser, J.J., Han, X., 2013. Response of the abundance of key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS ONE* 8, 1–10.
- Zinn, Y.L., Lal, R., Resck, D.V.S., 2005. Changes in soil organic carbon stocks under agriculture in Brazil. *Soil and Tillage Research* 84, 28–40.

Zheng, X., Wang, M., Wang, Y., Shen, R., Gou, J., Li, J., Jin, J., Li, L., 2000. Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agroecosystem in Southeast China. *Chemosphere - Global Change Science* 2, 207–224.