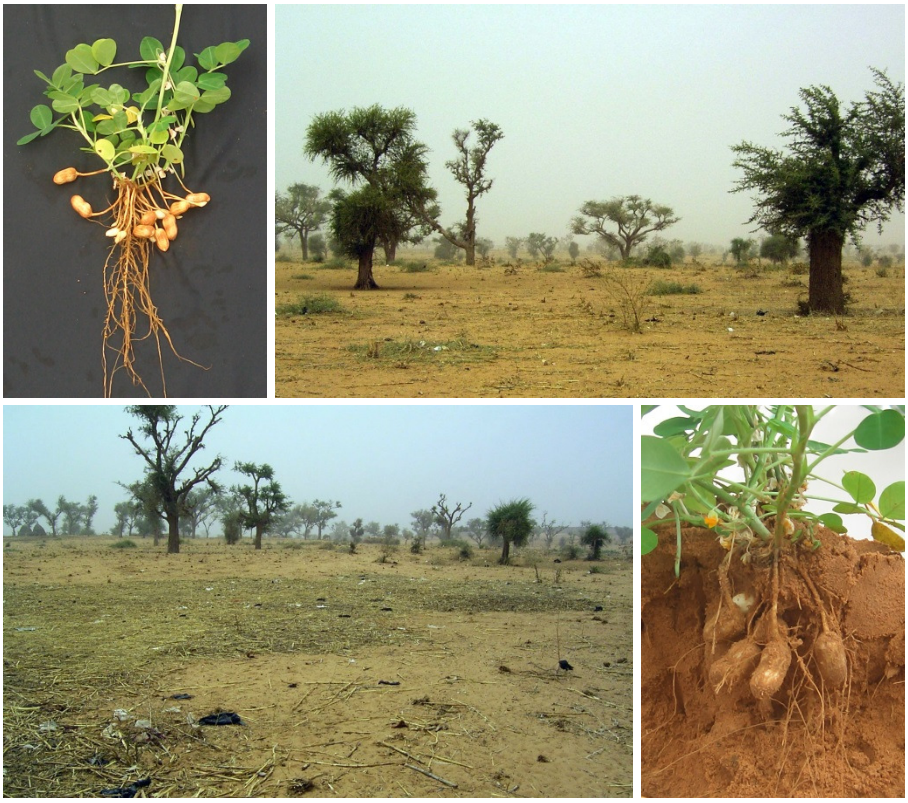
Causes of legume rotation induced yield increases in cereals grown on West African soils



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Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics

Soil Biology and Plant Nutrition

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Causes of legume rotation induced yield increases in cereals grown on West African soils

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Dedication

To

my mother and my sister for their love and support.

To the memory of

my father Hans Otto Formowitz and my grandmother Ruth Pfeiffer.

Preface

The present dissertation was made within the DFG-Project BU 1308/4-1 rooted in the two working groups ‘Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics” and “Soil Biology and Plant Nutrition” and submitted to the Faculty of Organic Agricultural Sciences (Fachbereich Ökologische Agrarwissenschaften, FB11) at the University of Kassel to fulfil the requirements for the degree: Doktor der Agrarwissenschaften (Dr. agr.).

This thesis is based on two papers which were accepted and are already published (2007 and 2009) in internationally refereed journals, one chapter which includes additional data of the second experiment and two chapters which will be submitted soon. These papers are found in chapters 4, 5, 7, 7 and 8. Chapter 1 gives a general introduction, chapter 2 introduces the objectives and hypotheses, and a short description of methods used is presented in chapter 3. General conclusions are drawn in Chapter 9 and an outlook is given in chapter 10. An English summary is included in chapter 11 and a German one in chapter 12.

The following papers are embedded in this dissertation:

Chapter 4

> Beate Formowitz, Mary-Catherine Schulz, Andreas Buerkert, Rainer Georg Joergensen (2007): Reaction of microorganisms to rewetting in continuous cereal and legume rotation soils of semi-arid Sub-Saharan Africa. Soil Biology & Biochemistry 39, p. 1512-1517. Doi:10.1016/j.soilbio.2006.12.033

Chapter 5

> Beate Formowitz, Rainer Georg Joergensen, Andreas Buerkert (2009): Impact of legume versus cereal root residues on biological properties of West African soils. Plant Soil 325, p. 145−156. DOI 10.1007/s11104-009-9964-6

Chapter 6

> Beate Formowitz, Andreas Buerkert, Rainer Georg Joergensen (to be submitted): Assessing P availability in continuous cereal soil influenced by mineral and organic amendments using an isotope method.

Chapter 7

> Beate Formowitz, Andreas Buerkert, Rainer Georg Joergensen (to be submitted): Plant growth of maize and sorghum on continuous cereal and legume rotation soils with different N and P application rates.

Chapter 8

> Beate Formowitz, Parva Zareitalabad, Andreas Buerkert, Rainer Georg Joergensen (to be submitted): Legume rotation induced differences in rhizosphere and bulk soil.

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Abbreviations

ADP	Adenosine diphosphate
AEC	Adenylate energy charge
AM	Arbuscular mycorrhiza
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
$BaCL_2$	Barium cloride
C	Carbon
^{13}C	Carbon isotope with the mass 13
C_{mic}	Microbial biomass carbon
C_{org}	Organic carbon
C_P	Amount of mobile ^{31}P ions in the soil solution
CC	Continuous cereal soil
CH_4	Methane
CL	Cereal legume rotation soil
CO_2	Carbon dioxide
CV	Coefficient of variation
DAS	Days after sowing
DGGE	Denaturation gradient gel electrophoresis
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
E_{1min}	Directly exchangeable (available) phosphorus within 1 minute
$E_{1min-1d}$	Exchangeable (available) phosphorus from 1 minute to 1 day
E_{1d-3m}	Exchangeable (available) phosphorus from 1 day to 3 months
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
K	Potassium

Abbreviations (continued)

k_{EC} and k_{EN}	Extractable part of the total amount of carbon (k_{EC}) and nitrogen (k_{EN}) bound in the microbial biomass
KH_2PO_4	Monopotassium phosphate
KOH	Potassium hydroxide
K_2SO_4	Potassium sulfate
N	Nitrogen
N_2	Gaseous nitrogen
N_{mic}	Microbial biomass nitrogen
N_{min}	Mineral nitrogen
NaOH	Sodium hydroxide
NH_4	Ammonium
NO_3	Nitrate
NH_4NO_3	Ammonium nitrate
OPA	Orthophthaldialdehyde
P	Phosphorus
^{31}P	Stable isotope of phosphorus with the mass 31
^{33}P	Phosphorus isotope with the mass 33
P_{tot}	Total phosphorus
P_{mic}	Microbial biomass phosphorus
R_0	Radioactivity added
r_t	Radioactivity remaining after certain time (t)
SPAD	Light absorption chlorophyll readings
USDA	United States Department of Agriculture
VAM	Vesicular-arbuscular mycorrhiza
WHC	Water holding capacity
w/w	weight per weight
Zn	Zinc

1 General Introduction

1.1 Situation of West African agriculture

In Sudano-Sahelian West Africa (SSWA) where rainfall is scarce and deficiencies in infrastructure and financial resources hamper the access to mineral fertilizers, agricultural production is largely restricted to the short rainy season. Thus it is not surprising that 30 of the 50 countries in the world where access to food is insufficient are in this region. In 2001 almost half of the population had to live on less than 1 US $ a day (UE 2008). According to the World Bank (2005) the number of poor people in SSWA nearly doubled in the past two decades to 40 % of the region's population due to low productivity of the agricultural sector. While agricultural productivity in some countries remained constant or even decreased from 2000 to 2004, countries such as Burkina Faso and Benin strengthened their agricultural sector which led to an annual increase of 3 % during this period (World Bank 2007). As agriculture accounts on average for one-third of the gross domestic product (GDP) and that around two-third of the workface is employed in this sector, a stable and productive agricultural sector is key for the development of sub-Saharan Africa (Dixon et al. 2001; One 2012).

Many soils in SSWA are sandy with a low pH, low organic matter, P availability, total N content and cation exchange capacity (Shetty et al. 1995). Thus farmers have to deal with low soil fertility and additionally with the risk of erosion and nutrient mining, both typical for all soils in SSWA. Most African farmers are smallholders whose whole family, especially the women, produce mainly rainfed food crops for auto-consumption (Livingston et al. 2011). With increasing rainfall the spectrum of cultivated crops becomes more divers from the northern Sahelian zone down to the Guinean zone (Shetty et al. 1995).

Traditionally, farmers grow cereals like pearl millet (***Pennisetum glaucum*** L.), sorghum (***Sorghum bicolor*** Moench) and maize (***Zea mays*** L.) in mixed cropping systems with legumes like groundnut (***Arachis hypogaea*** L.) and cowpea (***Vigna unguiculata*** Walp). In the beginning of the rainy season the cereals are sown after the first rainfall and a little later (after 15 to 30 days) legumes are intercropped, the time depending on rain evolution (Shetty et al. 1995). Normally cereals are used as food and the legume taken as fodder for livestock, the latter provides protein for human nutrition and increases farmers' income.

To intensify these production systems legume rotations in which a legume season is followed by cereals in the next season, have been introduced as a means to increase income for subsistence farmers.

1.2 Soil fertility, microorganisms and legumes – a brief overview

1.2.1 Natural and human made factors influencing soil fertility

Soils develop through the natural transformation of rock under site-specific climatic and vegetation conditions, controlled by soil building processes such as weathering, mineral formation, decomposition or degradation, humification, texture building and translocation

(Blume et al. 2010). Particle size and composition affect many of a soil's physical and chemical characteristics which in total determine its fertility. The granulation of a soil and its humus content affects water storage and binding, nutrient storage and cation exchange capacity, aeration, and biotic activity. The soil provides the basis for a living environment for plants, animals, humans, and soil organisms (flora and fauna). This so called ecosphere is characterized by multi-diverse material flows and nutrient cycles with various complex interaction systems (Blume et al. 2010). Soil microorganisms are involved in a soil's aggregate formation through recycling of plant residues and degradation of pollutants and agrochemicals, hence they govern many processes that are essential for soil fertility (Lupwayi et al. 1998; Mamilov and Dilly 2002). In natural habitats available nutrients allow crops to build up their biomass which in return provides organic material for the decomposer community for growth and humus formation. In addition to weather conditions, plant growth or vegetation establishment depend on the available space for rooting, aeration, water and nutrient availability as well as on pH (Kaltschmitt et al. 2009). These factors may vary immensely between different climatic zones or even within one field due to specific site conditions. According to Gregory (2006) roots are not only influenced by soil factors, but in turn modify soil properties through their growth, exudates and rhizodeposits that microorganisms and fungi feed on. The more biomass is produced the more organic material will be returned and degraded leading to substantial nutrient cycle in undisturbed ecosystems. However, a certain amount of substances from natural or anthropogenic origin is always brought in or removed from the ecosystem (Blume et al. 2010).

On arable land humus is degraded by agricultural production and nutrients are exported from the field through the removal of harvested material. Depending on the management system, cultivation practice, biomass usage or conversion pathway different amounts of foliar biomass are removed or returned. A rapidly growing world population increases the pressure on limited agricultural land, especially in areas, where the productivity of soils is insufficient to feed the local population (Baumer 1990). The intensive usage of arable land often leads to a reduction in soil fertility (Blume et al. 2010), which is often a slow and hardly noticeable process (Kibblewhite et al. 2008). Soil erosion, especially in many tropical countries, increases the problem through obvious soil and nutrient losses. Farmers can influence soil fertility through their cultivation practices, crops and machinery used, as well as protection measures against erosion. In semi-arid areas farmers often have to deal with inadequate soil moisture and the lack of financial resources to purchase inputs such as mineral fertilizers (Nyathi et al. 2003). Sandy soils in SSWA often reach soil temperatures > 40 °C and suffer from the effects of wind erosion (Bationo et al. 2003). These two factors may severely hamper crop establishment and expose the bare soil even more to wind and water erosion due to the reduction in vegetation cover. There is an urgent need to increase soil fertility in this region.

Although globally yield increases are heavily dependent on the targeted use of mineral fertilizers, organic amendments are the foundation for sustainable increases in soil fertility, building up soil organic matter, thereby leading to increased nutrient availability and fertilizer use efficiency (Murwira 2003). However, organic inputs, such as crop residues or manure, are often limited due to insufficient quantities or qualities or alternative uses

(Palm et al. 1997) and their application may be labour intensive. Hence there is a need for the introduction of higher yielding crops that produce residues of high quality leading to enhanced microbial reactions and maintenance or built-up of organic matter.

1.2.2 Soil microorganisms

A broad range of organisms inhabit each soil, from which typically only a small part is known. According to Lavelle (1996) more species have been identified in temperate than in tropical soils. While the taxonomic classification of soil organisms is controversial, a broader consense seems to exist for processes in which soil organisms are involved that affect soil quality and productivity of ecosystems (Lavelle 1996; Kibblewhite et al. 2008). The four major processes influencing soil health are (i) decomposition of organic material, (ii) recycling of nutrients, (iii) maintenance of soil physical structure and aggregate stability, and (iv) the control of pest and diseases (Swift et al. 2008; Kibblewhite et al. 2008):

(i) After soil animals, e.g. mites, earthworms or termites, have shredded organic residues, predominantly bacteria and fungi decompose it further until organic C is released as CO_2 and CH_4 or incorporated into the soil as stable soil organic matter (SOM).

(ii) Nutrient cycling, the availability of nitrogen (N), phosphorus (P) or sulphur (S), is closely related to decomposition processes for which again mainly microorganisms are responsible. Nutrient uptake by plants can be enhanced through symbiotic associations with e.g. rhizobacteria which forms root nodules for N_2-fixation or with mycorrhizae which penetrate into the roots and build up a mycelium in the near surrounding for nutrient and water acquisition. Free living nematodes that feed on soil bacteria or fungi play an important role in nutrient cycling and can therefore have strong effects on plant growth (Yeates and Bongers 1999).

(iii) The soil physical structure is mainly affected by plant roots, earthworms, ants and other soil macrofauna which move through the soil forming channels, pores, aggregates and transport particles. Therefore they are responsible for aeration, drainage and the creation of microhabitats for smaller organisms.

(iv) In soils with a high biological diversity, a wide range of microbial antagonistic interactions, microbivores and micropredators act on microbial and animal pests, respectively, to prevent outbreaks of soil-borne diseases and pests. In agriculture such epidemics are widely known and might occur due to a diminished soil biological diversity.

Microorganisms such as bacteria and protozoa tend to live in water films and thus are directly affected by reduced soil water contents. Fungi have the ability to grow across air-filled pores, which makes them more drought tolerant. All microorganisms are also indirectly affected by soil water contents through its influence on mass flow and diffusion for living soil organisms which is a critical point in West Africa with its wide range in rainfall. (Dick et al. 2001)

Through evolutionary adaptation microorganisms are adjusted to the harsh conditions in semi-arid West Africa characterized by regular wetting and drying cycles (Sparling et al. 1989) and might therefore react differently towards rewetting events than those in soils from humid temperate regions. Through air drying often only the strongest microorganisms may survive and build a (less diverse) new population (Sparling and Cheshire 1979). However, in general a lower microbial biomass can be expected to be found in many of the sandy soils in the Sudano-Sahelian zone with low nutrient and organic matter contents in addition to limited rainfall. This leads to a very restricted biomass production and thus less available organic residues that microbes can feed on (Dick et al. 2001).

Plant residues have direct (chemical composition) and indirect (e.g. soil faunal excrements) effects on the microbial community structure (Dick et al. 2001). Even pH values were found to have a more pronounced effect on fungal-to-bacterial ratio and hence on the structure of the microbial community than the substrate (Blagodatskaya and Anderson 1998). Plants and therefore substrate quality are able to affect the decomposer community. Rhizodeposition (e.g. released root cells, mucilage, root exudates, etc.) can alter the microbial community structure (Nardi et al. 2000; Benizri et al. 2002) and depend on differences in exudates or root border cells of different crop species and families (Rovira 1956; Hawes 1990; Wichern et al. 2007).

1.2.3 Leguminous crops

Legumes have high protein contents and therefore play an important role in animal and human nutrition. Especially in meat poor or vegetarian diets, grain legumes are practically indispensable for protein supply (Leterme 2002). Furthermore they are used in agricultural systems to improve soil fertility through e.g. deep rooting, enhancement of P acquisition, biological N_2 fixation and N sparing, humus accumulation and interrupting pest and disease cycles. Especially in developing countries where access to mineral fertilizers is often limited and in organic agriculture leguminous crops are the major N deliverer (Vance 2001; Graham and Vance 2003).

According to Bationo et al. (2003) cowpea and groundnut are the two grain legumes predominantly used in SSWA growing on around 6 and 2.7 Mio ha of arable land, respectively. Mainly cropped in mixed systems their yields are with 50 to 300 kg ha^{-1} very low on farmers' fields given low planting densities, pest and disease infestation, and low soil fertility, compared to more than 2000 kg ha^{-1} obtainable in research stations or by commercial enterprises (Ntare 1989; Reddy et al. 1992).

Legumes are known to live in a symbiosis with rhizo-bacteria that form root-nodules. In these nodules nitrogenase causes aerial N_2 to be transformed to NH_4 and subsequently to NH_3. The latter is directly bound and transformed to amino acids which are released into the soil (Richter, 2005). Quickly mineralised this N is available to microorganisms' and plants. The capacity of legumes to fix N_2 is strongly linked to adequate P availability (Kennedy and Cocking 1997) which is a major constrain to crop production on most sandy West African soils (Buerkert et al. 2001; Bationo et al. 2003). Legumes are able to secrete organic acids and acid phosphatase leading a mobilization of P for plants and microorganisms (Vance and Graham 2003; Richter 2005).

Numerous studies report cereal yield increases due to better N and P availability after growing a leguminous species in various cropping practices (Dakora and Keya 1997; Bagayoko et al. 2000; Soon et al. 2004). Available research on the effects of legume crop residues on N balances focused mainly on the above ground material that can be used as green manure. In West African agriculture almost all above ground biomass is used for grain and fodder (Bationo et al., 2003). Therefore N balances might be negative whenever major proportions of legumes are removed of the system. Over 90 % of total plant N was removed when the whole groundnut crop was harvested and even 60 to 70 % when only grain was taken (Norman et al. 1995). There is thus a risk to overestimate legume N inputs when the removal from above ground material is not taken into account, but fallen senescent leaves and roots also contribute to the N nutrition of the succeeding crop (Bado et al. 2006). According to Peoples et al. (1995) the amount of N accrued in the soil from legume derived organic inputs, such as green manure or leaf mulch, might be enough to meet N-demands of the following crop due to their contribution to readily mineralizable organic N accumulation in the soil.

Plant specific rhizodeposition affects the development of microbial communities in the rhizosphere. This has been shown for wheat, ryegrass, bentgrass and clover (Grayston et al. 1998) where discrimination between utilization of different carbon sources (carbohydrates, carboxylic acids and amino acids) by respective rhizospheral microbial communities was observed. This led to the assumption that exudation of these substances might differ between different plant species generating specific microbial communities. Other findings led to the suggestion that different microbial communities might strongly differ in substrate use efficiency meaning that different amounts of energy are needed to match the respective catabolic demands of each microbial biomass (Anderson and Domsch 1990).

2 Objectives and hypothesis

In previous experiments under West African field conditions legume rotations generated higher yields of the following cereal compared to continuous cereal plots and caused numerous changes in soil chemical and biological properties. These changes included a higher availability of N and P, an increased pH and early mycorrhizal infection rate, as well as decreased numbers of plant-parasitic nematodes (Bagayoko et al. 2000a/b; Buerkert 2000; Alvey et al., 2001). Under controlled conditions DNA extractions using denaturation gradient gel electrophoresis (DGGE) indicated site-specific microbial rotation effects due to differences in the rhizospheral bacterial and eukaryal community structure, higher microbial biomass N, and higher fungal biomass in rotation soils (Marschner et al, 2004). This provides solid evidence that soil microorganisms play an important role in improving cereal growth following legume rotations. But so far the processes underlying these effects are not fully understood (Marschner et al., 2004).

The objective of this study therefore was to investigate which biological and chemical factors cause the cereal yield enhancing effects of legume rotations on sandy, nutrient poor, West African soils.

The aim of the here described studies not only was to deliver additional insights into the role of legume residues and microorganisms for nutrient cycles on nutrient poor West African soils. The study also aimed to unravel if differences in substrate quality (e.g. root residues and root exudates) may cause changes in the microbial community structure and rhizospheral activity, and if differences in substrate use efficiencies exist between the specific developed microbial communities.

Four experiments were conducted to answer the following hypotheses:

1. Rewetting of heavily dried West African soils at the beginning of the rainy season causes strong growth dynamics (dying and growth processes) in the very small decomposer community
2. Different qualities of root residues, which are decomposed in the West African soils, change important soil characteristics, such as pH, mobilized P and N, as well as soil microorganisms, during decomposing processes and are thus responsible for cereal yield enhancing legume rotation effects
3. The higher nutrient concentrations in rotation soils, especially of N and P, are responsible for the yield increases in cereals following a leguminous crop
4. The increased nutrient availability, especially in N and P, after legume cultivation is caused by changes in the soil fauna and microorganisms (fungi, bacteria, nematodes) in the rhizosphere

3 Methodology

3.1 Description of the soils, their origin and confirmation of rotation effects in 2004

Many of the soils in SSWA are Arenosols with very low organic matter contents and low soil fertility. At several sites in Niger, Burkina Faso, and Togo, field experiments were conducted from 1996 - 1999 to compare different fertilizer regimes, the use of crop residues, and the cultivation of legume rotation ***versus*** continuous cereal plots. At the end of these field trials in Fall 1999 the upper soil layer (0-20 cm depth) was taken from cereal legume rotation (CL) and continuous cereal (CC) plots, air dried, sieved to 2 mm, packed into plastic bags, and shipped to Germany, where they were stored from then on at about 15 °C in the dark (Figure 1). For all experiments of the project only soils from Fada (Burkina Faso) and Koukombo (Togo) were taken except for fallow soils from other West African sites (Sadore and Gobery, Niger) which were used for root production in experiment 2 (chapter 5). At the end of 2004 the in chapter 2 mentioned yield increasing effects of legume rotations observed in previous studies were confirmed in greenhouse trials with soils from the two described sites, Fada and Koukombo. Even after 6 years of storage rotation effects on legume rotation soils were clearly visible leading to higher pearl millet yields (Figure 2).

Figure 1: Plastic containers filled with the experimental soils from West Africa in the basement of the University of Kassel, Witzenhausen – Steinstraße.

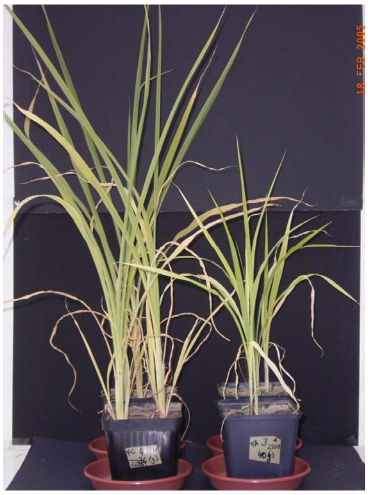

Figure 2: Pearl millet grown on continuous cereal (CC, right) and cereal legume rotation (CL, left) soils from Fada.

Fada n'Gourma (11°59 'N, 0°19 'E) is located in the Sudanian zone of Burkina Faso. The site's average temperature is about 28.3 °C a^{-1} with 850 mm a^{-1} rainfall during a single rainy season from May till October. According to the USDA soil taxonomy, the soil in Fada is an Alfisol (Haplustalf). An Alfisol is defined by its clay accumulation and exchangeable aluminium in the subsoil. Clay content, cation exchange capacity and organic C were 15 %, 28 µmol g^{-1} soil and 5.2 mg g^{-1} soil, respectively (Buerkert et al., 2000). Mean soil pH in water was 6.4 in both Fada soils used.

Koukombo (10°17 'N, 0°23 'E) is located in Northern Guinean zone of Togo. The site's average temperature is about 27.8 °C a^{-1} with > 1000 mm rainfall per year during a single rainy season from May till October. According to the USDA soil taxonomy, the soil in Koukombo is an Ultisol (Plinthic Kanhaplustult). Ultisols are characterized as soils with clay accumulation and a low base saturation. Clay content, cation exchange capacity and organic C were 5 %, 19 µmol g^{-1} soil and 3.7 mg g^{-1} soil, respectively (Buerkert et al., 2000). Mean soil pH in water was 6.2 in both Koukombo soils.

3.2 Experiment 1: Reaction of microorganisms to rewetting in continuous cereals and legume rotation soils of semi-arid Sub-Saharan Africa

In order to test hypothesis one (chapter 2) the first incubation experiment aimed at exploring the reaction of soils microorganisms towards rewetting simulating the onset of the rainy season after a long dry period.

Therefore soil samples (CL and CC) from Fada and Koukombo were taken, rewetted to 45 % water holding capacity (WHC) and incubated at 25 °C in the dark. To determine growth dynamics several soil microbial parameters were measured at 0 h, 0.5 h, 6 h, 12 h, 24 h, 48 h, 72 h, 100 h, 240 h and 480 h after rewetting soil. Microbiological indices measured were adenine nucleotides (ATP, ADP and AMP), microbial biomass C (C_{mic}) and N (N_{mic}), the fungal biomarker ergosterol and the fungal and bacterial cell wall components glucosamine and muramic acid. Furthermore CO_2-evolution was measured daily over the 20 days incubation experiment.

Detailed methodological and analytical descriptions are included in chapter 4.3.

3.3 Experiment 2a: Impact of legume versus cereal root residues on biological properties of West African soils

This experiment aimed at investigating the effects of legume root residues and their decomposition on important soil characteristics and cereal growth, addressing hypothesis 2 (chapter 2).

An incubation experiment and a greenhouse trial with continuous cereal soils from Fada and Koukombo were conducted. In both experiments CC-soil was mixed with root residues of three cereals (maize, sorghum, pearl millet) and two legumes (cowpea and groundnut) traditionally grown in West Africa. These treatments were compared to mineral amendments (P solely and P+N), applied at an equivalent amount to the respective P and N applied with legume roots, and a non-amended control.

During the greenhouse trial the soil water content was controlled daily. If necessary, water was added and pots were newly randomised. At the same frequency shoot length was measured until shoots and roots were harvested separately and analysed for their nutrient (N, P, K) concentrations. Furthermore nematodes were counted in soil samples of each pot.

For the incubation experiment soil samples mixed with root residues or mineral P were placed in glass bottles and incubated for 189 days at 25 °C in the dark. At five sampling dates (day 0, 7, 21, 63 and 189) a moist subsample from each glass was taken and analysed for its water content and different soil chemical and microbial parameters. If necessary, water was added to maintain 45 % WHC. Over the whole incubation period CO_2-evolution was measured regularly 22 times, initially every 2 to 4 days and later weekly. The soils' pH was measured in the samples to determine soil chemical changes. To observe changes in the microbial biomass again adenine nucleotides, C_{mic}, N_{mic} and ergosterol were measured.

Detailed methodological and analytical descriptions are shown in chapter 5.3.

3.4 Experiment 2b: Assessing P availability in continuous cereal soil influenced by mineral and organic amendments using an isotope dilution method

Using the isotopic dilution method this study aimed at assessing mobile P pools being available after adding mineral or organic P sources thereby addressing hypothesis 2 (chapter 2).

Among other treatments in the previous pot experiment (experiment 2, chapter 5) CC-soils from Fada and Koukombo were mixed with root residues of groundnut, sorghum or maize, or mineral P, and compared to a non-amended control. After harvesting soil samples of the remaining soil were dried at 60 °C, vacuum packed and send to ETH Zürich for ^{33}P analyses. There total P, mobile P ions in the soil solution, and the amount of radioactivity at t = 0 was measured. After the addition of carrier free ^{33}P-orthophosphate the decreasing radioactivity over time and isotopical P-exchange parameters were calculated.

Detailed methodological and analytical descriptions are shown in chapter 6.3.

3.5 Experiment 3: Plant growth of maize and sorghum on continuous cereal and legume rotation soils with different N and P application rates

To test hypothesis 3 (chapter 2) this greenhouse trial aimed at exploring whether the enhanced P and N availability after cultivation of legumes is the main factor causing the yield increases of the following cereal on the experimental soils.

In a first step contents of N_{min} and phosphorous (P-Bray) were determined in cereal legume rotation and continuous cereal soils from Fada and Koukombo. Subsequently, the difference between CL- and CC-soils for both nutrients was calculated and used to compute compensatory application rates. Thus both soils of each site were brought to the same level of respective P and N concentrations. Then sorghum was planted on Fada and maize on Koukombo soils. Again water content was controlled daily (adjustment to 10 % W/W) and shoot length measured at the same time. Before harvesting shoots and roots separately leaf SPAD values were determined. Above and below ground biomass was analysed for respective nutrient concentrations. In root sub samples mycorrhizal infection rate and root length were measured. Furthermore nematodes, P-Bray and P-water were determined in soil subsamples.

Detailed methodological and analytical descriptions are shown in chapter 7.3.

3.6 Experiment 4: Legume rotation induced chemical differences in rhizosphere and bulk soil

The last experiment of this study aimed at investigating whether legume induced changes in the soil fauna and microorganisms in the rhizosophere of the following cereal are driving forces for better nutrient availability, especially of N and P.

To this end soil of cereal legume rotation and continuous cereal plots from Fada and Koukombo were filled into root chambers. Each soil was planted with maize, sorghum and pearl millet. Additional treatments consisted of δ-radiation sterilized soils from both sites which were re-incubated with bacteria, inoculated with mycorrhizal spores, infected with plant parasitic nematodes. All variants with sterilized soil were planted with sorghum.

Over the growth period of 29 days, root chambers were watered and plant height determined daily. Non-destructive pH measurements took place 1, 2 and 4 weeks after planting using a self-made antimony-electrode followed by calculating the corresponding pH from the mV-readings obtained that were calibrated with buffer solutions followed by linear value interpretation. With an agar-plate placed on the opened root chamber, including a pH-indicator, differences in pH between rhizosphere and bulk soil were also visualized. Shoots and roots were harvested separately, dry mass determined and the sieved soil divided into bulk soil, rhizosphere soil and ‘rest-soil’. Subsequently, rhizosphere soil, root samples and separated $CaCl_2$-suspension to extract PO_4 and nitrate were frozen for further analyses. Bulk soil was also extracted with $CaCl_2$, but because no staning was possible to measure PO_4, P-Bray was measured instead in a few samples of particular interest.

More detailed description and discussion of the methodology and analyses are shown in chapter 8.3.

4 Reaction of microorganisms to rewetting in continuous cereals and legume rotation soils of semi-arid Sub-Saharan Africa

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4.1 Abstract

A 20-day incubation experiment with continuous cereals (CC) versus cereal legume (CL) rotation soils of two semi-arid Sub-Saharan sites (Fada-Kouaré in Burkina Faso, F, and Koukombo in Togo, K) were carried out to investigate the effects of rewetting on soil microbial properties. Site- and system-specific reactions of soil microorganisms were observed on cumulative CO_2 production, adenylates (ATP, ADP, and AMP), microbial biomass C and N, ergosterol, muramic acid and glucosamine. Higher values were found in the CL rotation soils and in the two soils from Fada-Kouaré. While the inorganic N concentration showed only a system specific response to rewetting, the adenylate energy charge (AEC) showed only a site-specific response. ATP recovered within 6 h after rewetting from ADP and AMP due to rehydration of microorganisms and not due to microbial growth. In this case, all NO_3 in the soil is immediately available to the plants avoiding the possible competition between plants and soil microorganisms. The fungal cell-membrane component ergosterol was three (CC) and five (CL) times larger at Fada than in the respective soils at Koukombo. The concentrations of the bacterial cell-wall component muramic acid were by 20 % and of mainly fungal glucosamine by 10 % larger in the CL rotation soils than in the CC soils. This indicates long-shifts in the microbial community structure.

Keywords: Adenylates; AEC; Ergosterol; Glucosamine; Muramic acid; Microbial biomass; CO_2 evolution rate

4.2 Introduction

In West Africa, where rainfall is irregular and often scarce, legume rotations with groundnut and cowpea have been reported to cause several changes in soil chemical and biological properties (Bagayoko et al., 2000a/b; Alvey et al., 2001). Legume rotations gradually increased soil pH, early mycorrhizal infection and N availability and decreased the number of phytoparasitic nematodes, thereby leading to an increase in total dry matter production of the following cereal crop (Buerkert et al., 2000; Bagayoko et al., 2000a). Under controlled conditions, differences in bacterial community structure, higher microbial biomass N and a higher fungal biomass in the rhizosphere of rotation soils are additional, site-specific microbiological rotation effects (Marschner et al., 2004). This indicates that soil microorganisms are an important component for improving plant growth by legume rotations.

A key-event is the mineralization pulse after rewetting. The responsible mechanisms have not been conclusively identified, especially for low organic matter soils from semi-arid Sub-Saharan regions, although numerous experiments have been carried out to analyze the effects of drying-rewetting cycles (Fierer and Schimel, 2003; Wichern et al., 2004; Wu and Brookes, 2005). The aim of the present experiment therefore was to analyse the effects of the onset of the rainy season after a long period of drought on the microbial community. Rewetting as a crucial event in the microbial life cycle was simulated in dried soils from semi-arid Sub-Saharan sites under continuous cereals and legume rotation cultivation. In these soils, microorganisms are adapted to repeated and sometimes long periods of drought (Marschner et al., 2004), and may react differently than microorganisms in soils from temperate humid climate (van Gestel et al., 1993; Fierer et al., 2003). The rewetting effects were monitored by measuring CO_2 evolution, inorganic N, adenylates (ATP + ADP + AMP), the fungal biomarker ergosterol, microbial biomass C and biomass N. Bacterial muramic acid and fungal glucosamine give also information on the long-effects of cropping systems on the microbial community structure due to their persistence as microbial cell-wall components.

4.3 Materials and methods

4.3.1 Soil and experimental layout

In December 1998 soil samples were taken from a sorghum/groundnut experiment in the Sudanian zone of Burkina Faso on a Haplustalf at Fada-Kouaré (11°59’N, 0°19’E; 850 mm mean annual rainfall and 28.3 °C mean annual temperature) and in December 1999 from a maize/groundnut experiments in the Guinean zone of Togo on an isohyperthermic Plinthic Kanhaplustult at Koukombo (10°17’N, 0°23’E; 1100 mm, mean annual rainfall and 27.8 °C mean annual temperature). The soil of each site was taken at 0-20 cm depth, air-dried and sieved to 2 mm, packed in plastic bags, shipped to Germany and stored in the dark at about 15 °C. Clay content, cation exchange capacity and organic C were 15 %, 28 µmolc g^{-1} soil and 5.2 mg g^{-1} soil in the Fada soil and 5 %, 19 µmolc g^{-1} soil and 3.7 mg g^{-1} soil in the Koukombo soil, respectively (Buerkert et al., 2000). Mean soil pH in water was 6.4 in the two Fada soils and 6.2 in two Koukombo soils. Eight replicates of the air-dried continuous cereals (CC) and cereal legume rotation (CL) soil samples from Fada-Kouaré (CC-F and CL-F) and Koukombo (CC-K and CL-K) were rewetted to 45 % water holding capacity with tap water and incubated at 25 °C in the dark. Soil biological indices were measured 0 h, 0.5 h, 6 h, 12 h, 24 h, 48 h, 72 h, 100 h, 240 h and 480 h after rewetting soil. CO_2 evolution was determined daily over a period of 20 days.

4.3.2 Analytical procedures

CO_2 evolution was determined in 50 g air-dried soil, placed in 80 ml incubation cylinders made of stainless steel nets, and transferred into 1000 ml incubation vessels containing NaOH solution at the bottom, rewetted with 5 ml of bidistilled water and incubated for 20 days at 25 °C in the dark with eight replicates. Evolved CO_2 was absorbed in 10 ml of 0.1 M, 0.05 M or 0.03 M NaOH, which was titrated daily with HCl of the same molarity. At 7 and 13 days after rewetting each sample was wetted again with 1 ml of bi-distilled H_2O to prevent the samples from drying.

Adenine nucleotides (ATP, ADP, and AMP) were extracted with an alkaline DMSO buffer according to Bai et al. (1988) as described by Dyckmans and Raubuch (1997) using a moist sample equivalent to 3 g oven-dry soil.

Microbial biomass C and biomass N were determined by fumigation extraction (Brookes et al., 1985) using pre-extraction to remove high background levels of organic C and inorganic N. A sample of 25 g soil (on an oven-dry basis) was pre-extracted with 100 ml of 0.05 M K_2SO_4 on a horizontal shaker at 200 rev min^{-1} for 30 min and centrifuged. Fumigated and non-fumigated portions of 10 g moist soil were taken from the remaining soil and extracted with 40 ml 0.5 M K_2SO_4 as described above. Microbial biomass C and N was calculated according to Wu et al. (1990) and Brookes et al. (1985). In the 0.05 M K_2SO_4 pre-extracts and in the 0.5 M K_2SO_4 extracts of the non-fumigated samples, NO_3-N and NH_4-N were determined using segmented flow analysis.

The fungal cell-membrane component ergosterol was extracted from 2 g soil with 100 ml ethanol by oscillated shaking at 250 rev min^{-1} for 30 min according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100 % methanol as the mobile phase and detected at a wavelength of 282 nm.

The fungal and bacterial cell-wall components glucosamine and muramic acid were determined according to Appuhn and Joergensen (2006) in 500 mg air-dried soil after hydrolysis with 6 M HCl. Fluorometric emission of amino sugar OPA (ortho-phthaldialdehyde) derivatives was measured at a wavelength of 445 nm with 340 nm as the excitation wavelength (Agilent 1100, Palo Alto, USA). Fungal glucosamine was recalculated into fungal C and muramic acid into bacterial C using the procedure and conversion values proposed by Appuhn and Joergensen (2006).

4.3.3 Statistical analysis

All results were tested for normal distribution of residuals using the Kolmogorov-Smirnov test. Soils were compared using a GLM-repeated measures ANOVA with site and system as between-subject factors. Extracted variables at the different times were taken as inner-subject factors, and means were separated using Tukey's HSD (honestly significant difference). All statistical analyses were performed with SPSS 11.5 (Backhaus et al., 2003).

4.4 Results

In the two Fada soils, cumulative CO_2 production was more than twice as high as in the Koukombo soils. This difference was especially strong in the two CL rotation soils. Throughout the incubation period the CO_2 evolution rate of two Fada soils was always larger than that of the two Koukombo soils, but this difference was largest immediately after rewetting (Fig. 1).

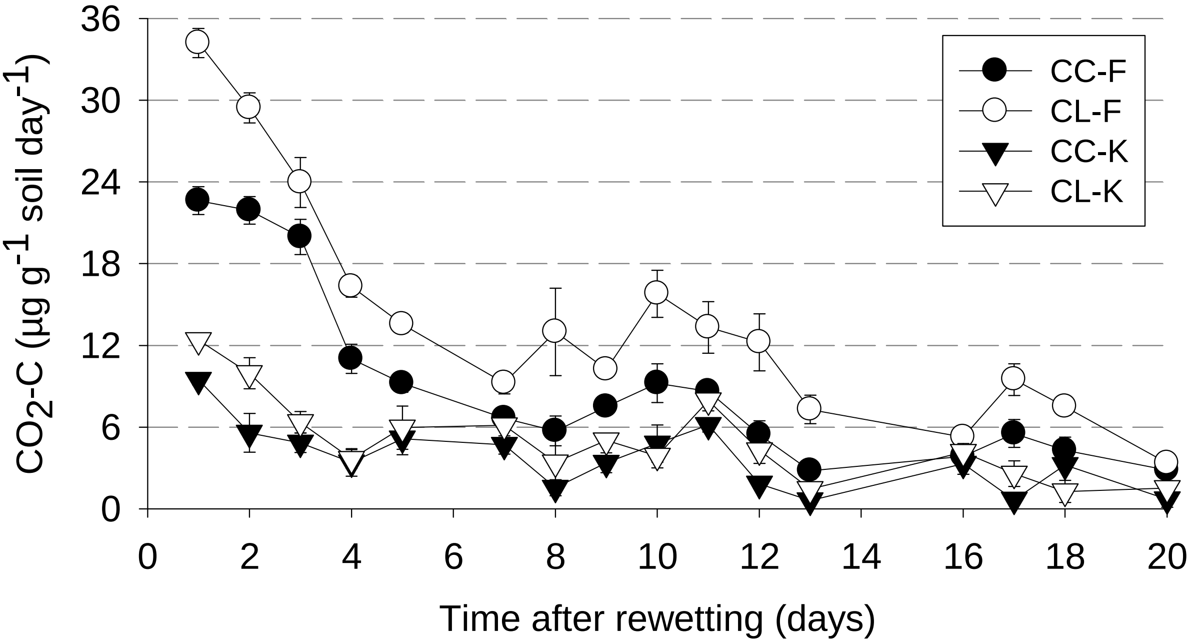

Figure 3: Basal respiration of rewetted (45 % WHC) continuous cereals (CC) and rotation soils (CL) from Fada (F) and Koukombo (K), vertical bars represent one standard error of the mean.

The CO_2 evolution rate showed a roughly 90 % decrease in all treatments during the 20-day incubation period with intermediate maxima around day 10 and day 17. In contrast to the cumulative CO_2 production, the amount of inorganic N after 20 days of incubation was similar in both soils, whereas across sites rotation led to significant increases (Table 1). The initial inorganic N concentration comprised between 30 % (CC-K) and 80 % (CL-F) of the final concentration at the end of the incubation period (Table 1).

Table 1: Cumulative CO_2-C production over 20-day incubation period at 25 °C, concentration of inorganic N (NH_4-N + NO_3-N) at the end of the 20-day incubation period, mean contents of adenylates (ATP + ADP + AMP) over all sampling dates (n = 10), adenylate energy charge (AEC) over all sampling dates excluding the sampling 0 and 6 h after rewetting (n = 8), contents of microbial biomass C and biomass N over all sampling dates (n = 10) in continuous cereal (CC) and cereal legume (CL) rotation soils from Fada (F) and Koukombo (K), probability levels of a two-way ANOVA for repeated measures**.**

	CO_2-C (μg g^{-1} soil)	Inorganic N (μg g^{-1} soil)	Adenylates (nmol g^{-1} soil)	AEC	Microbial biomass C (μg g^{-1} soil)	Microbial biomass N (μg g^{-1} soil)
CC-F	164	2.9	0.70	0.73	64	6.8
CL-F	247	4.7	0.75	0.71	112	10.6
CC-K	71	2.9	0.59	0.70	47	4.5
CL-K	96	4.2	0.75	0.74	45	4.4
Probability levels						
Time	<0.01	<0.01	<0.01	<0.01	0.01	0.04
Site	<0.01	0.48	<0.01	0.35	<0.01	<0.01
System	<0.01	<0.01	<0.01	0.28	0.02	0.05
Site x System	<0.01	<0.01	0.49	<0.01	0.01	0.03
CV (± %)	15	24	29	8	56	33

CV = mean coefficient of variation for the four treatments

The mean adenylate content of the rotation soils was similar for CL rotation soils at both sites, but it was significantly lower for the CC soil at Koukombo (Table 1). The adenylate content increased until 72 h after rewetting and declined roughly to the initial value at the end of the 20-day incubation period (Figure 4a). The AEC was 0.2 immediately after rewetting, increased to 0.45 within 30 min and reached the final value of 0.72 after 6 h without any soil or management system effect due to the transformation of AMP and ADP to ATP (Table 1, Figure 4b). The differences in microbial biomass concentrations between the soils reflected more the differences in CO_2 production than those in adenylates and inorganic N (Table 1). Microbial biomass C varied between 45 and 64 μg g^{-1} in most soils, but was nearly twice as high in CL rotation soil at Fada. Microbial biomass N followed biomass C with a relatively constant C-to-N ratio of 10.2.

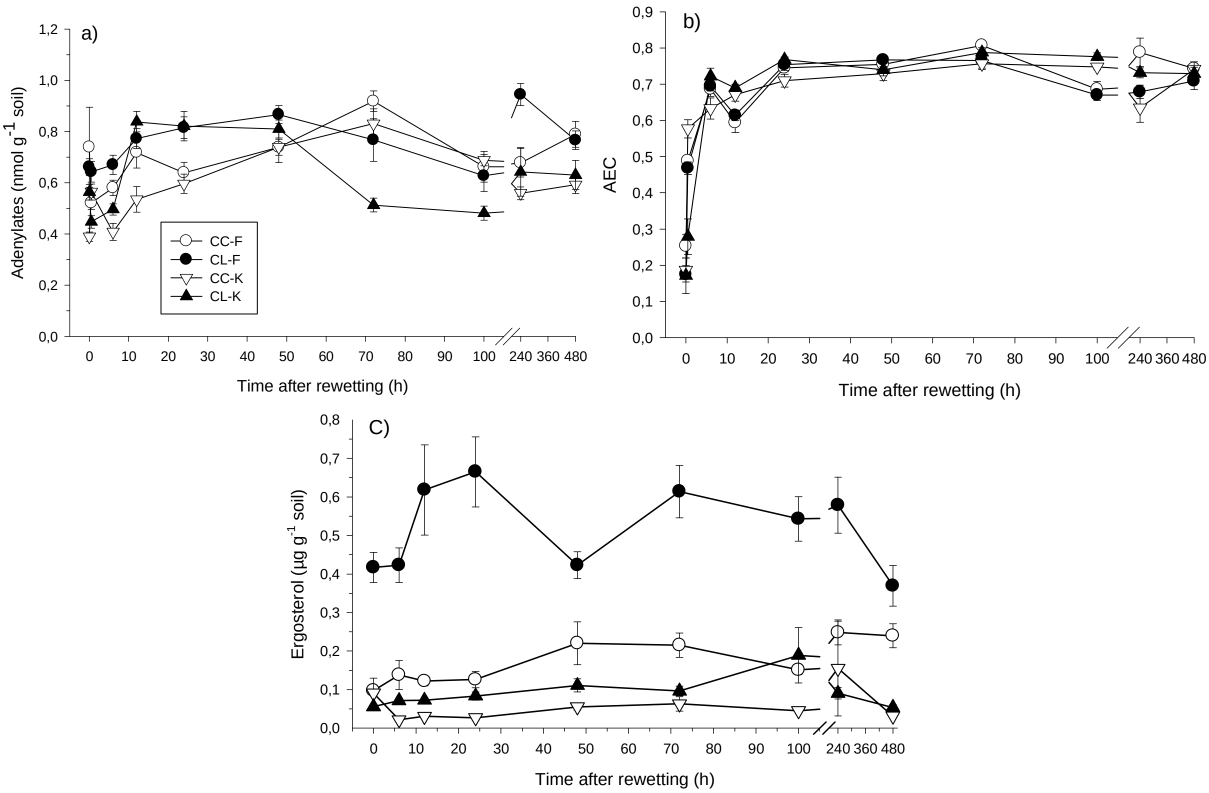

Figure 4: (a) Adenylates, (b) adenylate energy charge (AEC: (ATP + 0.5 x ADP) / (ATP + ADP + AMP)), and (c) ergosterol contents of rewetted (45 % WHC) continuous cereals (CC) and rotation soils (CL) from Fada (F) and Koukombo (K); vertical bars represent one standard error of the mean.

Table 2: Mean contents of ergosterol over all sampling dates (n = 10), the contents of muramic acid and glucosamine measured initially in air-dried soil, ratio of fungal C-to-bacterial C in continuous cereal (CC) and cereal legume (CL) rotation soils from Fada (F) and Koukombo (K), probability levels of a 2-way ANOVA for repeated measures.

	Ergosterol (µg g^{-1} soil)	Muramic acid (µg g^{-1} soil)	Glucosamine (µg g^{-1} soil)	Fungal C/bacterial C
CC-F	0.17	26	383	2.8
CL-F	0.52	31	404	2.4
CC-K	0.05	13	248	3.8
CL-K	0.09	16	290	3.6
Probability levels				
Time	0.25			
Site	<0.01	<0.01	<0.01	<0.01
System	<0.01	0.01	0.04	0.01
Site x System	<0.01	0.22	0.49	0.52
CV (± %)	10	3		5

CV = mean coefficient of variation within the four treatments

The fungal cell-membrane component ergosterol was three (CC) and five (CL) times larger at Fada than in the respective soils at Koukombo (Table 2). The ergosterol concentration increased slightly after rewetting and declined with some variation roughly to the initial value at the end of the 20-day incubation period (Figure 4c). The mean ergosterol-to-microbial biomass C ratio was 0.32 without any site and system effect. The concentrations of the cell-wall components muramic acid were by 100 % and of glucosamine by 50 % larger in the two Fada soils than in the two Koukombo soils (Table 2). The concentrations of muramic acid were by 20 % and of glucosamine by 10 % larger in the CL rotation soils than in the CC soils.

4.5 Discussion

ATP recovered within 6 h after rewetting. This recovery cannot be caused by growth processes of a small surviving community, which needs at least 10 to 12 h for glucose (Anderson and Domsch, 1978) and even longer for more complex substrates as microbial residues (Jenkinson, 1976). Our results are supported by Ahmed et al. (1982) and Ciardi et al. (1993), who observed a rapid increase in ATP within few hours after rewetting an air-dried soil. Growth processes leading to an increase of cell numbers would lead to strong immobilization of N in contrast to a simple rehydration process. However, this N immobilization has not been observed in the present experiment. Consequently all NO_3 in the soil is immediately available to the plants evading the possible competition between plants and soil microorganisms (Schimel et al., 1989). This facilitates rapid plant development under the harsh conditions of semi-arid regions with long periods of drought.

Microorganisms in these sub-tropical soils are able to dehydrate during drying and to reduce their metabolism accompanied by a transfer of ATP to ADP and AMP. This is in line with the observations of Raubuch et al. (2002), although the soils reached much lower water contents in the present experiment. However, with increasing storage period under air-dried conditions, microorganisms can also lose their ability to rehydrate. This has been shown in a rewetting experiment with soils stored air-dry for up to 103 years (De Nobili et al., 2006). Similar results were observed in an incubation experiment with abandoned terrace soils from four fallow age classes collected at a mountain oasis in Oman (Wichern et al., 2004). The respiratory response to rewetting and the microbial biomass declined with increasing fallow age and increasing dryness. The storage under air-dried conditions might be the reason that the microbial biomass C-to-soil organic C ratios of the present soils are relatively low in comparison with soils from other semi-arid subtropical areas (Khan and Joergensen, 2006). The same might be true for the mean ratios ATP-to-microbial biomass C and total adenylates-to-microbial biomass C, which were 6.9 and 10.4 µmol g^{-1}, respectively and thus in the lower range of the data available in the literature (Jenkinson, 1988; Dyckmans et al., 2003; Wu and Brookes, 2005).

The initial flush of CO_2 evolution after rewetting of the present experiment has been observed repeatedly (van Gestel et al., 1993; Fierer and Schimel, 2003; Wichern et al., 2004, Wu and Brookes, 2005). The most likely explanation is the use of organic components by rehydrated microorganisms that feed on soil organic matter physically released from soil colloids and microaggregates during drying (Fierer and Schimel, 2003; Wu and Brookes, 2005). The site-specific increase in respiration of the two Fada soils compared to the Koukombo soils may therefore be due to their higher soil organic C level. The present data suggest that an original microbial biomass killed by drying-rewetting processes is of minor importance as source for the initial CO_2 flush. Microbial death leads to an enormous loss of energy corresponding to approximately 60 % to 80 % of the biomass without any possibility to recover to the initial values (Jenkinson, 1988). The view of a killed microbial biomass as CO_2 source after rewetting is based on experiments with ^{14}C-labelled glucose (Bottner, 1985; Fierer and Schimel, 2003; Wu and Brookes, 2005). However, a large part of this easily available glucose is immediately transferred into the fraction of non-microbial residues (Chander and Joergensen, 2001), which could be preferentially decomposed in drying/rewetting cycles. This is in line with the observation of Fierer and Schimel (2003) that no lysis of microbial cells occurred during drying-rewetting.

Although ergosterol and glucosamine changed in similar directions, both indices drew a very different picture from fungal life in the present soils. If the ergosterol data were converted to fungal biomass C by the factor 90 (Djajakirana et al., 1996), fungi contributed 29 % to microbial biomass C in the four soils. According to fungal glucosamine and bacterial muramic acid, microbial residue C consisted of 72 % fungal C in the two Fada soils and of 79 % in the two Koukombo soils. Appuhn et al. (2006) did not find any indication for differences in turnover of bacterial and fungal tissue. They observed a close relationship between the composition of dead microbial residues (amino sugars) and the living fraction (ergosterol) in their soil. Fungal tissue dominates the microbial residues in both soils and likely also the microbial biomass. This suggests that the fungal communities in the present four Sub-Saharan soils contain less ergosterol than the cultivated fungal species summarized by Djajakirana et al. (1996). The lower fungal C-to-bacterial C ratio in the two CL rotation soils in comparison to the two CC soils might be caused by differences in the microbial colonization of plant roots (Marschner et al., 2004; Appuhn and Joergensen, 2006). In South African Highveld soils, a significant increase in the proportion of fungal residue C from 77 % to 84 % (recalculated according to Appuhn and Joergensen, 2006) was measured after cultivating native grassland (Amelung et al., 2002).

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5 Impact of legume versus cereal root residues on biological properties of West African soils

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5.1 Abstract

Many microbial turnover processes in acidic sandy subtropical soils are still poorly understood. In a 59-day pot and a 189-day laboratory incubation experiment with two West African continuous cereal soils, the effects of 2 mg g^{-1} root residues were investigated on growth of sorghum seedlings, soil microbial biomass and activity indices, using cowpea, groundnut, pearl millet, maize and sorghum. The effects of root residues were compared with mineral P or mineral P+N treatments and with a non-fertilized control treatment. On the Alfisol (Fada, Burkina Faso), shoot dry mass was always significantly higher than on the Ultisol (Koukombo, Togo). Highest shoot dry mass was observed after application of mineral P+N on the Alfisol and after mineral P alone on the Ultisol. The application of legume root residues led to small and non-significant increases in dry mass production compared to the non-amended control, whereas the application of cereal root residues led to a decline, regardless of their origin (millet, maize or sorghum). Contents of microbial biomass C, microbial biomass N and ergosterol were 75 to 100 % higher in the Alfisol than in the Ultisol, while ATP was only 36 % higher. Organic amendments increased ergosterol concentrations by up to 145 % compared to the control and mineral P application. Microbial biomass C and microbial biomass N increased by up to 50 % after application of root residues, but ATP only up to 20 %. After application of legume root residues, cumulative CO_2 production was similar in both soils with an average of 370 µg CO_2-C g^{-1} over 189 days. After application of cereal root residues, cumulative CO_2 production was higher in the Alfisol (530 µg g^{-1}) than in the Ultisol (445 µg g^{-1}) over 189 days.

Keywords: Legume rotations, Organic amendments, Microbial biomass, Ergosterol, ATP, Decomposition

5.2 Introduction

Low and irregular rainfall as well as deficient infrastructure and financial resources are major limiting factors for agricultural production in most West African countries leading to depletion in soil fertility (Bationo et al. 2007). Soil organic matter is a key component of soil fertility as sink and source for nutrients, habitat for soil organisms, but also for improving structural stability. Legume rotations have been proposed as a means to improve soil organic matter levels and thus soil fertility in comparison to the predominant production systems of continuous cereal monocultures (Boddey et al.; 1997), although evidence from long-term experiments is missing for arid Sudano-Sahelian West African soils (Anyanzwa et al. 2008).

The cultivation of legumes such as cowpea, soybean, and groundnut can cause numerous changes in soil chemical and biological properties (Horst and Hardter 1994; Adeboye et al. 2006; Jemo et al. 2006). Under field conditions, a leguminous crop led to a higher soil pH, higher N availability, higher early mycorrhizal infection and decreased numbers of phytoparasitic nematodes (Buerkert et al. 2000; Alvey et al. 2001; Bilgo et al. 2007). Extractions of soil microbial DNA in studies conducted under controlled conditions showed that the bacterial and eukaryal community structure in the soil, assessed by denaturing gradient gel electrophoresis (DGGE), differed between soils from legume rotation and continuous cereal sites (Marschner et al. 2004). Furthermore, higher microbial biomass C and N, higher fungal growth and a higher activity (respiration and concentrations of ATP) were found in rotation soils compared to continuous cereal soils from the same sites (Formowitz et al. 2007). However, the reasons for these differences are still not fully understood (Marschner et al. 2004; Formowitz et al. 2007).

Most studies looking at decomposition effects of organic materials to sustain soil productivity focused on shoot matter (Fox et al. 1990; Palm and Sanchez 1991; Muhammad et al. 2006) and less on decomposition effects of root residues (Ladd et al. 1981; Paré et al. 2000; Thippayarugs et al. 2008). As in Sudano-Sahelian West Africa, all shoot parts of legumes are typically harvested and removed from the field for human consumption (grain) and as fodder, the often reported cereal yield increase following legume cultivation may be largely due to rhizodeposition and decomposing root residues (Mayer et al. 2003). The objective of this study was to investigate the effects of an application of cereal versus legume root residues on the growth of sorghum seedlings and soil microbial biomass and activity indices.

5.3 Materials and methods

5.3.1 Soil characteristics and experimental layout

Samples from the upper 20 cm of two West African continuous cereal soils from Fada n’Gourma in Burkina Faso (11°59’N, 0°19’E) and Koukombo in Togo (10°17’N, 0°23’E) were collected in 1999, sieved (< 2 mm), air-dried and shipped to Germany, where the present experiments started in 2005. The soil from Fada was an Alfisol (Haplustalf) according to the USDA soil taxonomy, had a pH in water of 6.1 and contained 4.9 mg soil organic C g^{-1} soil as well as 17 µg and 42 µg water extractable P and K g^{-1} soil, respectively. The soil from Koukombo was an Ultisol (Plinthic Kanhaplustult), had a pH in water of 5.9 and contained 1.9 mg soil organic C g^{-1} soil as well as 11 µg and 16 µg water extractable P and K g^{-1} soil, respectively. Both soils were rewetted to 45 % water holding capacity (WHC = 10 % water g^{-1} dry soil) and pre-incubated for 20 days at 25 °C in the dark.

Root residues of six-week-old plants of the two legumes cowpea (***Vigna unguiculata*** Walp) and groundnut (***Arachis hypogaea*** L.), and of the three cereals pearl millet (***Pennisetum glaucum*** L.), maize (***Zea mays*** L.), and sorghum (***Sorghum bicolor*** Moench), were produced in pots on a West African fallow soil. This timing was chosen to produce sizeable amounts of roots in a reasonable amount of time. After analysing their nutrient concentrations (Table 3), 2 mg root residues g^{-1} soil of each crop were applied to each of the two soils, whereby four replicates were used. These treatments were compared to mineral P applied as KH_2PO_4 in an amount equivalent to the mean P applied through legume root residues (i.e. 0.28 µg P g^{-1} soil), an application of the legume-equivalent amount of mineral P plus N applied as NH_4NO_3 (i.e. 0.28 µg P + 38 µg N g^{-1} soil), and a non-amended control treatment.

Table 3: Nutrient concentrations of the root residues applied.

	C	N	C/N	P	K
	(mg g^{-1})			(mg g^{-1})	
Groundnut	380	18	21	0.13	4.9
Cowpea	350	20	17	0.15	8.0
Pearl millet	340	13	27	0.12	5.5
Maize	360	9	40	0.10	3.3
Sorghum	350	10	37	0.10	5.0

For the plant experiment, 2 kg of soil were placed in 2 L pots and planted with 3 days pre-germinated sorghum seedlings (Sorghum bicolor). The growth conditions in the climate chamber were set at 40 % humidity, 14 h light at 30 °C and 10 h darkness at 25 °C. Every day, water content was gravimetrically controlled and maintained by adding water. Also every day, shoot length measurements were conducted until harvest at day 59. Then, shoots and roots of all plants were separated, dried, weighed, ground and analysed for their concentrations of N, P, and K. Total numbers of nematodes were determined according to the Bearmann funnel method, whereby four aliquots of a suspension of 100 g soil were filtered through milk filters for 24 h (Decker 1969). Subsequently, an aliquot of 5 ml was taken and sub-samples analysed for nematode numbers using a microscope at a magnification of 100 x for counts and 400 x to separate phytoparasitic nematodes by their stylet from non-phytoparasitic ones.

For the incubation experiment, initially 880 g soil at 45 % WHC was placed for each treatment in quadruplicate into 3 l glass bottles and incubated for 189 days at 25 °C in the dark. At five sampling times (day 0, 7, 21, 63 and 189), 160 g moist soil of each treatment soil was taken for the determination of water content and microbial biomass indices (see below). The water content was maintained at 45 % by adding distilled water after each sampling day if necessary. Measurements of soil pH were conducted in a suspension of 10 g soil mixed with 25 ml of water. Carbon dioxide production was determined during the incubation using 5 ml 1 M NaOH solution for treatments without organic amendments throughout the experiment and initially 50 ml 1 M NaOH for the other treatments. In these treatments, 20 ml 0.5 M NaOH was used after 6 days of incubation, then 15 ml and finally 5 ml 0.5 M NaOH. The amount of NaOH solution was adjusted so that between 20 and 40 % was converted to Na_2CO_3. The remaining NaOH solution was back-titrated with 1 M HCl after addition of $BaCl_2$ solution.

5.3.2 Analytical procedures

Total N was analysed using an FP-328 N-analyser (LECO, St Joseph, MI, USA). Following combustion at 550 °C, the ash was dissolved in 20 ml HCl (32 %) and filled up to 100 ml after 12 h in the dark with bi-distilled water. Subsequently, total P was measured by photospectrometry using the vanadate-molybdate-method (Gericke and Kurmies 1952). Potassium was measured using a flame photometer.

Adenine nucleotides (ATP, ADP, and AMP) were extracted with an alkaline DMSO buffer according to Joergensen and Raubuch (2005), using a moist sample equivalent to 2 g oven-dry soil. After adding chloracetaldehyde and derivatisation, the adenine nucleotides were determined by ion-pair reversed-phase HPLC. The adenylate energy charge (AEC) was calculated on a molar basis as follows: (ATP + 0.5 ADP) / (ATP + ADP + AMP), representing the physiological alertness of soil microorganisms.

Microbial biomass C and microbial biomass N were determined by fumigation extraction (Brookes et al. 1985, Vance et al. 1987) using pre-extraction to remove high background levels of organic C and inorganic N (Widmer et al. 1989; Mueller et al. 1992). A sample of 25 g soil (on an oven-dry basis) was pre-extracted with 100 ml of 0.05 M K_2SO_4 on a horizontal shaker at 200 rev min^{-1} for 30 min and centrifuged. Fumigated and non-fumigated portions of 10 g moist soil were taken from the remaining soil and extracted with 40 ml 0.5 M K_2SO_4 as described above. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as EC / k_{EC}, where EC = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al. 1990) to account for the non-extractable part of microbial biomass C. Total N in the extracts was measured after combustion at 850 °C by a Dima-N (Dimatec) chemoluminescence detector. Microbial biomass N was calculated as EN / k_{EN}, where EN = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al. 1985) to account for the non-extractable part of microbial biomass N.

The fungal cell-membrane component ergosterol was extracted from 2 g soil with 100 ml ethanol (96 %) according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100 % methanol as the mobile phase and detected at a wavelength of 282 nm.

5.3.3 Statistical analysis

All results were tested for normal distribution of residuals using the Shapiro-Wilks test. To analyse the shoot dry matter data of the plant experiment, values of plant total N were transformed with log10, of plant total K with 1 / (x + 1) and total numbers of nematodes with square-root. Phosphorus data were analysed without transformation, even if they were not normally distributed as any transformation use did not improve normality of distribution. Data of microbial biomass N were transformed with square-root, those of ergosterol with Ln (logarithm to the base e), of ATP with 1 / (x + 1), of adenylates with square-root(x + 3) and of AEC with e-x. Site, amendment and time effects were tested using the GLM-Univariate procedure. Significance of treatment effects was analysed with the Tukey-HSD (honestly significant difference) post-hoc test. All statistical analyses were performed with SPSS 11.5 (Backhaus et al. 2006).

5.4 Results

5.4.1 Plant experiment

The application of mineral P alone and in combination with N increased plant height significantly, producing plants up to 35 cm taller on the Ultisol from Koukombo (Figure 5) and up to 28 cm taller on the Alfisol from Fada compared to the respective non-amended control (results not shown). Legume root residues increased plant height significantly compared to the control and the cereal root residue treatments, leading to up to 6 cm taller plants (Figure 5). On the Alfisol, shoot dry mass was always significantly higher than on the Ultisol (Figure 6): 42 % after application of mineral nutrients, 48 % after application of legume root residues, 83 % after application of cereal root residues and 70 % on the non-amended control. Highest shoot dry mass was observed after application of mineral P+N on the Alfisol and after mineral P alone on the Ultisol. The application of legume root residues led to small and non-significant increases in dry mass production compared to the non-amended control, whereas the application of cereal root residues led to a decline, regardless of their origin (millet, maize or sorghum).

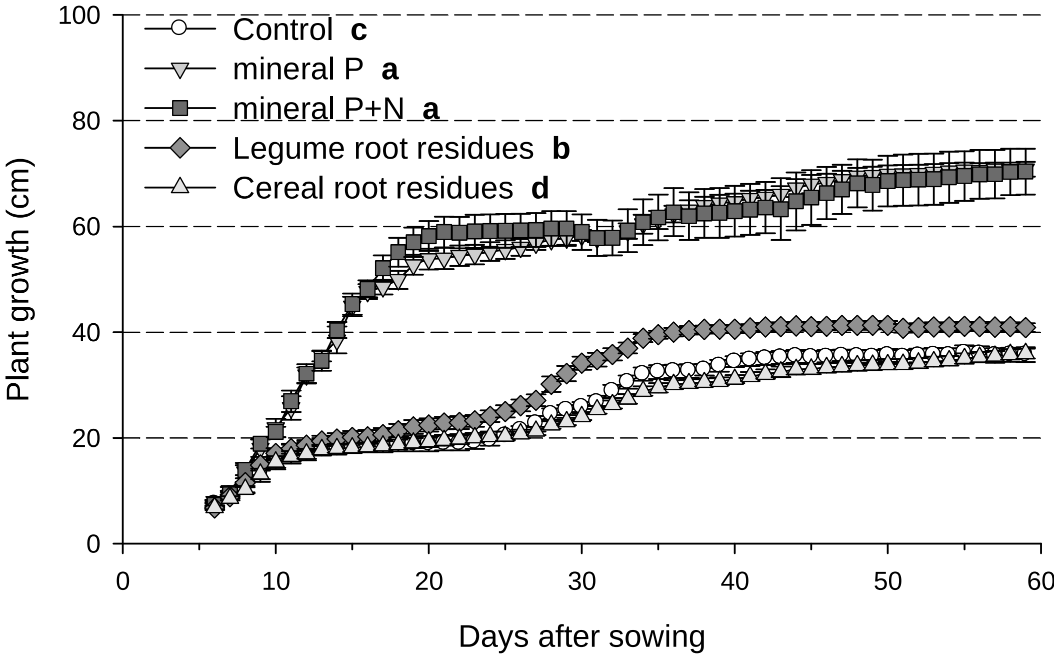

Figure 5: Plant height of sorghum on an Ultisol (Koukombo, Togo; CC-K) after application of root residues from legumes (means of cowpea and groundnut), cereals (means of pearl millet, maize und sorghum), compared to mineral P alone, mineral P+N and a non-amended control; bars represent ±one standard error of mean; treatment means with different letters are significantly different at the end of the pot experiment (P < 0.05, Tukey-HSD).

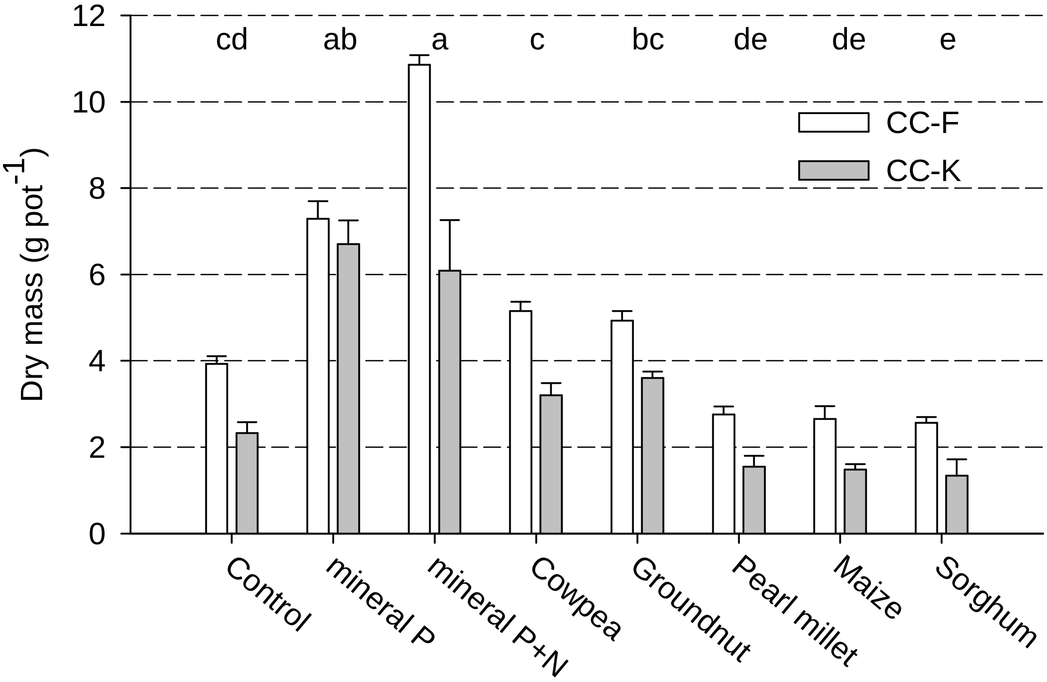

Figure 6: Shoot dry matter of sorghum grown on an Alfisol (Fada, Burkina Faso; CC-F) and an Ultisol (Koukombo, Togo; CC-K) after application of root residues from cowpea, groundnut, pearl millet, maize and sorghum, compared to mineral P alone, mineral P+N and a non-amended control; bars represent ±one standard error of mean; treatment means over both soils with different letters are significantly different (P < 0.05, Tukey-HSD).

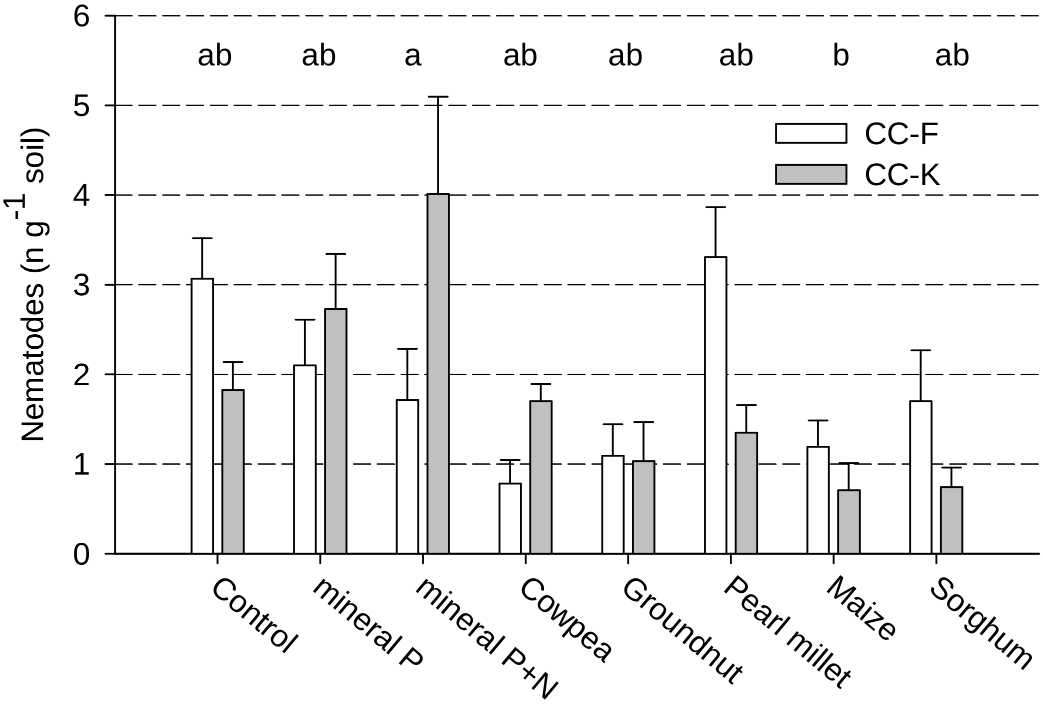

Figure 7: Numbers of nematodes in an Alfisol (Fada, Burkina Faso) and an Ultisol (Koukombo, Togo) planted with sorghum after application of root residues from cowpea, groundnut, pearl millet, maize and sorghum, compared to mineral P alone, mineral P+N and a non-amended control; bars represent ±one standard error of mean; treatment means over both soils with different letters are significantly different ($P < 0.05$, Tukey-HSD).

Concentrations of N, P, and K in harvested sorghum shoots were slightly higher on the Ultisol, except for K where concentrations were higher in plants grown on the Alfisol (Table 4). Nitrogen concentrations were increased by 63 % after application of mineral P+N, increased by 14 % after application of legume root residues and decreased by 16 % after application of mineral P alone in comparison with the non-amended control soils. Both treatments with applicaton of mineral nutrients highly increased P concentrations (7.2 mg g^{-1}) compared to the other treatments, with an average P concentration of 0.6 mg g^{-1}. Also the K concentration was significantly increased, especially by application of mineral P alone with a 200 % increase, but also with application of mineral P+N with only 60 % increase. Significant soil × amendment interactions indicate soil-specific growth reaction and nutrient acquisition of the sorghum plants.

The application of root residues reduced in most cases the total number of nematodes to an average of 1.4 g^{-1} soil, compared to 2.6 g^{-1} soil determined in the control soils and after application of mineral nutrients (Figure 7). However, these differences were only significant for mineral P+N with a mean of 2.9 g^{-1} soil and maize root residues with a mean of 1.0 g^{-1} soil.

Table 4: Soil- and amendment-specific mean nutrient concentrations of sorghum shoots grown on an Alfisol (Fada, Burkina Faso; CC-F) and an Ultisol (Koukombo, Togo; CC-K) mixed with legume and cereal root residues compared to mineral P alone, mineral P+N, and a non-amended control; probability values for a two-way ANOVA.

	N	P	K
	in mg g^{-1}		
Soil			
Alfisol (CC-F)	6.2	1.9	12.4
Ultisol (CC-K)	7.4	2.5	10.2
Amendment			
Control	6.2	0.6	8.8
Mineral P	5.2	7.6	25.8
Mineral P+N	10.1	6.9	13.4
Legume root residues	7.1	0.5	8.1
Cereal root residues	6.3	0.6	8.7
Probability values			
Soil	<0.01	<0.01	<0.01
Amendment	<0.01	<0.01	<0.01
Soil × amendment	<0.01	<0.01	0.03
CV (± %)	8.3	27.7	14.7

CV = mean coefficient of variation between replicate pots

5.4.2 Incubation experiment

Significant site effects were found for pH and all microbial indices (Table 5). Contents of microbial biomass C, microbial biomass N and ergosterol were 75 to 100 % higher in the Alfisol, while ATP was only 36 % higher compared to the Ultisol. For this reason, the ATP-to-microbial biomass C ratio was 33 % higher in the Ultisol (8.3 µmol ATP g^{-1} microbial biomass C) than in the Alfisol (6.2 µmol g^{-1}). In contrast, the mean microbial biomass C/N ratio of 12 and the mean ergosterol-to-microbial biomass C of 0.23 % did not differ significantly between the two soils (results not shown). Also, the average cumulative respiration was similar in both soils for the control and mineral P treatment, with an average CO_2 evolution of 165 µg g^{-1} (Figure 8).

All amendments led to pH increases even if this effect was only significant for cereal root residues compared to the non-amended control (Table 5). From initially 6.1, the pH decreased gradually over time to 5.8 at harvest. Organic amendments increased ergosterol concentrations by up to 150 %, microbial biomass C and microbial biomass N by up to 50 %, but ATP only by up to 20 % compared to the non-amended control and mineral P treatments. Significant soil × amendment interactions indicate soil-specific reactions solely of microbial biomass C and not of the other microbial biomass indices.

Table 5: Soil-, amendment-, and sampling day-specific means of pH, contents of microbial biomass C, microbial biomass N, and ergosterol as well as the ratio of ergosterol-to-microbial biomass C in an Alfisol (Fada, Burkina Faso; CC-F) and an Ultisol (Koukombo, Togo; CC-K) mixed with legume and cereal root residues compared to mineral P alone and a non-amended control; probability values for a two-way ANOVA for repeated measures.

	pH	ATP	Microbial biomass C	Microbial biomass N	Ergosterol	Ergosterol/ microbial biomass C
	(H_2O)	in nmol g^{-1}	in µg g^{-1} soil			in %
Soil						
Alfisol	6.0	0.53	88	7.5	0.13	0.14
Ultisol	5.9	0.39	49	4.3	0.06	0.13
Amendment						
Control	5.8	0.42	55	4.4	0.05	0.09
Mineral P	6.0	0.38	53	4.4	0.05	0.09
Legume roots	5.9	0.46	75	6.4	0.12	0.15
Cereal roots	6.1	0.50	73	6.6	0.11	0.16
Sampling day						
0	6.1	0.44	60	4.2	0.08	0.12
7	6.2	0.52	69	5.8	0.13	0.21
21	5.9	0.59	72	7.0	0.09	0.14
63	5.9	0.37	90	7.7	0.12	0.13
189	5.8	0.39	49	4.9	0.06	0.13
Probability values						
Soil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Amendment	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Sampling day	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Soil × amendment	0.02	0.81	0.01	0.33	0.78	<0.01
Soil × day	0.01	<0.01	<0.01	<0.01	0.03	<0.01
Amendment × day	<0.01	<0.01	0.04	0.13	<0.01	<0.01
CV (± %)	1.9	24	29	35	39	

CV = mean coefficient of variation between replicate incubations

While ergosterol concentrations varied over the experimental period without clear trends, highest contents of ATP were measured at day 21 and highest contents of microbial biomass C and microbial biomass N were determined at day 63 of the incubation followed by a decline. At day 189, ATP, microbial biomass C, and ergosterol were below the initial values at day 0. The mean AEC of 0.71 over all sampling days and treatments and also the mean microbial biomass C/N ratio of 12 were only slightly affected by the application of root residues (results not shown). In contrast, 75 % higher ergosterol-to-microbial biomass C ratios were found after application of root residues compared to the control and the mineral P treatments (Table 5). Significant soil × sampling day and amendment × sampling day interactions were found for soil pH and all microbial biomass indices, indicating complex soil-specific and amendment-specific reactions of soil microorganisms during the decomposition of the organic amendments. After application of the legume root residues, cumulative CO_2 production was similar in both soils, with an average of 370 µg CO_2-C g^{-1} (Figure 8). After application of the cereal root residues, cumulative CO_2 production was higher in the Alfisol (530 µg g^{-1}) than in the Ultisol (445 µg g^{-1}).

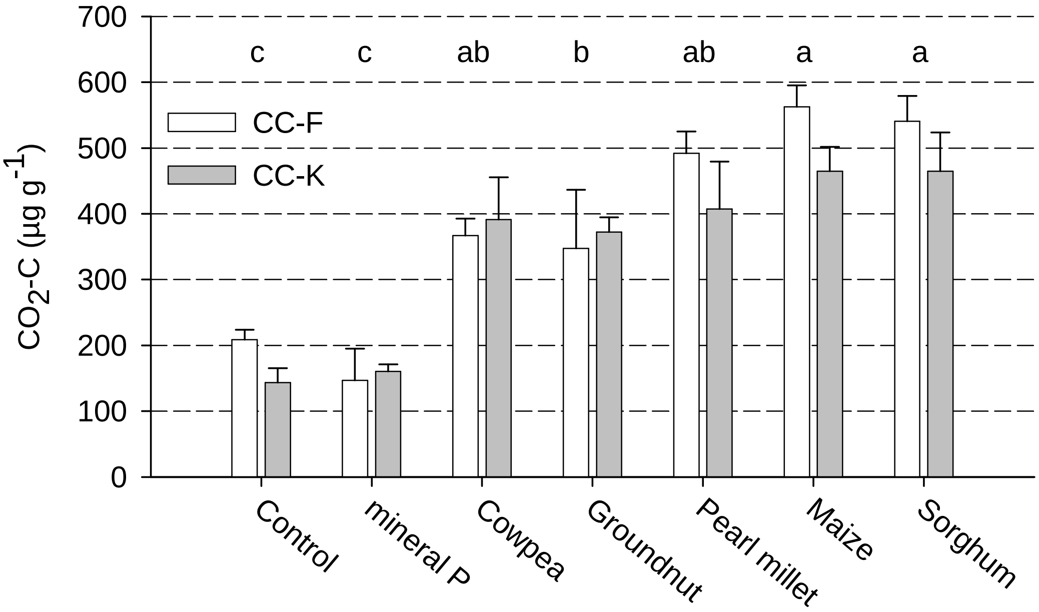

Figure 8: Cumulative respiration over 189 days incubation at 25°C in an Alfisol (Fada, Burkina Faso; CC-F) and an Ultisol (Koukombo, Togo; CC-K) mixed with root residues from cowpea, groundnut, pearl millet, maize and sorghum, compared to mineral P alone and a non-amended control; bars represent ±one standard error of mean; means over both soils with different letters are significantly different ($P < 0.05$, Tukey-HSD).

5.5 Discussion

The application of mineral fertilizers (P and N+P) led to immediate increases in plant growth, while growth increasing effects of legume root residues became significant only after 18 days (Figure 5). This is in agreement with results of Ladd et al. (1981) and Azam et al. (1985) who concluded that legume residues did not serve as an immediate N source for plants, but contributed to an N pool in the soil with a long-term release. The application of cereal roots had no effect on plant height (Figure 5), but reduced shoot dry mass production (Figure 6), which reflects an immobilization of N or P by the microbial decomposer community. This can be explained by the wider C/N ratio found in the cereal root residues (average 35) compared to legume root residues (average 19) (Powlson et al. 1999). However, the positive effects of legume root residues are also smaller than those usually observed in the literature (Paré et al. 2000; Glasener et al. 2002; Da Varennes et al. 2007), presumably caused by the low overall fertility of the two West-African soils (Bationo et al. 1992, 2007). This is especially true for the Ultisol from Koukombo (Togo), where sorghum plants had higher N and P concentrations (Table 4), but smaller dry matter than those grown on the Alfisol from Fada (Burkina Faso), and where the application of N did not result in further growth promotion in comparison to mineral P alone (Figure 6). This suggests that nutrients other than N and P or other growth conditions, e.g. mycorrhizal infection (Tarafdar and Marschner 1993; Marschner et al. 2005), have limited growth of sorghum seedlings on the Ultisol.

In this study the counted nematodes were predominantly free living species and the numbers of plant-parasitic nematodes were negligible, contrasting the field data of the Bagayoko et al. (2000), where significant numbers (0.8 to 4.6 g^{-1} soil) of plant-parasitic nematodes were found. This is probably caused by long-term desiccation and absence of host plants during storage. Free living nematodes that feed on soil bacteria or fungi play an important role in the nutrient cycling and can therefore have strong effects on plant growth (Yeates and Bongers 1999). It is an interesting feature of the present results that the organic amendments reduced the number of nematodes that feed on soil bacteria or fungi (Figure 7). This might be an additional reason that the soil microbial biomass was increased after the application of root residues (Table 5).

The measured pH changes during the incubation experiment, an initial rapid increase followed by a slow decline (Table 5), corresponds to the pattern repeatedly observed by others after amendment of organic residues (Tang et al. 1996; Yan et al. 1996; Yan and Schubert 2000). Tang and Yu (1999) explained the initially observed pH increase with decarboxylation of organic anions and ammonification, the subsequent decrease in pH with nitrification of mineralised residue N over the 100-day incubation period.

The higher content of microbial biomass in the Alfisol than in the Ultisol (Table 5) is in line with an earlier study on the effects of rewetting using the same soils (Formowitz et al. 2007), indicating higher soil fertility in the Aflisol. The level of microbial biomass in these two soils is markedly lower than the world wide average of 330 µg g^{-1} soil proposed by Wardle (1998). It is also at the lower end of the microbial biomass C range determined in West Africa, where mean values of 70 µg g^{-1} soil (Bilgo et al. 2007) 120 µg g^{-1} soil (Wick et al. 1998), and 150 µg g^{-1} soil (Koné et al. 2008) have been observed, or other semi-arid regions of the world (Torres et al. 2005; Bastida et al. 2006, 2008; Khan and Joergensen 2006). The large microbial biomass C/N ratio of 12 is also in line with other observations in semi-arid subtropical (Khan and Joergensen 2006) and humid tropical soils (Dinesh et al. 2003; Salamanca et al. 2006). The reasons for the relatively large microbial biomass C/N ratios in these subtropical and tropical soils compared to arable soils from humid climates are still not fully understood (Joergensen and Emmerling 2006).

A sometimes stated explanation for large microbial biomass C/N ratios is the possibility of fungal dominance in soils (Dilly et al. 2003). However, the ratio of the fungal cell-membrane component ergosterol to microbial biomass C, which is an indicator for living saprotrophic fungi (Joergensen and Wichern 2009), is relatively low in comparison with arable soils from temperate humid climates (Djajakirana et al. 1996). The fraction of microbial residues on the basis of fungal glucosamine and bacterial muramic acid contains 74 % fungal residues in the Alfisol compared to 79 % in the Ultisol, which is close to the world wide average of 75 % for arable soils (Joergensen and Wichern 2009). Consequently, a shift towards fungi is an unlikely explanation for the observed wide microbial biomass C/N ratios. In some cases, P limitation has been demonstrated as a possible explanation (Salamanca et al. 2006). However, the application of mineral P had no effect on the microbial biomass C/N ratio in the present incubation experiment, so that other reasons remain to be found. Also the ATP-to-microbial biomass C ratio, which is usually very low in P limited soils (Dinesh et al. 2003; Salamanca et al. 2006), is within the range obtained by the present methodological approach (Dyckmans et al. 2003).

The mean microbial biomass C to soil organic C ratio was 1.8 % in the Alfisol soil and 2.6 % in the Ultisol, suggesting a better availability of the organic matter to the soil microbial community in the latter soil (Anderson and Domsch 1989). This is in line with the observation that the basal respiration rate was identical in both soils, although the Ultisol had a roughly 60 % lower soil organic C content and a roughly 40 % lower microbial biomass C content than the Alfisol. This indicates that the soil microbial community of the Ultisol is less efficient in substrate use, i.e. more energy is necessary to match the catabolic demand of its biomass (Anderson and Domsch 1990). High organic matter availability and low substrate use efficiency apparently led to the very low soil organic C level in the Ultisol.

Assuming that the addition of the root residues did not affect the decomposition of soil organic matter, around 25 % of the added legume root residue C and 40 % of the added cereal root residue C were respired as CO_2. The mineralization rates of the cereal root residues are in line with the data reported from other incubation experiments (Gorissen and Cotrufo 2000; Lu et al. 2003; Johnson et al. 2007), but those of the legume root residues were considerably lower (Paré et al. 2000). This led to the unexpected result that less legume root residues were mineralized to CO_2 compared to cereal root residues. However, a similar, but even more pronounced difference in the decomposition of wheat root residues (41 % loss) and pea root residues (19 % loss) has been observed after an eleven-month field experiment (Soon and Arshad 2002). On the other hand, the lower CO_2 production from the legume root residues does not necessarily reflect a lower decomposition rate. The CO_2 production does not fully assess the decomposition of root residues, because it does not take the formation of non-biomass microbial residues into account, which might form a large fraction during the decomposition of easily available legume residues (Jensen 1996; Mayer et al. 2003). Additionally, the amendment of N-poor cereal root residues might have caused a positive priming effect to match the N-demand of decomposing microbial community (Kuzyakov et al. 2000).

Another characteristic feature is the significantly lower decomposition rate of the cereal root residues in the Ultisol, although it contained a higher percentage of fungal residue C. However, the ergosterol content was significantly lower in the Ultisol, suggesting a lower percentage of saprotrophic fungi in this soil (Joergensen and Wichern 2009). On the other hand, the difference in CO_2 production between the two soils (Figure 8) might be simply the result of differences in priming action after amendment of the cereal root residues, as explained above.

5.6 Conclusions

Sorghum growth reacted mainly to the amendment of mineral P in the Ultisol (Koukombo, Togo) and the availability of P and N in the Alfisol (Fada, Burkina Faso) added by mineral fertilizer. The application of legume root residues had small effects and that of cereal root residues even had negative effects on shoot dry mass production. Treatment effects did not vary significantly between the two legume species (groundnut and cowpea) or between the three cereal species (millet, maize, and sorghum) used for the two experiments. In contrast to the growth of sorghum seedlings, the microbial community did not react to the application of mineral fertilizers, but only to the application of energy with organic amendments. However, the relative difference in shoot dry mass between the two soils was much smaller than the differences in the contents of soil organic matter and microbial biomass. The repeated C-input by legume root residues might have contributed to these differences in soil fertility.

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6 Assessing P availability in continuous cereal soil influenced by mineral and organic amendments using an isotope dilution method

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6.1 Abstract

In West African agricultural production systems almost all foliar biomass is harvested and used as fodder. Thus, cereal yield increasing effects of legume rotations may be mainly based on rhizodeposition and root turnover. The effect of legume root residues on available P pools was examined by using continuous cereal (CC) soils from Fada (Burkina Faso) and Koukombo (Togo) in a greenhouse trial. Treatments of this experiment consisted of legume root residues (groundnut), cereal root residues (maize or sorghum), mineral P and an unamended control. After harvest soil samples were taken and send to ETH Zürich for ^{33}P analyses. With R/r_1 ratios ranging between 1.1 and 2.1 the buffering capacity was found to be low in all treatments. Soils with a calculated ***n*** value close to 0 indicate a good P status, which was only found with application of mineral P showing an ***n*** value of 0.04. The same was found for total P with highest amounts in the mineral P treatment (229 - 246 mg P kg^{-1} soil) while the control and organic treatments ranged between 39 - 47 mg P kg^{-1} soil. The amount of P ions in the soil solution (C_P) and directly available P (E_{1min}) followed the same pattern with higher values found in the mineral P treatment. While the application of groundnut root residues led to E_t ($E_{1min-1d}$ and E_{1d-3m}) increases with time from 8 to 73 mg P kg^{-1} soil (Fada) and from 1 to 6 mg P kg^{-1} soil (Koukombo), the opposite was observed for the mineral P treatment (Fada = from 23 to 18 mg P kg^{-1} soil; Koukombo = from 21 to16 mg P kg^{-1} soil). This may indicate that P becomes slowly available from decomposing root residues, while applied mineral P is taken up by plants or fixed in the soil. Due to the fact that plant growth in the greenhouse trial reacted mainly to the application of mineral P, but the microorganisms reacted solely to the organic amendments, the combination of both seems to be the best measure for a sustainable increase of crop production in West Africa.

6.2 Introduction

To enhance the productivity of agricultural production system in West Africa integrated soil fertility improvement strategies, including legume rotations, were often advocated as they can lead to large yield increases of the following cereals (Bagayoko 2000; Buerkert et al. 2000a+b). Higher nutrient availability, higher early mycorrhizal infection, decreased numbers of nematodes and higher soil pH after legume cultivation were reported to be major factors enhancing cereal production (Buerkert et al. 2000b; Alvey et al. 2001; Bilgo et al. 2007). Under controlled conditions a different microbial community structure, higher microbial biomass C and N, higher ergosterol concentration, and a higher microbial activity were found in rotation soils compared to continuous cereal ones (Marschner et al. 2004; Formowitz et al. 2007). Since most of the above ground biomass in West Africa is used for human consumption or fodder (Bationo et al. 2003), the yield increasing effects of legume rotations might largely be based on rhizodeposition and root residues that are decomposed by the microbial community (Mayer et al. 2003). In an experiment with applied cereal or legume root residues compared to mineral P and N fertilization, soil microorganisms reacted mainly to the organic amendments, while plant growth was strongly increased by mineral P and N input (Formowitz et al. 2009; chapter 5). Mineral P fertilizers, crop residue application, and legume cultivation have all been shown to enhance P availability on the predominantly sandy West African soils (Alvey et al. 2001, Buerkert et al. 2001a; Sinaj et al. 2001).

Several studies assessed mobilization of P ions comparing different mineral P fertilization strategies (Salcedo et al. 1991; Fardeau and Zapata 2002) or cropping systems and organic amendments (Sinaj et al. 2001; Buehler et al. 2003) using the isotopic exchange kinetics technique for P ions (Fardeau 1996). When radioactive $^{33}PO_4$ ions are added to a soil-water suspension in steady state, an isotopic exchange takes place between $^{33}PO_4$ ions in solution and stable $^{31}PO_4$ ions on the solid phase. Measuring the ^{33}P to ^{31}P ratio in the solution allows calculating the isotopically exchangeable P (that is plant available P) for any given time of exchange. The ratio between the radioactivity introduced in the system and the radioactivity remaining in solution after one minute of exchange (R_0/r_1) is well correlated with soil P-fixing capacity (Frossard et al. 1993).

By using the isotopic dilution method this study aimed at assessing mobile P pools available for plant uptake after adding mineral or organic P sources. This will deliver more insights whether root residues staying in the soil and decomposing can partly explain the cereal yield enhancing legume rotation effects on West African soils.

6.3 Materials and methods

6.3.1 Greenhouse trial and soil sampling

Samples from the 0 - 0.2 m layer of two West African continuous cereal (CC) soils from Fada n'Gourma in Burkina Faso (11°59'N, 0°19'E) and Koukombo in Togo (10°17'N, 0°23'E) were collected in 1999, sieved (< 2 mm), air-dried, shipped to Germany and stored in a dry place at 10 - 20 °C, where the present experiments started in 2005. Before the greenhouse trial started, samples of about 50 g air dried continuous cereal (CC) and legume rotation (CL) soils were vacuum packed and send to ETH Zürich for ^{33}P analyses.

In the greenhouse experiment with four replicates (Formowitz et al. 2009; chapter 5) 2 mg root residues g^{-1} soil of six-week-old groundnut, sorghum or maize plants were applied to both soils that had been incubated at 45 % water holding capacity for 24 hours. These treatments were complemented by an application of mineral P as KH_2PO_4 at an amount equivalent to the mean P applied with legume root residues (that is 0.28 µg P g^{-1}) soil, and an un-amended control. On pots ***Sorghum bicolor*** was grown at 40 % air humidity, 14 h light at 30 °C and 10 h darkness at 25 °C. After 59 days of growth, shoots and roots were harvested. Samples of about 50 g were taken from the remaining soil, carefully dried at 60 °C, vacuum packed, and also send to ETH Zürich for ^{33}P analysis.

6.3.2 Determination of isotopically exchangeable P

To measure total P concentration, 1 g of 2 mm sieved soil was incinerated in the muffle furnace at 550°C for 1 h. The ashed soil samples were transferred into plastic bottles and mixed with 50 ml of 0.5 M H_2SO_4. After 16 hours shaking end over end, the solution was filtered at 0.2 µm and total P content was determined photometrically using the malachite green method (Ohno and Zibilsky 1991). For ^{33}P analyses according to Bühler et al. (2003), 10 g of 2 mm sieved soil were mixed with 99 ml of deionized water and shaken for 16 hours end over end. After equilibration, the suspension was continuously stirred at 350 rpm on a stirring plate. Subsequently, 1 ml of ^{33}P solution was added and 1, 10, 30 and 60 minutes thereafter 5 to 10 ml of suspension were extracted with a plastic syringe, filtered at 0.2 µm and collected in scintillation vials. Radioactivity (r_t) was measured in aliquots of the solution, and the ^{31}P concentration (C_P) was photometrically determined using the malachite green method (Ohno and Zibilsky 1991). The radioactivity in 1 ml of the ^{33}P solution was also measured to assess the amount of radioactivity added at t = 0 (R_0). After application of carrier-free ^{33}P-orthophosphate to the soil suspension, two models to determine decreasing radioactivity over time were used (Fardeau and Jappe 1980; Frossard et al. 1994; Salcedo et al. 1991).

Model 1 (simplified Fardeau model): $r_t/R_0 = r_1/R_0\ (t + (r_1/R_0)^{(1/n)})^{-n}$

Model 2 (power model): $r_t/R_0 = r_1/R_0{*}t^{-n}$

After that, data was compared using the statistical program STATRAPH. The r_t/R_0, ***n***, asymptotic standard deviation and R^2 were estimated and seemed not to differ much between the two models. For this reason, only results from the simplified Fardeau model are presented in this thesis. Furthermore, the ratio between the radioactivity introduced into the system and the radioactivity remaining in solution after one minute of exchange (R_0/r_1) correlated well with the soils' P-fixing capacity. Assuming that the orthophosphate concentration in the soil is constant, a decrease in radioactivity is due to the exchange of added ^{33}P and soluble ^{31}P from soil colloids, calculated with the equation: $E_t = 10C_p{*}R_0/r_t$, where the factor 10 results from the soil to water ratio of 10 g soil in 99 ml of deionized water.

6.3.3 Statistical analysis

All results from the analysis of the soil samples of the greenhouse trial were tested for normal distribution of residuals using the Shapiro-Wilk test. Treatment effects were tested using GLM-Univariate and means were separated using Tukey's HSD (honestly significant difference) at $P < 0.05$. All statistical analyses were performed with SAS 9.2.

6.4 Results and discussion

Concentrations of total P (P_{tot}) in soils taken from the storage containers were higher in Fada than in Koukombo soils and higher in CL soils (Fada = 59 mg kg^{-1}; Koukombo = 42 mg kg^{-1}) compared to CC ones (Fada = 42 mg kg^{-1}; Koukombo = 39 mg kg^{-1}) of both sites. Contrarily, the amount of soluble P in the soil solution (C_P) was in the same range of 0.13 mg kg^{-1} for all treatments, except for the CL soil from Koukombo with 0.19 mg kg^{-1}. Total P concentrations in the greenhouse trial did not show any effects of organic amendments compared to the control, ranging between 39 mg kg^{-1} at Koukombo and 46 to 47 mg kg^{-1} at Fada, thus staying below values measured in the rotation soils. Only the mineral P application increased total P concentrations significantly in soils of both locations to 229 and 246 mg kg^{-1} soil from Koukombo and Fada, respectively (Table 6). Measured C_p values followed the same order ranging between 0.09 and 0.12 mg P kg^{-1} for the control and organic amendments, while they reached around 62 mg P kg^{-1} in the mineral P treatment of both soils.

According to Frossard et al. (1993) soils with R/r_1 ratios below 2.5 are generally of very low buffering capacity. Sinaj et al. (2001) described strong site differences in West African soils, while R/r_1 ratios at Gaya (Niger) ranged between 4.0 and 8.0, soils from Goberi (Niger), Kara Bedji (Niger) and Fada (Burkina Faso) had ratios between 1.0 and 2.0. Our R/r_1 ratios ranging between 1.1 and 2.1 were comparable to those of the latter three sites. Only the rotation soil from Fada had with 2.7 a slightly higher buffering capacity. While the organic amendments did not affect this factor as compared to the control, mineral P application tended to decrease the ratio. The same was true for calculated ***n*** values, which were with 0.04 lowest in the mineral P treatment and with 0.50 highest for applied groundnut root residues in the soil from Fada in the greenhouse trial (Table 6). According to Fardeau et al. (1991) soils with ***n*** values close to 0.5 are fixing large amounts of applied mineral P, while soils with ***n*** values close to 0 are already well saturated with P. This would indicate that only the mineral P treatment led to a satisfactory P status for plant uptake, whereas most of the organically applied P might be fixed.

Table 6: Isotopic exchange kinetic parameters according to the simplified Fardeau Model (Frossard et al. 1994) in continuous cereal (CC) and cereal legume rotation (CL) soils from Fada (Burkina Faso) and Koukombo (Togo) taken directly from the storage, and CC soils from both sites used in the greenhouse trial amended with root residues of maize, sorghum, or groundnut, mineral P, or without amendments (control). Values with different letters per site are significantly different at $P < 0.05$ (Tukey HSD).

Site	System and amendments	R_o/r_1	***n***	P_{tot}	C_P	E_{1min}	$E_{1min-1d}$	E_{1d-3m}
				in mg P kg^{-1} soil				
Stored soil								
Fada	CC	1.93	0.27	42	0.13	0.25	1.59	4.47
	CL	2.70	0.28	59	0.14	0.37	2.36	6.70
Koukombo	CC	1.59	0.22	39	0.13	0.20	0.77	1.60
	CL	1.41	0.19	42	0.19	0.27	0.83	1.52
Soils from the greenhouse trial with stored soil								
Fada	CC control	1.92^a	0.39^{ab}	46^b	0.10^b	0.20^b	3.29^{ab}	17.10^a
	CC sorghum	2.05^a	0.35^b	47^b	0.12^b	0.25^b	2.99^b	14.03^a
	CC groundnut	2.10^a	0.50^a	47^b	0.11^b	0.25^b	8.36^{ab}	73.44^a
	CC mineral P	1.07^b	0.04^c	246^a	60.78^a	65.21^a	22.73^a	17.89^a
Koukombo	CC control	1.62^a	0.26^b	39^b	0.10^b	0.17^b	0.89^b	2.26^b
	CC maize	1.62^a	0.34^a	39^b	0.09^b	0.14^b	1.63^b	7.53^b
	CC groundnut	1.68^a	0.33^{ab}	39^b	0.09^b	0.15^b	1.49^b	5.72^b
	CC mineral P	1.05^b	0.04^c	229^a	62.56^a	65.74^a	20.84^a	16.14^a

The E_{1min} values represent P ions which are directly water soluble without initial chemical transformation (Frossard and Sinaj 1997). Soils with E_{1min} below 5 mg P kg^{-1} are poor in available soil P (Fardeau et al. 1995). Except for the mineral P treatment (E_{1min} = 65 mg P kg^{-1}), E_{1min} values in the present study ranged between 0.14 and 0.20 mg P kg^{-1}. This is even below values found in six acid soils by Owusu-Bennoah et al. (2002), who stated that the isotopically exchangeable P was generally very low in those soils. Again, all E values were higher for Fada than for Koukombo indicating the generally higher P availability in the soil from Burkina Faso. The $E_{1min-1d}$ values represent the isotopically exchangeable P from 1 minute to 1 day which is equivalent to the period of P uptake during the lifetime of a single root, whereas the exchangeable P in a time equivalent to the active P uptake by the whole root system of annual crops is characterized by E_{1d-3m} values (Sinaj 2001). While the $E_{1min-1d}$ and E_{1d-3m} values decreased for the mineral P treatment in both soils, the exchangeable P in the organic treatments increased with time (Table 6). This can be P slowly released from the plant debris when it is decomposed by soil microorganisms, while the applied mineral P is taken up by plants or fixed in the soil. The slightly higher P concentrations applied with legume root residues (Formowitz et al. 2009; chapter 5) might be the reason for the strong increases in exchangeable P (E_{1min} to $E_{1min-1d}$ and E_{1d-3m}) shown for the soil from Fada mixed with groundnut root residues. Although slight yield increases were found with applied legume root residues, no differences of P concentrations in shoots and roots between organic amendments and the control were noted (Formowitz et al. 2009; chapter 5.4.1).

However, the crop residue induced decrease of the intensity factor (C_P) and the isotopically exchangeable P within 1 minute (E_{1min}) under field conditions as reported by Sinaj et al. (2001) were not found under the controlled conditions of this study. Solely the mineral P treatment caused increases of C_p and E_{1min} in both studies, thereby leading to higher dry matter yields and higher P concentrations in harvested biomass, but only when applied as single superphosphate (SSP) in the study of Sinaj et al. (2001). This confirms the importance and effectiveness of such P application, which has been repeatedly reported to strongly limit plant growth on West African soils (Buerkert et al. 2001b, Bationo et al. 2003). Continuously applied crop residues were found to reduce P sorption to a larger extent than any application of mineral P (Bationo and Buerkert 2000). Furthermore, in contrast to crops soil microorganisms did not react to the application of mineral P, but strongly to C additions from root residues (Formowitz et al. 2009; chapter 5.4.2). Assuming that biological factors play a major role for yield increases following legume rotations, the combination of mineral and organic amendments seems to be most effective to sustainably enhance crop production on poorly buffered West African soils.

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7 Plant growth of maize and sorghum on continuous cereal and legume rotation soils with different N and P application rates

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7.1 Abstract

To determine whether the yield increases of a cereal following a legume rotation are mainly caused by increased P and N availability, differences in those nutrient concentrations between the respective cereal legume rotation (CL) and continuous cereal (CC) soils from Fada (Burkina Faso) and Koukombo (Togo) were calculated and taken as application rates to both soils a pot experiment conducted under controlled conditions. After the application of five times the difference of P, N and both nutrients as mineral fertilizers to continuous cereal soil and four times to rotation soils, sorghum (***Sorghum bicolour*** Moench) was planted on Fada soils and maize (***Zea mays*** L.) on Koukombo soils.

Soil amendments increased plant growth and biomass production by up to 32 % on Fada soils and by up to 15 % on Koukombo soils in the following order mineral P+N > mineral P > control > mineral N, with significantly higher values on rotation soils compared to the respective treatments on CC-soils. Plants grown on CC-soils showed a 7 % higher total root length, 26 % higher P and 4 % higher K concentrations. On rotation soils mycorrhizal infection and N concentrations were increased by 46 % and 10 %, respectively and numbers of nematodes almost reduced by half.

In conclusion, increased N and P availability are not the only reasons for yield increases of cereals following field-planted leguminous crop. Soil biological factors have also to be taken into account such as increased mycorrhizal infection and decreased numbers of nematodes.

Keywords: legume rotations, mineral amendments, mycorrhizal infection, nematodes

7.2 Introduction

In most agricultural systems plant production has to cope with soil degradation and losses of soil fertility as a consequence of increasing population pressure whose effects have to be countered with an increased use of soil amendments. However, cash-poor smallholders are often unable to purchase mineral fertilizers (Sanginga 2003). In this context the use of legumes in rotation systems plays a major role (Wani et al. 1995; Oikeh et al. 1998). N_2 fixing leguminous crops generally take up less amounts of inorganic N from the soil than cereals, which is called the N-sparing effect (Chalk 1998), even if for grain legumes N requirements and harvest removal may exceed biological N_2 fixation (Peoples and Craswell 1992). Legumes are also well known to be able to increase P availability in the rhizosphere through an excretion of organic acids, enhanced early mycorrhizal infection and an increased phosphatase activity in the rhizosphere (Alvey et al. 2001; Buerkert et al. 2001a). For West African soils previous studies indicated that changes in the amount and activity of soil microorganisms can play an important role in cereal yield increases of cowpea and groundnut rotations (Buerkert et al. 2000; Marschner et al. 2004; Formowitz et al. 2007).

This study therefore aimed at investigating if the increases in N and P availability after a leguminous crop are the main reasons for yield increases of a following cereal on West African soils. To this end the differences in P and N concentrations between continuous cereal and cereal legume rotation soils from two West African locations were taken as application rates for mineral P and N supply.

7.3 Materials and Methods

7.3.1 Soil and experimental layout

Soil samples from the upper 20 cm of two West African continuous cereal (CC) and rotation (CL) soils from Fada in Burkina Faso (11°59’N, 0°19’E; F) and Koukombo in Togo (10°17’N, 0°23’E; K) were collected, shipped to Germany and stored at 15 - 20 °C in the dark. Chemical analysis showed that pH, C, N_{min}, P (P-Bray) and K concentrations were higher in soils from Fada (Table 7).

Table 7: Soil pH, C_{org}, N_{min} and P-Bray and K of continuous cereal (CC) and cereal legume rotation (CL) soils from Fada (Burkina Faso; CC-F and CL-F) and Koukombo (Togo; CC-K and CL-K).

	pH	C	N_{min}	P-Bray	K
		(mg g^{-1})		(μg g^{-1})	
CC-F	6.1	4.9	16	17	42
CL-F	6.3	2.3	17	23	80
CC-K	5.9	1.9	6	11	16
CL-K	6.2	1.7	10	15	30

The differences in P-Bray and N_{min} concentrations between continuous cereal and rotation soils of each site were calculated and used to compute P and N application rates.

The treatments for continuous cereal soils consisted of 5 times and for rotation soils of 4 times the difference in P (Fada = 6.0 mg kg^{-1}; Koukombo = 3.3 mg kg^{-1}), the difference in N (Fada = 1.6 mg kg^{-1}; Koukombo = 3.7 mg kg^{-1}), and the combination of both nutrients. These treatments allowed adjusting both soils of each site to the same level of P, N and P+N. Phosphorus was thoroughly mixed with 2 kg of each soil as KH_2PO_4 and N as NH_4NO_3 after pre-incubation at 45 % water holding capacity for 24 hours at 25 °C in the dark. Sorghum (***Sorghum bicolor*** Moench) was planted on the Fada soils and maize (***Zea mays*** L.) on the Koukombo soils and thinned at 5 days after sowing (DAS) to 1 plant. Over a period of 53 days plant height was measured daily and SPAD values were determined on the day of harvest. After harvest plants were dried, ground and analysed for their nutrient concentrations. Mycorrhizal infection rate and root length were determined. In the sieved soils the numbers of nematodes, P-Bray and P-water were measured.

7.3.2 Analytical procedures

Light absorption-based chlorophyll readings (SPAD-502 chlorophyll meter, Konica-Minolta Corporation, Osaka, Japan) of seven measurements of the youngest expanded leaf on each plant were taken at 52 DAS.

Total N in plant tissue was analysed using an FP-328 N-analyser (LECO, St Joseph, Mi, USA). Following combustion at 550 °C, the ash was dissolved in 20 ml HCl (32 %) and filled to 100 ml after 12 h in the dark with bi-diionized water. Subsequently total P was measured by spectrophotometry (U-2000 spectrophotometer, Hitachi, Tokyo, Japan) using the vanadate-molybdate-method (Gericke and Kurmies 1952). Potassium was measured using a flame photometer (Laboratory Instrument 543, Lexington, USA). After staining roots with cooking in a 10 % KOH and 5 % acidic acid ink solution mycorrhizal vesicles in root tissue were counted and the percent mycorrhizal colonisation was measured with the line intersection method of Kormanik and McGraw (1984).

Soil phosphorus was determined as water soluble P and P-Bray (Olsen and Sommers 1982). For water soluble P a soil samples of 5 g was extracted with demineralised H_2O on a horizontal shaker at 200 rev min^{-1} followed by centrifugation at 10000 rev for 8 minutes and 15 °C. Aliquots of 15 ml were mixed with 5 ml Ammonium-vanadat-moblybdat and 1 ml Zn-solution. The same procedure was used for P-Bray measurements with Bray I solution as an extraction agent and 10 ml extract for colorization. Additionally aliquots of 10 ml extract were mixed with 400 µl reagent A and 800 µl reagent B. Subsequently P was measured at 660 nm after the Zn-colorization and at 882 nm after colorization with reagent A and B using a spectrophotometer (UVIKON 930, Kontron Instruments, Neufahrn, Germany).

For nematode counts in Fada soils, aliquots of 100 g soil were filtered using 4 milk filters for more than 24 h over a funnel (Bearmann funnel method; Decker 1969). Subsequently an aliquot of 5 ml was taken and subsamples analysed using a microscope under which the nematodes were counted.

7.3.3 Statistical analysis

All results were tested for normal distribution of residuals using the Shapiro-Wilk test. Dry mass and N were transformed with 1/(x+1) while SPAD and P-Bray colorized with reagent A and B were transformed with LG10. Data of total root length, VAM infection rate, P, K, P-Bray with Zn colorization and nematodes were transformed with SQRT. Site, system and amendment effects were tested using GLM-Univariate. Means were separated using Tukey's HSD (honestly significant difference). All statistical analyses were performed with SPSS 11.5 (Backhaus et al. 2003).

7.4 Results

The application of mineral P alone increased growth of sorghum seedlings by up to 32 % on Fada soils and growth of maize seedlings by up to 15 % on the Koukombo ones compared to the respective unamended control. Mineral P+N addition increased plant growth on Fada soils by up to 54 % and on Koukombo soils by up to 25 % (Figure 9). The application of mineral N alone decreased plant growth by about 4 to 16 % on all soils except for the rotation soil of Koukombo on which plant growth was nearly similar to the respective control (Figure 9).

Sorghum leaves contained up to 3.5 times more chlorophyll (as indicated by the SPAD values) than maize leaves. On all soils higher SPAD values were detected in the control and mineral N treatment compared to mineral P only and mineral P+N (Figure 10). However, on Fada soils plant dry matter of all treatments of the rotation soil were significantly larger than of the respective treatments of the continuous cereal soil. The same was true for the Koukombo soils except for the mineral N treatment for which plant dry matter was similar on both soils. Dry matter differences were only significant for mineral P and mineral P+N of the continuous cereal soil compared to all other treatments (Figure 11).

Dry matter production was significantly increased by the application of mineral P and P+N compared to the control and mineral N on all soils. This effect was especially pronounced for sorghum plants on the rotation soil from Fada with 5 to 8 times higher dry matter compared to those of the control in both continuous cereal soils (Figure 11).

An 11 % higher total root length, 43 % higher VAM infection and 10 % higher N concentrations were found with sorghum plants grown on Fada soils, while 19 % higher P and 7 % higher K concentrations were found in maize plants grown on Koukombo soils (Table 8). For all plants grown on continuous cereal soils 7 % higher total root length, 26 % higher P and 4 % higher K concentrations were observed while VAM infection was 46 % and N concentrations 10 % higher on rotation soils (Table 8).

Compared to the unamended control root growth was increased by 16 % with the application of P alone and by 3 % with P+N. The application of N alone decreased root growth by 5 %, but increased mycorrhizal infection rate by 65 % while P alone and P+N both increased VAM infection by 23 % compared to the respective control (Table 8).

Nitrogen concentrations were increased by 51 % with N application and decreased by 36 % with application of P alone and by 7 % with application of P+N. Both P application treatments strongly increased P concentrations in plant tissue (mineral P by 48 %; mineral P+N by 41 %) compared to the control while they increased by only 4 % after application of N alone (Table 8). Potassium concentrations were increased by 12 % with P alone and decreased by 4 % and 6 % with the application of P+N and N alone, respectively (Table 8).

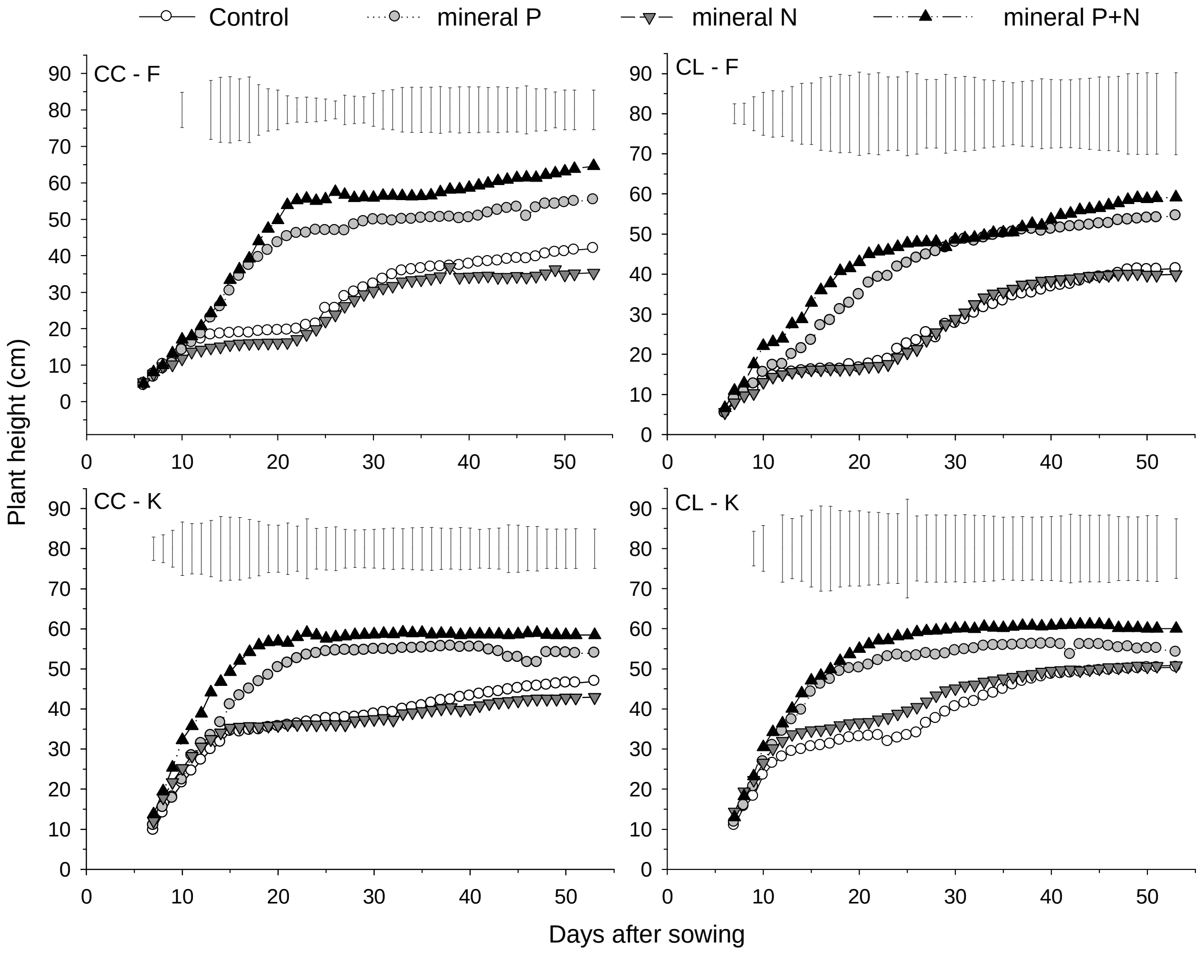

Figure 9: Growth of sorghum grown on continuous cereal (CC) and rotation (CL) soils from Fada (CC-F; CL-F) and maize grown on CC and CL soils from Koukombo (CC-K; CL-K) with different levels of mineral P, mineral N and mineral P+N. Vertical bars represent the 95 % confidence interval of the Least Significant Difference (LSD) whenever significant treatment effects were detected.

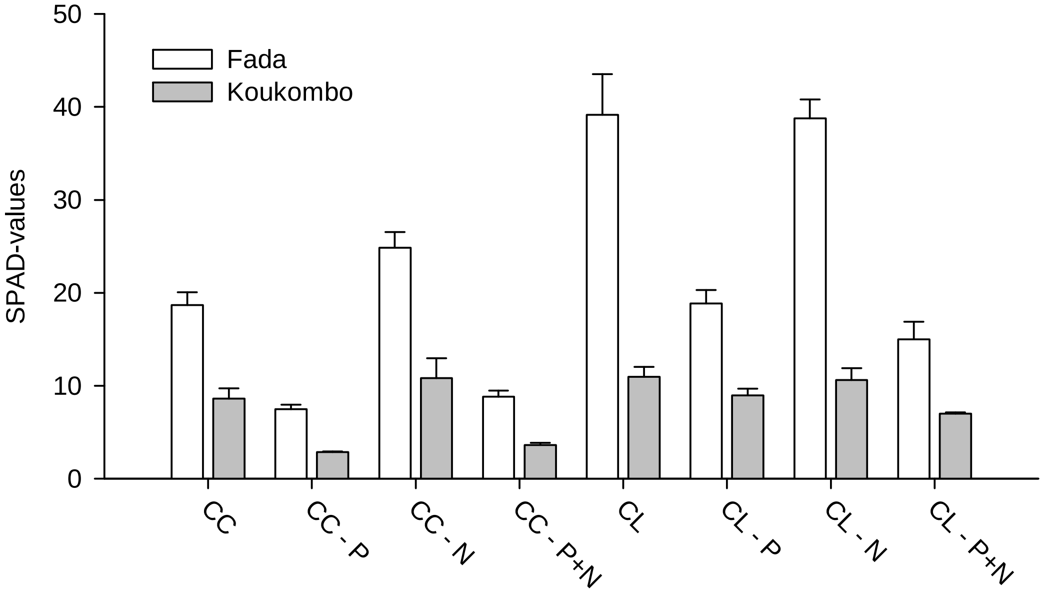

Figure 10: SPAD values of the youngest fully expanded leaf of sorghum grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Fada and maize grown on CC and CL soils from Koukombo fertilized with different levels of mineral P, mineral N and mineral P+N. Error bars represent standard errors of the mean.

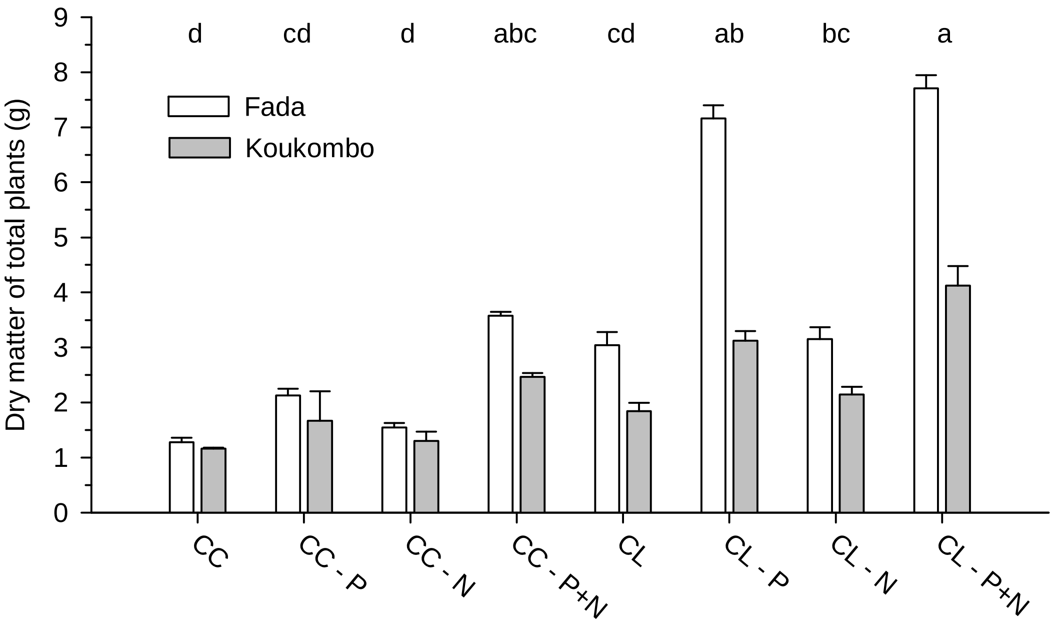

Figure 11: Average dry matter per plant (shoot and root) of sorghum grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Fada and maize on CC and CL soils from Koukombo with different levels of mineral P, mineral N, or mineral P+N. Treatments (means over both soils) with different letters are significantly different at ***P*** < 0.05 (Tukey HSD)***.***

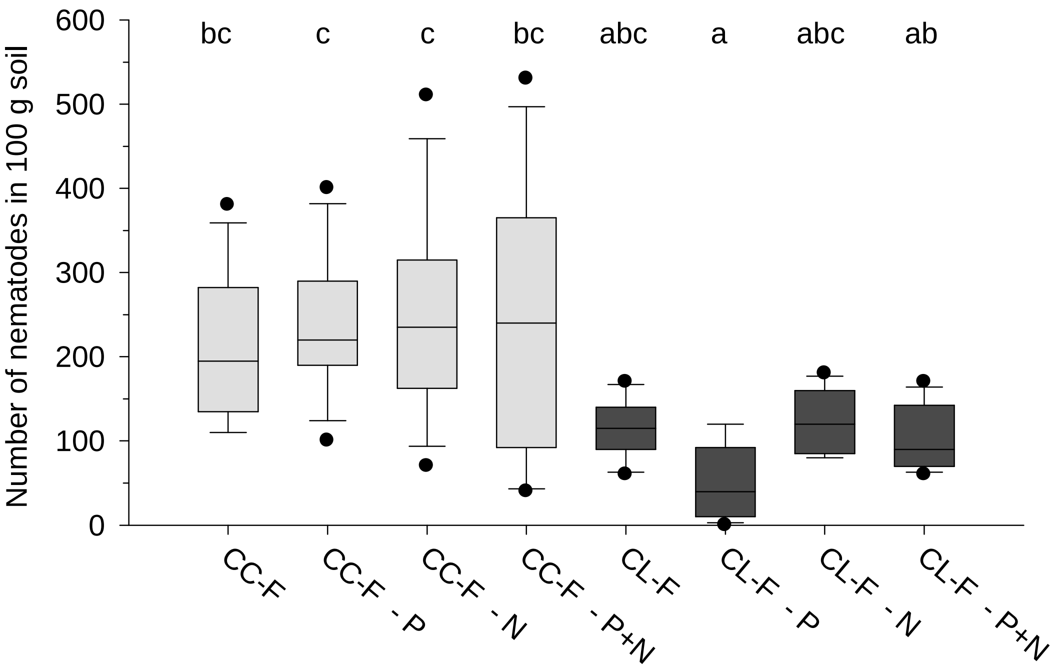

Figure 12: Counts of total nematodes in continuous cereal (CC) and cereal legume rotation (CL) soils from Fada (CC-F; CL-F) with different levels of mineral P, mineral N and mineral P+N. Treatments with different letters are significantly different at ***P*** < 0.05 (Tukey HSD)*.*

While P-Bray concentrations were in general 43 % (Zn colorization) and 75 % (CAL colorization) higher in the soils from Fada, P-water was 10 % higher in the Koukombo soils. P-Bray concentration according to the Zn colorization method was 10% higher in the rotation than the continuous soil while it was 12 % to 27 % higher in the continuous cereal soils for P-Bray CAL and P-water, respectively. Both mineral P applications strongly increased the P concentrations in all soils with an average P concentration of 4.7 µg g^{-1} compared to an average of 1.4 µg g^{-1} after mineral N application and 1.1 µg g^{-1} in the control (Table 8).

In Fada twice as many nematodes were found in the continuous cereal soil (236 nematodes per 100 g soil) compared to the rotation soil (98 nematodes per 100 g soil). No treatment differences on nematode were detected regardless of the location, except for the rotation soil where numbers of nematodes decreased by 56 % after application of mineral P alone (50 nematodes per 100 g soil) compared to all other treatments with an average of 114 nematodes per 100 g soil.

Table 8: Total root length, VAM infection, nutrient concentrations of total plants and P-Bray and P-water concentrations in the soil of a plant experiment where continuous cereal (CC) and cereal legume rotation (CL) soils from Fada and Koukombo were mixed with different levels of mineral N, P and N+P and planted with sorghum (Fada) and maize (Koukombo). To normalize the data, VAM, total root length, P, K, N and P-Bray Zn values were transformed with SQRT while P-Bray CAL was transformed with LG10 and N with 1/(x+1)

	Total root length	VAM infection	N	P	K	P-Bray Zn	P-Bray CAL	P-Water Zn
	(cm)	(%)	(mg g^{-1})			(µg g^{-1} soil)		
Site								
Fada	1809	10	7.7	2.3	12	3.2	6.6	0.9
Koukombo	1636	7	7.0	2.8	12	2.3	3.8	1.0
System								
CC	1779	7	7.0	2.8	12	2.6	5.5	1.1
CL	1666	10	7.7	2.2	12	2.9	4.9	0.8
Amendment								
Control	1665	7	7.2	2.1	12	1.0	1.6	0.6
Mineral N	1585	11	10.9	2.1	11	1.4	2.0	0.7
Mineral P	1927	8	4.6	3.0	13	4.4	9.0	1.3
Mineral N+P	1712	8	6.7	2.9	12	4.2	8.2	1.2
F-Probability								
Site	0.14	<0.01	0.16	<0.01	0.05	<0.01	<0.01	0.28
System	0.23	<0.01	0.02	<0.01	0.31	<0.01	<0.01	0.01
Amendment	0.16	0.02	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
Site*System	0.31	0.08	<0.01	0.01	0.04	<0.01	<0.01	<0.01
Amendment*Site	0.10	0.24	0.01	0.19	0.44	<0.01	<0.01	<0.01
Amendment*System	0.55	0.09	0.15	0.03	<0.01	<0.01	<0.01	<0.01
CV (± %)	21	45	20	20	13	12	8	39

CV = mean coefficient of variation between replicate incubations

7.5 Discussion

Growth of sorghum and maize on all soils strongly increased with the application of mineral nutrients, particularly the combination of N and P which underlines the often reported plant nutritional limitations on the sandy West African soils investigated (Bationo et al. 1992; Formowitz et al., 2009). Sorghum yield increases observed in the present study were in the same range as observed by Bagayoko et al. (2000b) who applied nearly the same rate of P (10 mg kg^{-1}) to a sandy soil from Niger. The decrease in plant growth on both CC soils after N application might indicate an N immobilization through the microbial community (Formowitz et al., 2009) or an even stronger P limitation (dilution effect). A positive N effect was only observed in the presence of an adequate P supply or an overall better nutrient state in case of the rotation soils (Table 7), which is in line with other studies on West African soils (Bationo and Mokwunye 1991). Our findings confirm the suggestion that the often reported yield declines on West African soils are not mainly caused by low N availability (Buerkert et al. 2001b). The vigorous growth of plants on the control and mineral N treatments after 25 days of growth, except for the continuous cereal soil of Koukombo, may back this argument. This increase in plant growth might be due to an increasing age and development of the root system leading to a higher phosphatase activity (Tarafdar and Marschner 1993) or to increased mycorrhizal infection and an increased phosphatase activity in the presence of mycorrhizal hyphae (Tarafdar and Marschner 1993; Marschner et al. 2005).

The lower amounts of P in plants grown on the rotation soils compared to the higher SPAD values at lower plant dry matter production on the control and after N application on all soils reflect a dilution effect due to the increased plant biomass production on the rotation soils (Bagayoko et al. 2000b). This argument might be backed by the higher P concentrations found in continuous cereal soils after harvest indicating the lower uptake of P by the plants compared to the ones grown on rotation soils.

Soil P-Bray and P-water concentrations after harvest were higher in Fada soils due to their higher initial P concentrations. Additionally the plants may have directly mobilized nutrients through root exudation or indirectly by stimulating microbial activity through rhizodeposition (Wichern et al. 2007; Paterson 2003) which can sometimes exceed plant uptake (Häussling and Marschner 1989). That might explain the higher residual P-Bray and P-water concentrations found in treatments with higher root growth as observed in the present study. The significantly higher P remains in the treatment with N only compared to the control might be attributed to the higher mycorrhizal infection and their hyphospheral phosphatase activity which has been reported to release P from organic and inorganic sources (Tarafdar and Marschner 1994; Koide and Kabir 2000).

On all soils N application significantly increased VAM infection which is in line with observations of Furlan and Bernier-Cardou (1989) who stated that root colonisation with VAM was stimulated through N fertilization and spore production promoted by plants fertilized with K. The significantly higher mycorrhizal infection rate may thus have additionally increased the N concentrations of sorghum seedlings on CL compared to CC soils from Fada through the transfer of N via the hyphae to the host (Ames et al. 1983; Johansen et al. 1993). The symbiosis between mycorrhizae and the host plant is based on substantial C-flows from the host towards the fungus (Bago et al. 2000). Therefore the higher mycorrhizal infection after application of N alone may have negatively affected plant growth (C drain into the formation of the mycorrhizal mycelium), even if Fitter et al. (1998) claimed that the 10 % C cost of mycorrhizal colonization may not alter plant performance or fitness directly.

In this study most of the counted nematodes were free living soil nematodes and the numbers of plant parasitic nematodes were negligible. Nevertheless, the lower numbers of nematodes found in the rotation soil of Fada compared to the CC-soil can be explained by the already reduced nematode population observed in the field (Bagayoko et al. 2000a; Alvey et al. 2001) probably leading to a lower egg density in the rotation soils. Additionally enhanced microbial activity found in rotation soils (Formowitz et al. 2007) may have hampered nematode infestation through decomposition of organic materials into protein and amine rich compounds with low C/N ratios (Rodriguez-Kabana et al. 1987).

7.6 Conclusions

Cereal dry matter production was significantly higher on rotation soils compared to continuous cereal soils even if the soils were brought to the same level of P and N concentrations. Enhanced N and P availability following a leguminous are therefore not the only reasons to explain yield increases of the adjacent cereal. Other causes such as higher microbial activity of the specific decomposer community establishing after legume cultivation, a higher VAM infection and the decreased number of nematodes in rotation soils have also to be taken into account.

7.7 Acknowledgements

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8 Legume rotation induced chemical differences in rhizosphere and bulk soil

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8.1 Abstract

The present study was conducted to measure pH gradients along the root-soil-interface of millet, maize and sorghum grown on non-sterilized and 36 kGy gamma sterilized continuous cereal (CC) and cereal legume rotation (CL) soils of West Africa. Some sterilized soils were re-inoculated with bacteria solution, inoculated with mycorrhizae (***Glomus mossae species***) or infected with nematodes (***Pratylenchus penetrans***). Subsequently, millet, maize and sorghum varieties from West Africa were grown for 29 days and non-destructive pH measurements with an antimony microelectrode were performed at 3 dates.

Plant growth was larger on CL than on CC soils in both years in which the experiment was conducted, but effects were only small, probably due to general limitation of plant growth in the small root chambers used. Higher biomass yields on sterilized soils may be attributed to nutrient mineralization following radiation-based sterilization. Mycorrhizal inoculation enhanced plant growth through P mobilization by slightly increased pH values that may have led to higher P-Bray concentrations in these soils. pH values were higher on CL than CC soil from Fada while the opposite was true for the Koukombo soils. Site specific differences in N contents and microbial community structures that led to varying plant performances may explain the contrasting findings. More research into the highly complex microbe-root-soil interactions is needed to enhance our understanding of the rhizosphere-system as it is affected by and affects cropping systems.

8.2 Introduction

Legumes are used in many regions of the world to enhance soil structure, increase nutrient availability and supply N into the soil through biological N_2-fixation (Lupwayi et al. 2011). Complex cation-anion-exchange relationships regulate electrical charges of roots to maintain electroneutrality at the soil-root-interface and cause pH changes if imbalance or over-uptake of one or the other occurs (Haynes 1990). Many legumes are known to acidify the soil through the release of protons due to an excess of cation uptake during N_2-fixation or exudation of organic acids. In comparison to wheat, pearl millet and sorghum acid phosphatase secretion by legumes was up to 72 % higher (Yadav and Tarafdar 2001). In P fixing acid sandy soils of Sub Saharan Africa leguminous crops can contribute to P mobilization through root induced pH increases and stimulation of microorganisms (Bagayoko et al. 2000a; Alvey at al. 2001). In another study increased decomposition of legume roots containing higher N contents was reported to cause more vigorous growth of young cereal plants followed by higher micorrhizal colonization rates (Bagayoko et al. 2000b). Koide and Kabir (2000) found extra-radical hyphae of ***Glomus intraradices*** to be very efficient in mobilizing P, taking it up and in transporting it to host roots. In competing for carbon (C) from root exudates and other nutrients with the rhizospheral microorganisms, also mycorrhizae can affect their population through varying decomposition rates of root and fungal root exudates (Kaman et al. 2011). Because of the reaction of microorganisms to fungal and plant specific root exudation and rhizodeposition (Morgan et al. 2005; Wichern et al. 2007), chemical, biological and physical properties of the rhizosphere often largely differ from bulk soil characteristics (Ndakidemi 2006; Hinsinger et al. 2009). Previous findings indicate that the microbial community structure, its changes and activity on rotation soils can lead to large differences between soil compartments and thus contribute to the yield promoting legume effects on West African soils (Marschner et al. 2004; Formowitz et al. 2007). Increased activity of ammonium- and nitrate-oxidizing bacteria leads to an acidification in their surrounding (Hinsinger et al. 2009). In the cereal legume rotation soils higher pH gradients between rhizosphere and bulk soil compartments might be expected compared to pH gradients in continuous cereal soils.

Therefore this study aimed at exploring whether the previously detected higher microbial activity in rotation soils causes larger pH gradients between rhizosphere and bulk soil of millet, maize and sorghum than in continuous cereal soils from West Africa. Gamma irradiation causes less chemical and physical changes in dry sandy soils than other sterilization methods (Salonius 1967; Berns et al. 2008). The comparison of gamma irradiated soils to sterilized soils re-inoculated with bacteria, inoculated with mycorrhiza or infected with phytopathogenic nematodes may therefore provide information on the impact of these organisms to the yield enhancing cereal legume rotation effects.

8.3 Materials and methods

8.3.1 Soil and experimental layout

Soil samples from the upper 20 cm of two West African continuous cereal (CC) and cereal legume (CL) rotation soils from Fada in Burkina Faso (11°59’N, 0°19’E; F) and Koukombo in Togo (10°17’N, 0°23’E; K) were collected, air-dried, shipped to Germany and stored at 15 - 20 °C in the dark for subsequent use. Higher pH, higher C_{mic}, N_{mic}, P and K concentrations were measured in the respective soils from Fada than from Koukombo (Formowitz et al. 2009).

In the first experimental year, for the plant experiment 800 g of each continuous cereal (CC) and rotation (CL) soil from Fada (CC-F and CL-F) and Koukombo (CC-K and CL-K) were filled into root chambers (25 x 10 x 2 cm) and planted with three days old pre-germinated seedlings of pearl millet (***Pennisetum glaucum*** L.), maize (***Zea mays*** L.) and sorghum (***Sorghum bicolor*** Moench). Furthermore, dry soil samples were sterilized (st) with gamma radiation (39 kGy) and planted with 3 days pre-germinated sorghum seedlings. Additional treatments consisted of sterilized soil re-inoculated with bacteria (sorghum st. +Bac; collected from a soil suspension), sterilized soil inoculated with mycorrhizal granulate of ***Glomus mossae*** (sorghum st. +AM) and sterilized soil infected with nematodes of the species ***Pratylenchus penetrans*** (sorghum st. +Prat.), propagated with maize roots grown on a Murashige and Skoog medium (Murashig-Skoog Basal medium, M-5519, Sigma-Alderich).

To obtain the bacteria solution for re-inoculation, soil samples of 10 g were mixed with 200 ml bi-distilled water for 2 hrs with a magnetic stirrer. Subsequently 80 ml were added to the sterilized soil in the root chambers. After a pre-incubation of about 12 hours seedlings were planted. Mycorrhizal granulate (MYKOTOWN®Arabia; MYKOTOWN®Biotechnology AG, Dessau, Germany) consisting of different ***Glomus*** species was applied using 3 g granulate per root chamber. To propagate nematodes, maize seeds were sterilized with 95 % Ethanol (40 sec.) and 2.5 % sodium hypochlorite solution before they were placed on a Murashige and Skoog medium (M+S mixed with saccharose, agar and bi-distilled water). After incubation of 8 days at 21 to 22 °C in the dark, the seedling was removed and nematodes (***Pratylenchus penetrans***) obtained from stock cultures maintained on sterile roots were placed on the grown maize roots. After 6 to 8 weeks of incubation at 21 to 22 °C the M+S medium (with maize roots and nematodes) was placed on milk filters according to the Baermann funnel method, normally used for nematode counts in soil samples (Decker 1969). In this study the method was used to gain nematodes for infection. Subsequently, an aliquot of 2 ml solution containing around 1000 nematode individuals was added to the sterilized soil.

In the subsequent year the experiment was repeated with non-sterilized (sorghum) and sterilized (sorghum st.) CC and CL soils from Koukombo only, planted with 3 days pre-germinated sorghum seedlings. As additional treatments sterilized soil re-inoculated with bacteria (sorghum st. +Bac) and sterilized soil inoculated with mycorrhizal granulate of Glomus mossae (sorghum st. +AM) as described above were added.

In both experiments, plants were grown for 29 days on the soils watered at 10 % weight/weight whereby plant height to the largest leaf was measured daily. At three sampling times (after 1, 2 and 4 weeks) non-destructive pH measurements in rhizosphere and bulk soil took place using a self-made antimony-microelectrode for pH detection in mV on the spot (Häussling et al. 1985). Buffer solutions of pH 3 to 9 were mixed with the soil and a linear equation calculated to transform the measurements into the corresponding pH values. To visualize differences in pH between rhizosphere and bulk soil, an agar-plate including a pH indicator (Bromkresolpurpur) was placed on the opened root chambers (Marschner et al. 1982; Dinkelacker et al. 1989).

After harvest the above ground biomass was dried at 60 °C, roots were sieved and the soil separated into rhizosphere soil, bulk soil and “rest”-soil. Roots with adhering soil (rhizosphere soil) were mixed with 0.01 M $CaCl_2$ in 500 ml flasks, incubated overnight, extracted for 30 min at 150 rev min^{-1} on the following day before roots were hand picked with tweezers. The remaining suspension was centrifuged for 8 min at 10.000 rev min^{-1}. Ten g of bulk soil were extracted with 0.01 $CaCl_2$ as described above to measure PO_4 and nitrate. Because no colorization for P measurements was possible probably due to very small amounts of P in the solutions obtained, P-Bray (Olsen and Summers 1982) was measured in some representative samples. To this end 2.5 g of oven dry soil were extracted with 50 ml Bray I solution on a horizontal shaker at 200 rev min^{-1} for 30 minutes. Aliquots of 20 ml were mixed with 200 µl 4.5 M H_2SO_4 to obtain a pH of 1.5. Afterwards aliquots of 10 ml extract were mixed with 400 µl reagent A (ammonium hepta molybdate + potassium antimony tartrate solution) and 800 µl ascorbic acid solution (reagent B). Subsequently P was measured at 882 nm after colorization with reagent A and B using a spectrophotometer (UVIKON 930, Kontron Instruments, Neufahrn, Germany).

8.3.2 Statistical analysis

All data were tested for normal distribution of residuals using the Shapiro-Wilk test. System, soil compartment, time and treatment effects were tested using GLM-Univariate. Means were separated using Tukey’s HSD (Honestly Significant Difference). All statistical analyses were performed with SAS 9.2.

8.4 Results and discussion

In both experiments for Koukombo plant growth on CL-soils was larger than on CC-soils (Figure 13 and Figure 14), while data were more inhomogeneous for Fada soils (data not shown). The differences in plant growth on Koukombo soils were significant, but not as large as previously found (chapter 7.4), probably due to a general hampered plant growth caused by the very limited size of the root chambers and temporal problems with water supply. In the second experimental year root chambers were covered with an aluminum-foil to reduce evaporation which generally increased plant growth (Figure 14).

According to Salonius et al. (1967) δ-irradiation of a dry soil, as it was done in this experiment, causes a much smaller release of NH_4-N than autoclaving while the opposite is true for wet soils. However, through irradiation small amounts of nutrients can be mobilized mainly from dead microbial cells, to a lesser extend from humus, stimulating plant growth (McLaren 1969). This might explain the increased plant growth on almost all irradiated Koukombo soils during this study which is backed with higher P-Bray concentrations found in sterilized soils compared to non-sterilized variants of the first experimental year (Table 9). On the severely nutrient poor West African soils already small amounts of nutrients mobilized can contribute to large yield increases (Bagayoko et al. 2000b).

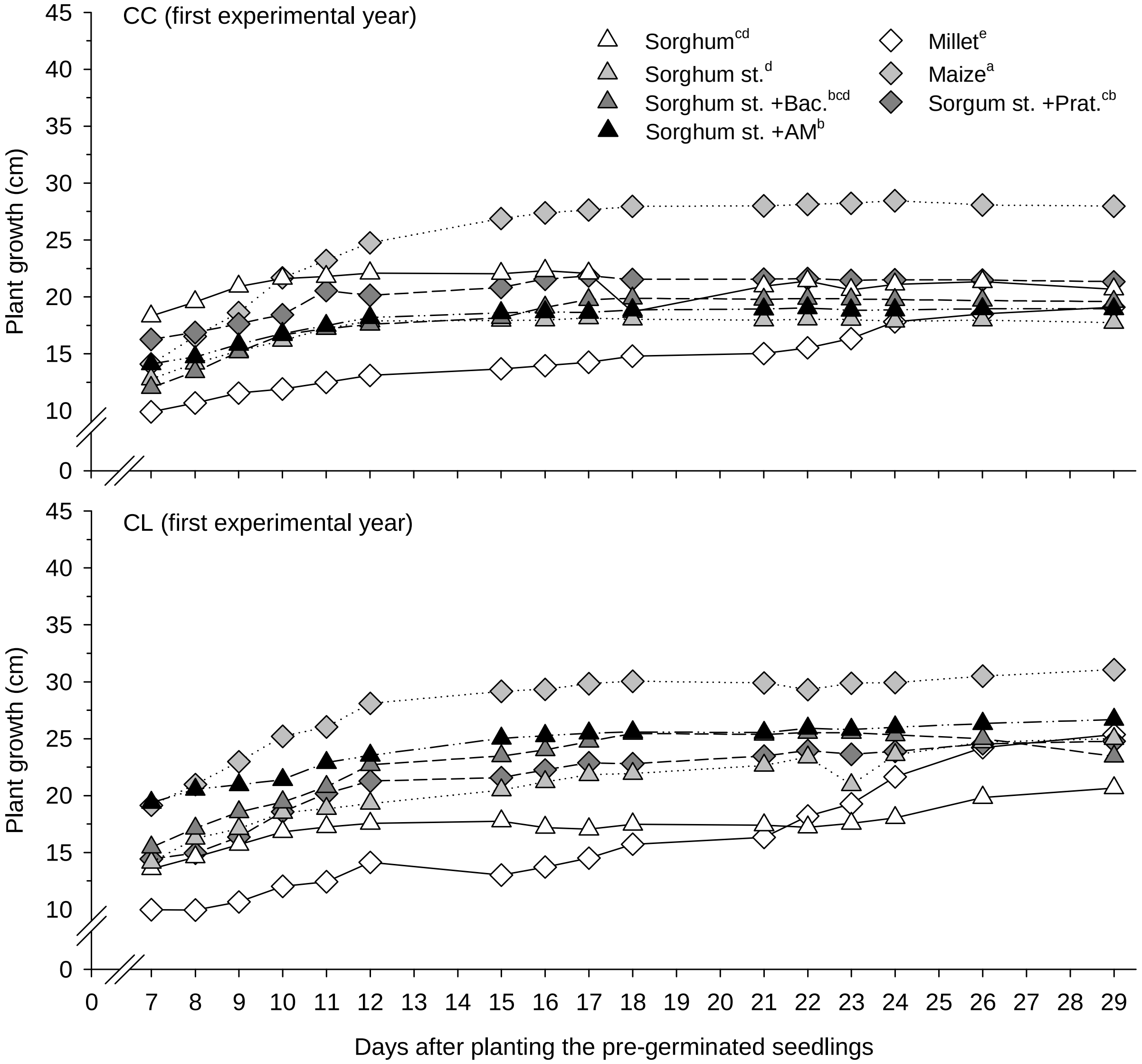

Figure 13: Plant growth of millet, maize and sorghum grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Koukombo in the first experimental year. Additional sorghum treatments consisted of with δ-radiation sterilized (st.) soil re-inoculated with bacteria (st. +Bac.), inoculated with mycorrhiza (st. +AM) and infected with nematodes (st. +Prat.). Treatments with different letters are significantly different at $P < 0.05$ (Tukey HSD).

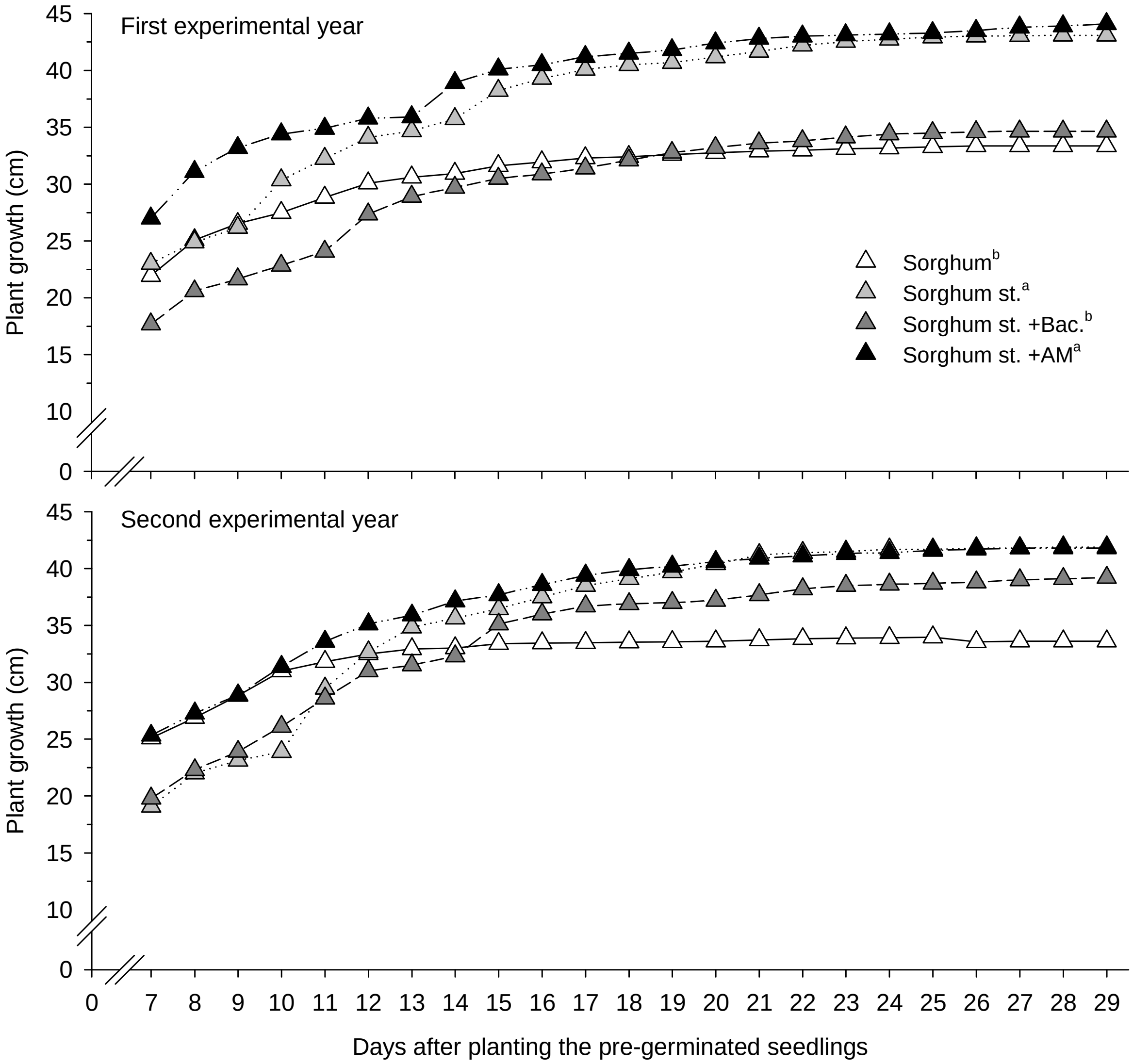

Figure 14: Plant growth of sorghum grown on continuous cereal (CC) soil from Koukombo in the first and second experimental year (sorghum). Additional sorghum treatments consisted of with gamma radiation sterilized (st.) soil re-inoculated with bacteria (st. +Bac.) and inoculated with mycorrhiza (st. +AM). Treatments with different letters are significantly different at P < 0.05 (Tukey HSD).

In the first experimental year biomass production on rotation soils from Koukombo were significantly increased by 54 % or even tripled compared to the respective treatments of the continuous cereal soils, except for sorghum grown on non-sterilized soils (Figure 15). With 0.30 g dry matter on CC-soil and 0.46 g dry matter on CL-soil maize yields in the first experimental year were much larger than those of the other plants or treatments. In the following year rotation effects were only minor with a yield increase of 1 to 5 % compared to the respective treatments on continuous cereal soils (Figure 16).

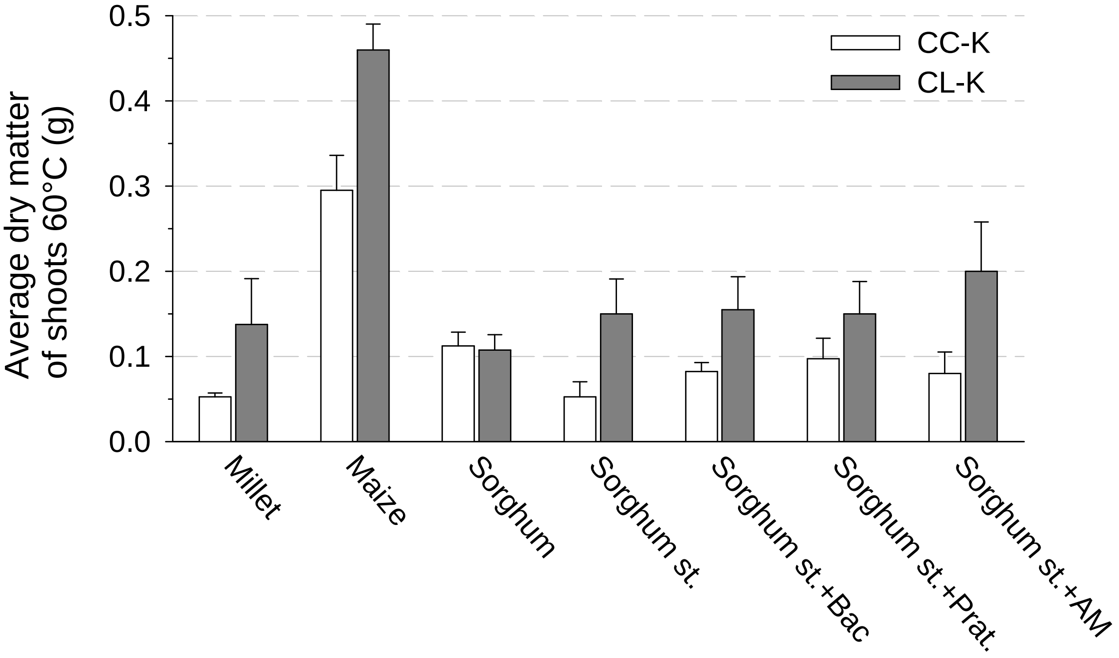

Figure 15: Average dry matter of millet, maize and sorghum shoots per treatment grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Koukombo in the first experimental year. Additional sorghum treatments consisted of with gamma radiation sterilized (st.) soil re-inoculated with bacteria (st. +Bac.), inoculated with mycorrhiza (st. +AM), or infected with nematodes (st. +Prat.). Error bars represent +/- standard error of the mean. Treatments with different letters are significantly different at P < 0.05 (Tukey HSD).

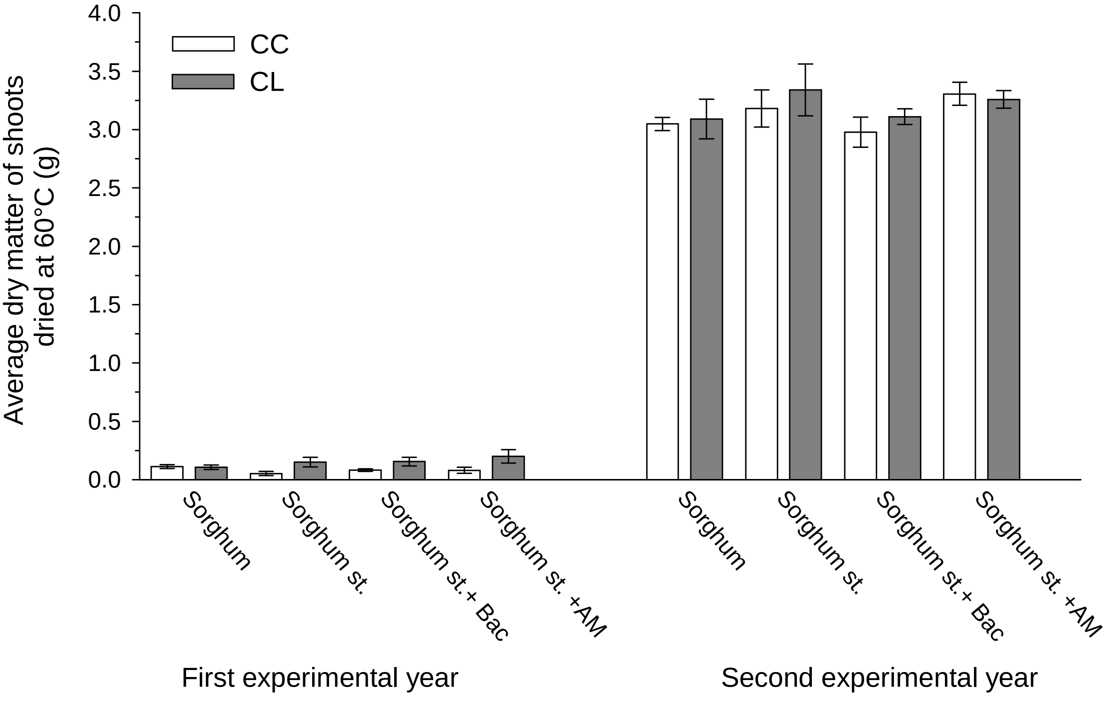

Figure 16: Average dry matter of sorghum shoots grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Koukombo in the two experimental years. Error bars represent +/- one standard error of the mean. Additional sorghum treatments consisted of with gamma radiation sterilized (st.) soil re-inoculated with bacteria (st. +Bac.) and inoculated with mycorrhiza (st. +AM). Treatments with different letters are significantly different at P < 0.05 (Tukey-HSD).

The relatively good performance of sorghum grown on sterilized soil re-infected with the migratory endoparasitic nematode ***Pratylenchus penetrans*** (Figure 15; Table 9) might be explained by nutrients that were mobilized while extracting nematodes from the M+S media and applied to the soils with the nematode-solution. Potential negative effects on sorghum performances through plant parasitic nematodes might thus have been masked by stimulating nutrients at that early growth stage. Furthermore temporal drought stress in the small root chambers could have evoked anhydrobiosis, a known constraint to soil nematodal survival under harsh conditions such as the extremely arid environment of the Mojave Desert site at the Rock Valley, Nevada (Freckmann et al. 1977) and Antarctic Dry Valleys (Treonis and Wall 2005). At this stage nematodes show a coiled and almost "water-free" body accompanied by cessation of metabolic activity that will change rapidly after re-hydration in the presence of water (Crow et al. 1992). This survival strategy could have disabled added nematodes penetrating the roots allowing normal sorghum growth comparable to the other sorghum treatments.

Sorghum growth on Koukombo soils was significantly increased through mycorrhizal (AM) inoculation compared to sorghum grown on non-sterilized soil in both years, and sorghum on sterilized soil and millet in the first year, as well as sorghum re-inoculated with bacteria solution in the second year (Figure 13; Figure 14). Dry matter yields backed these findings about growth promoting effects of mycorrhiza even though differences were not significant (Figure 3, Figure 16; Table 1). Yield differences between AM-treated rotation and continuous cereal soil were bigger than for almost all other sorghum variants. Bagayoko et al. (2000b) stated that mycorrhizal infection contributes to the yield increasing effects of legume rotations on West African soils. In a pot experiment using the same P deficient soil collected from the West African experimental site and VAM-colonized maize roots with adhering soil as inoculum, increased sorghum growth was dependent on the interaction of phosphorus application and mycorrhizal root infection (Bagayoko et al. 2000c). The same was true for maize grown on an acidic soil which performed best with application of rock phosphate plus inoculation with arbuscular mycorrhizal fungal (AM) ***Glomus clarum*** compared to exclusive P fertilization or solely inoculation with mycorrhiza (Alloush and Clark 2001).

Many studies report mycorrhizal induced increase in P uptake by plants (Bolan 1991; Lekberg and Koide 2005; Gong et al. 2012) through making organically and anorganically bound P available by enhanced hyphospheric phosphatase activity (Joner et al. 2000; Koide and Kabir, 2000). But in contrast to N_2-fixing bacteria bringing N into the soil-system, mycorrhizas do not add P into the soil (Cardoso and Kuyper 2006). Thus the more than doubled sorghum yields on AM-treated rotation soils during this study can be attributed to the overall higher nutrient concentrations found in rotation soils as shown by their 0.9 $\mu g\ g^{-1}$ higher PO_4-P concentrations compared to CC-soils (Table 9). These positive effects of mycorrhizal inoculation on plant growth could have been raised through a slight enhancement in phosphorus availability through δ-irradiation (Thompson 1989; McNamara et al. 2003).

Table 9: Dry mass of pearl millet, maize and sorghum, as well as pH and P-Bray in continuous cereal (CC) and cereal legume rotation (CL) soils from Koukombo (Togo). Some of the soils were sterilized with δ-radiation and re-inoculated with bacteria (st. +Bac.), inoculated with the mycorrhizal species ***Glomus mossae*** (st. +AM), infected with nematodes of the species ***Pratylenchus penetrans*** (st. +Prat.) and compared to an unamended sterilized variant (steril). The sterilized soils were all planted with sorghum.

Measured parameter	Dry mass 60 °C in g	Dry mass 60 °C in g	pH	pH	P-Bray PO_4-P in µg g^{-1}
Year	First year	Second year	First year	Second year	First year
System					
CC	0.11	2.92	5.5	5.4	2.7
CL	0.19	3.15	4.9	5.0	3.6
Soil compartment					
Rhizosphere			4.6	4.8	
Bulk soil			5.7	5.6	3.4
Treatments					
Pearl millet	0.10		5.0		2.8
Maize	0.38		5.0		2.4
Sorghum	0.11	3.07	4.8	5.2	2.8
Sorghum st.	0.10	3.25	5.3	5.2	3.4
Sorghum st. +Bac.	0.12	3.03	5.4	5.4	3.2
Sorghum st. +AM	0.14	3.28	5.4	5.0	3.6
Sorghum st. +Prat.	0.12		5.3		3.7
Time					
Week 1			5.4	5.3	
Week 2			5.2	5.4	
Week 4			5.0	4.9	
F-Probability					
System	<0.001	0.409	<0.001	<0.001	0.001
Soil compartment			<0.001	<0.001	
Treatment	<0.001	0.092	<0.001	< 0.001	
Time			0.001	<0.001	
System*Treatment	0.177	0.824	0.264	0.965	

Measured pH values in Koukombo soils were in the same range in both years, with 0.8 to 1.1 pH units lower values in the rhizosphere than in the bulk soil. Furthermore the pH was 0.4 to 0.6 pH units lower in rotation soils compared to continuous cereal ones (Table 9; Figure 17). Over 29 days pH values were reduced by 0.4 pH units in both years. However, pH values in Fada soils were higher in rotation soils (average pH = 6.3) than in continuous ones with an average pH of 5.6 (Figure 17). Similar results were obtained by Alvey et al. (2001) with higher pH values on rotation soils compared to continuous cereal ones and additionally an increase in rhizosphere pH. Comparable results were also found with destructive pH measurements under field conditions whereby pH values increased from the bulk soil to the rhizosphere and rhizoplane by up to 0.3 pH units for millet (Bagayoko et al. 2000a). Both groups of authors attributed these pH increases largely on increased NO_3^- uptake which might have been also the case for Fada soils during our study, containing generally higher N-contents than the less buffered Koukombo soils. The pH rise in Fada rotation soils may have also contributed to a change in the microbial community structure with a higher fungi occurrence and activity (Formowitz et al. 2007) leading to increased decarboxylation rates of organic anions. The latter was found to result in pH increases (Yan et al. 1996), especially when organic anions are extruded with an accompanying cation, such as K^+, inhibiting roots to release protons (Hinsinger et al. 2003). However, root induced changes of rhizosphere pH strongly depend on root induced cation-anion balances to regulate electrical charges of root cells with compensatory exudation of H^+ if more cations (e.g. NH_4^+) are taken up or with release of OH^- in case of raised anion (e.g. NO_3^-) uptake. This can even differ between different zones along one root (Hinsinger et al. 2003).

Although not all interactions are well understood, root exudates and rhizodeposition play an enormous role in changing directly and indirectly chemical parameters such as pH in the rhizosphere influencing nutrient availability and microbial communities (Dakora and Phillip 2002; Rengel and Marschner 2005; Hinsinger et al. 2009). Thus the large release of C through root exudates reaching estimates of 2 to 10 % (Broeckling et al. 2008) or 5 to 20 % (Walker et al. 2003) of the total photosynthetically fixed C can have large effects on bacterial and fungal populations (Broeckling et al. 2008; Walker et al. 2003). Through various soil-plant-microorganism-interactions microbes can also contribute to soil acidification through e.g. production of organic acids or high proton release through nitrification (Casarin 2003; Xu and Coventry 2003). Fierer and Schimel (2002) stated that a NH_4-flush after rewetting coupled with low mortality rates of a nitrifier community might lead to their proliferation and higher activity. Rotation soils of this study generally contained more N than the continuous ones which may have led to higher N-flushes after rewetting. If these N-flushes consisted mainly of NH_4 in Koukombo soils coupled with higher microbial biomass C in rotation soils (Formowitz et al. 2007), the nitrifier community may have contributed to the measured pH decreases in those soils. In Fada soils the higher amount of fungal biomass, especially in the rotation soil compared to soils from Koukombo (Formowitz et al. 2007), may react differently to N-flushes hence influencing the bacterial community that feed on fungal exudates rather than direct on root exudates (Buée et al. 2009). If true, this may increase bacterial decarboxylation of fungal released organic anions supporting an increase of pH values. However, with increased pH values Al concentrations are reduced leading to enhanced N, P, Ca and Mg availability and a better uptake of latter by millet grown on acid sandy West African soils (Bagayoko et al. 2000).

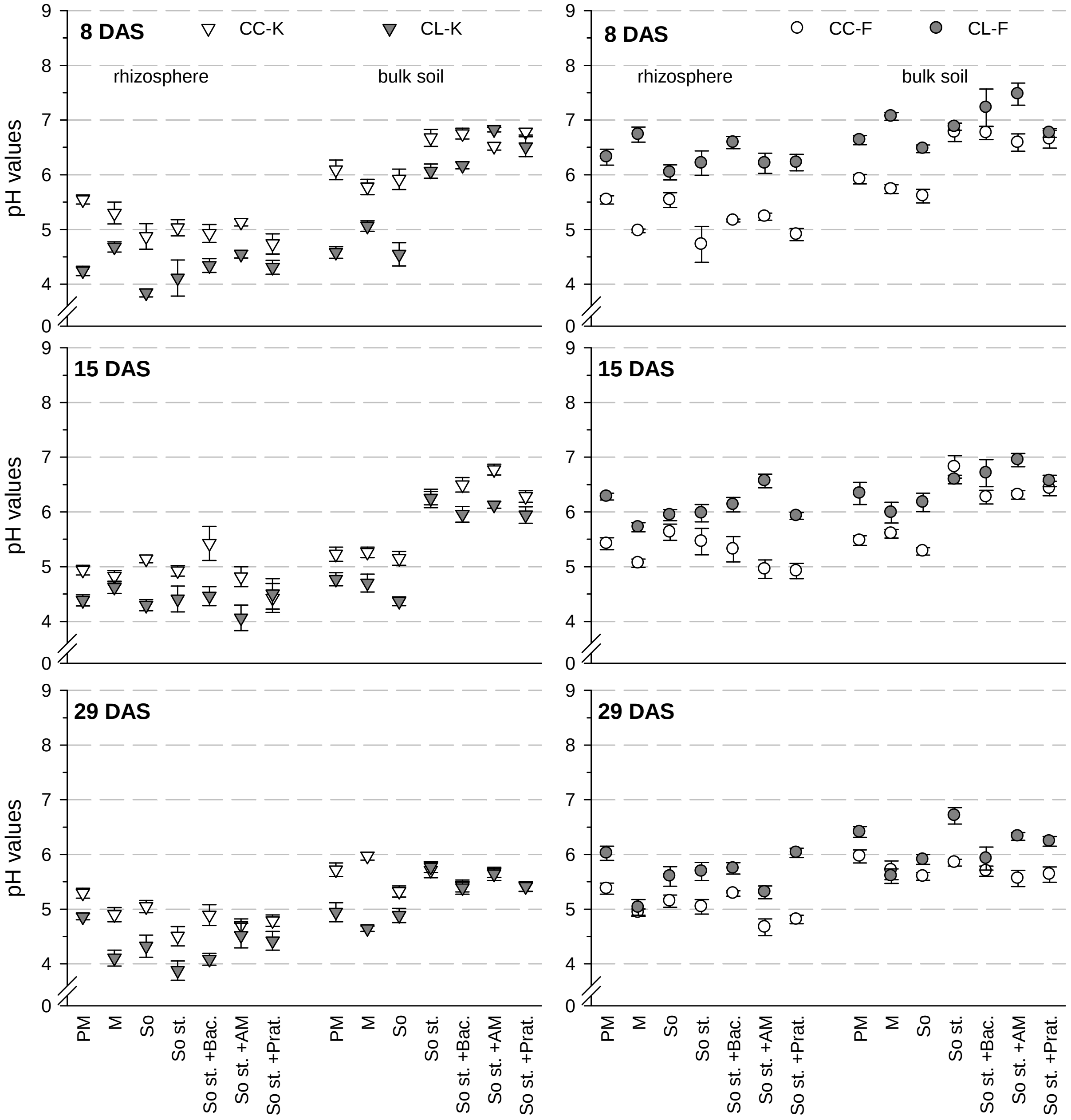

Figure 17: pH values measured at three times in the rhizosphere and bulk soil of millet, maize and sorghum grown on continuous cereal (CC) and cereal legume rotation (CL) soils from Koukombo (CC-K; CL-K) and Fada (CC-F; CL-F) in the first experimental year (DAS = days after sowing). Additional sorghum treatments consisted of δ-irradiated soil (st.) re-inoculated with bacteria (st. +Bac.), inoculated with mycorrhiza (st. +AM) and infected with nematodes (st. +Prat.). Error bars represent +/- one standard error of the mean.

A decrease of soil pH by up to 0.4 units directly after partial sterilization with *δ*-irradiation of 5 to 20 kGy in a wet vertisol (50 % moisture content) was attributed to mobilized N nitrified to NO_3^- by Thompson (1990). Gamma irradiation of wet soils can lead to a plant growth stimulating ammonia flush through N release from soil organic matter and killed microorganisms (Stribley et al. 1975) inducing proton accumulation in soil due to either anion-cation balances and/or nitrification (Hinsinger et al. 2003). Irradiation of dry soils during our study led to an increase of pH values from 5.3 to 5.4 in all sterilized variants compared to 4.8 to 5.0 in non-irradiated soils (Table 9). This increase was even more pronounced in bulk soil (Figure 17). Gamma-sterilization is effective in killing microorganisms or at least inhibiting their growth already at lower or similar radiation doses as used for sterilization in this study (McNamara et al. 2003; Berns et al. 2008; Buchan et al. 2011). The higher pH in our sterilized soils may thus be explained by the absence of ammonium- and nitrite-oxidizing bacteria. To gain complete sterility, doses above 50 kGy are needed, depending on soil properties (McNamara et al. 2003). Thus decreased pH values at the end of the experiment (Table 9; Figure 17) might indicate activity of recovered surviving microorganisms.

8.5 Acknoledgements

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9 General Conclusions

The introduction of legume rotations to West African agriculture is an important component in holistic soil fertility management. In previous field experiments in West Africa, yield increasing effects and changes in biochemical and biological soil parameters were found on many rotation soils. After a 6-year storage of air-dried continuous cereal (CC) and cereal legume rotation (CL) soils, these effects could still be reproduced under controlled conditions during this study. Even though the application of mineral P and the combination of P + N caused yield increases on both CC and CL soils, crop growth was most vigorous on CL soils. Thus the increased nutrient availability after cultivating a leguminous crop definitely contributes to the legume rotation induced yield increases of the following cereal.

All comparisons of continuous cereal (CC) and cereal legume rotation (CL) soils showed higher yields and higher contents of microbial parameters (C_{mic}, N_{mic}, ergosterol, glucosamine, muramic acid, mycorrhizal infection) on rotation soils. The rapid rehydration of microbial cells after rewetting without N-immobilization in addition to the overall higher microbial biomass with its higher activity in rotation soils leads to higher indirect nutrient mobilization enhancing crop development. Initial findings of higher ergosterol, glucosamine and muramic acid were assumed to indicate shifts of the microbial community structure. In contrast, according to the findings of the second experiment, the microbial residue fraction resembled the world-wide average range of arable soils. The shifts in microbial community structure are thus an insufficient explanation for the wide C_{mic}/N_{mic} ratios, which are normally attributed to a shift towards a more fungal based microbial communities. However, the microbial biomass in Koukombo soils seems to be less efficient in substrate use demanding higher energy input to match the catabolic requirements of its biomass. At both sites, microorganisms did not react to the application of mineral fertilizers, but only to the application of energy in form of the organic amendments (root residues). Thus, the repeated C-input by legume root residues might have contributed to differences in soil fertility. Other studies using the same soils highlighted the importance of mycorrhizal root colonization to increase crop establishment. Yield performances could be confirmed repeatedly in this study by positive correlations between mycorrhizal colonization and plant growth.

Although mineral fertilizer might create even higher dry matter yields of cereals and not all root-soil-microorganism interactions were uncovered, the contribution of legume rotations to soil fertility in Sudano-Sahelian West Africa was highlighted. In times of fertilizer shortage and climate change, legume rotations are simple measures to complement mineral fertilizer applications in the predominantly nutrient-limited agro-ecosystems, thereby reducing farmers' input costs and strengthening their income through optimized yields. Stable and higher yields may lead to higher investments in land care thereby reducing soil erosion and decline of organic C. In the long term, this may contribute to a better soil structure and enhanced water holding capacity as well as an increased and more active microbial community adapted to long recurrent annual drought conditions.

10 Outlook

The present study backed earlier findings that higher nutrient availability and enhanced microbiological factors are major causes of yield increases by legume rotations. Although it could be shown that continuous C input by leguminous root residues contributed to the rotation effects, the latter seem to rely more on rhizodeposition and its changes in the rhizosphere and microbial community.

^{13}C and ^{15}N labeling might be a good opportunity to verify the amount of C and N that is released by rhizodeposition under field and controlled conditions. Labeling could be done with double labeling using the wick method (Wichern et al. 2007 and 2010) or using rhizoboxes for plant cultivation, while plants are simultaneously labeled with gaseous $^{13}CO_2$-C in a gastight labeling chamber and ^{15}N via the wick method (Schenck zu Schweinsberg-Mickan et al. 2010). Labeling crops with ^{13}C and ^{15}N will provide information about the quantitative rhizodeposition of these important nutrients into different soil pools depending on the distance from the root surface. Simultaneously measured microbial parameters will show if the increased N availability after legume rotations strongly affects the rhizospheral microbial community.

The causes of contrasting pH measurements in the rhizosphere and bulk soil differing between the soils from Fada and Koukombo have not been revealed during this study. Measurements of microbial parameters such as C_{mic}, N_{mic}, P_{mic}, ergsterol concentrations, phosphatase activity and ATP in the different soil compartments would help to obtain a more detailed picture of crop-microorganism interactions. The additionally combined measurement of muarmic acid and glucosamine compared with findings from C_{mic} and ergosterol may show if a shift towards a more fungal or more bacterial based microbial community is responsible for the different pH values. On the other hand ATP and phosphatase activity would allow drawing conclusions if a more active microbial community in rhizosphere and bulk soil is found to be responsible for site differences.

Although groundnut and cowpea both represent host plants for phytopathogenic nematodes such as ***Pratylenchus penetrans***, they seem to suppress nematode infestation. Therefore pot experiments with artificial nematode infection using higher amounts of soil than in this study should be established to prevent fast drying of the soil and allow nematode propagation. For unknown reasons the cultivation of groundnut roots on M+S media during this study failed. If growing groundnut roots on an agar-media could be achieved, it would allow direct inoculation with nematodes from a starter culture (e.g. maize roots). It could then be observed if nematodes will multiply on the groundnut roots. Additionally, analyses of groundnuts could focus on their root exudation which might directly control nematodes, or on their rhizodeposits, which might be transferred into controlling compounds during their degradation.

11 Summary

In West Africa, where agricultural production is very limited and restricted to a short rainy season, the introduction of legume rotations presents a promising complement within a holistic soil fertility management approach to intensify the local agricultural production. In previous experiments under field conditions, the preceding cultivation of cowpea (***Vigna unguiculata*** Walp) and groundnut (***Arachis hypogea*** L.) led to yield increases in the succeeding cereals (***Pennisetum glaucum*** L.; ***Sorghum bicolor*** Moench; ***Zea mays*** L.), increased soil pH, early mycorrhizal infection and decreased the number of phytoparasitic nematodes. Additionally, indicators for a specific shift in the microbial community structure were found, but still these changes are not fully understood.

This study was therefore conducted to investigate soil biological and chemical factors that give rise to the cereal yield enhancing effects of legume rotations on sandy, nutrient poor West African soils. The aim was not only to gain more information on the role of legume residues and microorganisms in the soil nutrient cycle. But the study aimed at evaluating if differences in substrate qualities (e.g. root residues) cause changes in the microbial community structure due to specific and highly complex microbe-root-soil interactions.

The comparison of continuous cereal (CC) and cereal legume rotation (CL) soils from two West African sites (Fada-Kouaré in Burkina Faso, F, and Koukombo in Togo, K) allowed to confirm assumptions of previous studies that site and system specific differences in soil chemical and biological parameters play an important role in explaining the yield increasing effects of legume rotations.

The onset of rainy season was simulated in an incubation experiment by rewetting CC and CL soil samples from Fada and Koukombo. Site and system specific reactions of microorganisms were observed. Higher respiration rates, higher amounts of microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) as well as higher ergosterol, muramic acid, glucosamine and adenylate (ATP, ADP and AMP) concentrations were measured in CL soils of Koukombo and in both soils from Fada. The immediate increase in ATP concentrations after rewetting was likely caused by rehydration of microbial cells where N was not immobilized and, thus, available for plants facilitating their rapid development. Soil microorganisms seemed to contribute to the yield increasing rotation effects through high mineralization and, thus, through increased nutrient release during the first days after rewetting. The site-specific high respiration rate of Fada soils was likely due to high soil organic C levels that serve as the main source of energy for rehydrated microorganisms. Higher amounts of the fungal cell-membrane component ergosterol and mainly fungal glucosamine in addition to higher concentrations of the bacterial cell-wall component muramic acid in CL soils indicate shifts in the microbial community structure.

Effects of cereal and legume root residues on sorghum growth and microbial turnover processes were observed in a 59-day pot and a 189-day laboratory experiment. Therefore, 2 mg g^{-1} of root residues were mixed with CC soils from Fada and Koukombo and compared to mineral treatments (mineral P or mineral P+N) as well as to a control without any amendments. Sorghum growth was higher for all treatments on the Fada soil and significantly enhanced by mineral nutrient supply. Legume root residues led only to slightly better plant performances compared to the control, while the application of cereal roots reduced seedling growth. Soil microbial indices were 75 to 100 % higher in the Fada soil compared to the soil from Koukombo. In contrast to sorghum seedlings, the microbial community did not react to the mineral treatment. Thus the energy supply in form of organic amendments increased ergosterol, C_{mic}, N_{mic} and ATP concentrations compared to mineral P application and the control. Interestingly, the number of free living nematodes was reduced through root residue application. This may have been an additional reason for the increased microbial biomass C in those treatments. The fraction of microbial residues (fungal glucosamine and bacterial muramic acid) ranged between 74 and 79 %, which is similar to the world wide average of 75 % for arable soils. Consequently, shifts towards fungi or P limitations are unlikely explanations for the large microbial biomass C/N ratio of 12 found in soils during this study. The results of basal respiration rates, C_{mic} and C_{org} levels indicate that the microbial community in the soil from Koukombo is less efficient in substrate use compared to microorganisms in the soil from Fada. However, the continuous carbon input by legume root residues might have contributed to these differences in soil fertility.

The ^{33}P isotopic exchange method was used to compare the effects of legume and cereal root residues compared to a mineral P treatment on P availability in the above described pot experiment. Irrespective of treatments a low buffering capacity was detected in both soils. Total P as well as all isotopic exchange parameters indicated a good soil P status solely with application of mineral P (P_{tot} = 229-246 mg kg^{-1}; n = 0.04; C_P = 61-63 mg kg^{-1}; E_{1min} = 65-66 mg kg^{-1}) while the directly available P pool (E_{1min}) in the other treatments was extremely low ranging between 0.20 to 0.25 mg kg^{-1} (Fada) and 0.14 and 0.17 mg kg^{-1} (Koukombo). Calculated E values increased over time (E_{1min} to $E_{1min-1d}$ and E_{1d-3m}) with application of groundnut root residues while the opposite was found for mineral P. This indicates a slowly release of P due to root turnover while applied mineral P is taken up by plants or fixed to the soil. Due to the fact that sorghum growth reacted mainly to the application of mineral P and the microorganisms solely to the organic inputs, the combination of both amendments seems to be the best approach to a sustainable increase of crop production on many nutrient-poor, sandy West African soils.

In a 53-day pot experiment CC and CL soils from Fada and Koukombo were adjusted to the same level of P and N concentration through mineral N and P application rates equal to the differences between respective nutrient concentrations of CC and CL soils. The treatments consisted of mineral P, mineral N or mineral P+N, compared to a control without any amendments. With the application of both nutrients (mineral P+N), dry matter production of sorghum (Fada) and maize (Koukombo) was increased by 32 % and 15 %, respectively, compared to the control or to solely mineral N addition. Significantly higher values were found on CL soils, compared to the respective treatments on CC soils. Mycorrhizal infection of roots was increased by 46 % and N concentration of plant tissue was 10 % higher on rotation soils. Furthermore, the number of nematodes, predominantly free living nematodes, was almost halfed. In conclusion, increased nutrient availability (especially P and N) through the introduction of legumes is not the only reason for the observed yield increasing effects. Soil biological factors, such as mycorrhizal infection, seem to play an important role as well.

Millet, maize and sorghum were grown on non-sterilized and sterilized (36kGy gamma irradiation) CC and CL soils of Fada and Koukombo. Some sterilized soils were re-inoculated with bacteria solution, inoculated with mycorrhizae (***Glomus mossae*** species) or infected with nematodes (***Pratylenchus penetrans***). The pH gradient along the root-soil-interface was measured at 3 times in 2007 using an antimony microelectrode. The trial was repeated with reduced treatments in 2008. Plant growth was slightly increased on CL compared to CC soils. Although only small amounts of nutrients are released through δ-irradiation in sandy soils, the increased dry matter production on sterilized soils can be attributed to this sterilization induced effect. Infection with nematodes did not hamper plants performance probably due to temporally occurring drought stress in the small root chambers that also hampered nematode’s metabolic activity. Again, positive correlations of mycorrhizal infection rates and plant growth were observed that might be explained by P mobilization through slightly increased pH values. For Fada soils, pH values were higher on CL than CC soils while the opposite was true for the Koukombo soils. Site-specific differences between Fada and Koukombo soils in N content and microbial community structures might have created varying crop performances leading to the contrasting pH findings. However, the mechanisms involved in this highly complex microbe-root-soil interaction remain unclear.

12 Zusammenfassung

In West Afrika, wo die landwirtschaftliche Produktion stark eingeschränkt und an kurze Regenzeiten gebunden ist, stellt die Einbindung von Leguminosen in Fruchtfolgen eine vielversprechende Möglichkeit dar, um die lokale Agrarproduktion zu intensivieren. In vorangegangenen Forschungsarbeiten unter Feldbedingungen in West Afrika führte der Anbau von Augenbohne (***Vigna unguiculata*** Walp) und Erdnuss (***Arachis hypogea*** L.) in einer Saison zu Ertragssteigerungen bei nachfolgendem Getreide (***Pennisetum glaucum*** L.; ***Sorghum bicolor*** Moench; ***Zea mays*** L.), einer Erhöhung des pH-Wertes im Boden, einer früheren Wurzelinfektion mit Mykorrhiza und einer Unterdrückung von phytopathogenen Nematoden. Darüber hinaus gab es Hinweise auf eine spezifische Veränderung der Mikroorganismenpopulation, allerdings sind die Mechanismen dieser Veränderungen noch nicht vollständig geklärt.

In der vorliegenden Arbeit wurden die bodenchemischen und bodenbiologischen Faktoren näher untersucht, die für die ertragssteigernden Effekte von Leguminosenrotationen verantwortlich sind, welche auf überwiegend sandigen, nährstoffarmen Böden aus Westafrika beobachtet wurden. Das Ziel der Studie war zunächst das Erlangen von zusätzlichen Informationen über die Rolle von Pflanzenresten und Mikroorganismen auf den Nährstoffkreislauf der Böden. Des Weiteren sollten unterschiedliche Substratqualitäten (z.B. Wurzelreste) hinsichtlich ihrer Wirkung auf das komplexe Zusammenspiel von Wurzel, Boden und Mikroorganismen beleuchtet werden.

Der Vergleich von Monokultur- (CC) und Rotationsböden (CL) zweier westafrikanischer Standorte (Fada-Kouaré in Burkina Faso, F, und Koukombo in Togo, K) konnte die Annahmen früherer Untersuchungen bestätigen, dass die standort- und managementspezifischen Unterschiede bodenchemischer und bodenbiologischer Messgrößen eine wichtige Rolle spielen um die ertragssteigernden Effekte von Leguminosenrotationen zu erklären.

In einem Inkubationsversuch mit CC- und CL-Böden aus Fada und Koukombo wurde das Einsetzen der Regenzeit als zentrales Stressereignis für die Mikroorganismen mittels Wiederbefeuchten simuliert. Dabei konnten standort- und managementspezifische Reaktionen der Bodenmikroorganismen beobachtet werden. Höhere Atmungsaktivitäten und erhöhte Gehalte an mikrobiell gebundenem Kohlenstoff (C_{mik}) und Stickstoff (N_{mik}), Ergosterol, Muraminsäure, Glukosamin und Adenylaten (ATP, ADP und AMP) wurden in den Rotationsböden gemessen, sowie zusätzlich im CC-Boden aus Fada verglichen mit jenem aus Koukombo. Der sofortige ATP-Anstieg nach Wiederbefeuchtung ist vermutlich auf die Rehydrierung der mikrobiellen Zellen zurückzuführen. Bei diesem Prozess wird kein Stickstoff immobilisiert, der somit den Pflanzen für eine schnelle Jugendentwicklung zur Verfügung steht. Daher scheinen die Mikoorganismen zu den ertragssteigernden Effekten beizutragen, indem sie gerade in den ersten Tagen nach Wiederbefeuchten für eine hohe Mineralisation und somit hohe Nährstoffmobilisierung sorgen. Die standortspezifisch höheren Atmungsraten der Fada-Böden können mit den höheren Kohlenstoffgehalten des Bodens erklärt werden, die von den „rehydrierten“ Mikroorganismen überwiegend als Nahrungs- bzw. Energiequelle genutzt werden. Die höheren Anteile an Ergosterol (Zellwandbestandteil von Pilzen), dem überwiegend pilzlichen Glykosamin sowie der Muraminsäure (Zellbestandteil von Bakterien) in Rotationsböden deuten auf eine Veränderung der spezifischen Zusammensetzung der Zersetzergemeinschaft hin.

Der Einfluss von im Boden verbleibenden Getreide- und Leguminosenwurzeln auf das Pflanzenwachstum von Sorghum und auf mikrobielle Abbauprozesse im Boden wurde in einem Topfversuch (über 59 Tage) und einem Inkubationsversuch (über 189 Tage) geprüft. Dafür wurden je 2 mg Wurzelresiduen (Mais, Perlhirse, Sorghum, Augenbohne, Erdnuss) pro Gramm Boden dem Monokulturboden aus Fada und Koukombo untergemischt und mit Sorghum bepflanzt. Vergleichsvarianten bestanden aus mineralisch verabreichtem Phosphor (P) oder mineralischem P + N, jeweils in mengenmäßig vergleichbarer Höhe zu den Nährstoffen der Wurzelresiduen, sowie einer unbehandelten Kontrolle. Pflanzenwachstum und Trockenmasseproduktion aller Varianten des Fada-Bodens waren signifikant höher im Vergleich zum Boden aus Koukombo. Während die Mineraldüngervarianten die signifikant höchsten Erträge erzielten, erhöhten Leguminosenwurzeln die Trockenmasseerträge noch leicht im Vergleich zur Kontrolle. Die Getreidewurzeln brachten dagegen geringere Erträge als die Kontrolle hervor. Im Boden aus Fada konnten 36 bis 100 % höhere Gehalte an bodenbiologischen Messgrößen (ATP, C_{mik}, N_{mik}, Ergosterol) erfasst werden als in dem Koukombo-Boden. Im Gegensatz zum Pflanzenwachstum rief die Zugabe von mineralischem P keine Reaktion der Mikroorganismen hervor. Im Vergleich dazu und zur Kontrolle, führte die Zugabe des organischen Materials (Wurzelresiduen, egal welcher Pflanze) wiederum zu gesteigerten Gehalten an C_{mic}, N_{mic}, Ergosterol und ATP. Interessant zu erwähnen ist die durch Wurzelresiduen reduzierte Anzahl an freilebenden Nematoden, die sich von Bodenbakterien und -pilzen ernähren, was ein zusätzlicher Grund für die erhöhten Gehalte an mikrobieller Biomasse entsprechender Varianten sein könnte. Die Fraktionen mikrobieller Residuen (pilzliches Glukosamin und bakterielle Muraminsäure) lagen mit 74 bis 79 % dicht am weltweiten Mittel für Ackerflächen. Somit können die weiten C_{mik}/N_{mik}-Verhältnisse nicht durch eine Veränderung der Bodenfauna zu einer von Pilzen dominierten Zersetzergemeinschaft erklärt werden. Messungen der Basalatmung unter Berücksichtigung der C_{mik}- und C_{org}-Gehalte deuten auf eine weniger substrateffiziente Zersetzergemeinschaft im Koukombo-Boden hin. Die wiederholte Kohlenstoffzugabe über Leguminosenwurzeln scheint zu den unterschiedlichen Bodenfruchtbarkeiten beizutragen.

Mittels der ^{33}P isotopenaustausch Methode wurde die Wirkung von Getreide- und Leguminosenwurzeln auf die Phosphorverfügbarkeit im oben beschriebenen Topfversuch untersucht. Als Vergleich dienten eine Behandlung mit mineralischem P und eine unbehandelte Kontrolle. Bei beiden Böden wurde unabhängig der Behandlungen eine geringe Pufferkapazität festgestellt. Der Gesamtgehalt an Phosphor (P_{tot}) sowie alle Parameter der isotopenaustausch Methode deuten nur in der mineralisch gedüngten Variante auf eine gute P-Versorgung des Bodens hin (P_{tot} = 229-246 mg kg^{-1}; n = 0,04; C_P = 61-63 mg kg^{-1}; E_{1min} = 65-66 mg kg^{-1}), während z. B. die Menge des direkt verfügbaren P (E_{1min}) der anderen Behandlungen extrem niedrig ausfiel mit Werten zwischen 0,20 bis 0,25 mg kg^{-1} (Fada) und 0,14 bis 0,17 mg kg^{-1} (Koukombo). Im Laufe der Zeit erhöhte sich die P-Verfügbarkeit (E_{1min} zu $E_{1min-1d}$ und E_{1d-3m}) nach Zugabe der Leguminosenwurzeln. Das Gegenteil war für die mineralische P-Düngung der Fall. Dies deutet auf eine, durch den Abbau der Wurzeln hervorgerufene erhöhte P-Verfügbarkeit hin, während der mineralisch gedüngte Phosphor von den Pflanzen aufgenommen oder im Boden festgelegt wird. Da das Wachstum von Sorghum vor allem durch mineralischen Phosphor angeregt wurde, die Mikroorganismen jedoch ausschließlich auf die Wurzelresiduen reagierten, scheint die Kombination aus beiden Behandlungen die beste Maßnahme für eine nachhaltige Steigerung der Pflanzenproduktion in West Afrika zu sein.

In einem weiteren Topfversuch über die Dauer von 53 Tagen wurden die Monokultur- und Rotationsböden aus Fada und Koukombo durch mineralische Zugabe von P, N oder P+N auf ein jeweils gleiches Nährstoffniveau gebracht und mit einer unbehandelten Kontrolle verglichen. In den mineralischen Behandlungen mit P oder P+N erzielte Sorghum bis zu 32 % höhere Trockenmasseerträge (Fada-Böden) und Mais bis zu 15 % höhere Trockenmasseerträge (Koukombo-Böden) verglichen mit der Kontrolle oder der alleinigen Zugabe von mineralischem Stickstoff. Verglichen mit der gleichen Behandlung des Monokulturbodens erzielten alle Varianten auf den Rotationsböden höhere Erträge. Gleichermaßen war die Mykorrhiza-Kolonisationsrate im Rotationsboden um 46 % und die N-Konzentration um 10 % höher, während nur etwa halb so viele freilebende Nematoden vorgefunden wurden. Schlussfolgernd lässt sich festhalten, dass nicht nur die erhöhte Nährstoffverfügbarkeit (N+P) nach Leguminosen-Rotationen zur Ertragssteigerung bei nachfolgendem Getreide führt, sondern auch biologische Kenngrößen, wie z.B. die Mykorrhiza-Kolonisationsraten, eine wesentliche Rolle spielen.

In einem letzten Topfversuch wurden im Jahr 2007 sterilisierte (36 kGy Gammastrahlung) und nicht-sterilisierte Monokultur- und Rotationsböden aus Fada und Koukombo in Wurzelkästen gefüllt und mit Perlhirse, Mais und Sorghum bepflanzt. Zusätzlich wurden sterilisierte Böden einmal mit einer Bakterienlösung wieder-beimpft, mit Mykorrhiza (***Glomus mossae*** Spezies) inokuliert oder mit Nematoden (***Pratylenchus penetrans***) infiziert. Mittels einer Antimon-Mikroelektrode wurden die pH-Werte in Rhizosphäre und wurzelfernem Raum punktgenau zu drei Terminen gemessen. Der Versuch wurde 2008 mit reduzierten Varianten wiederholt. Ein leicht erhöhtes Pflanzenwachstum auf den Rotationsböden konnte auch in diesem Versuch festgestellt werden. Auch wenn nur geringfügige Nährstoffmengen durch die Gamma-Bestrahlung von sandigen Böden freigesetzt werden, können damit die auf sterilisierten Böden gesteigerten Pflanzengewichte erklärt werden. Die Infektion mit pflanzenpathogenen Nematoden führte vermutlich deshalb zu keinerlei negativen Erscheinungen an den Pflanzen, da kurzzeitig auftretender Trockenstress in den kleinen Wurzelkästen den Metabolismus und die Aktivität der Fadenwürmer einschränkte. Erneut konnten jedoch positive Korrelationen zwischen Mykorrhiza-Kolonisationsrate und Pflanzenwachstum beobachtet werden, welches zusätzlich an einer gesteigerten P-Mobilisierung aufgrund leicht erhöhter pH-Werte liegen kann. Während die pH-Werte des Rotationsbodens aus Fada höher lagen als jene im Monokulturboden des gleichen Standorts, war genau Gegenteiliges für Koukombo der Fall. Die standortspezifischen Unterschiede bezüglich der N-Gehalte und Zusammensetzung der Mikroorganismenpopulation könnten zu unterschiedlichem Pflanzenwachstum und schließlich zu den kontrastreichen pH-Messungen geführt haben. Jedoch bleiben die in dem hoch komplexen Mikroorganismen-Wurzel-Boden-Interaktionssystem beteiligten Mechanismen weiterhin ungeklärt.

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Affidavit – Eidesstattliche Erklärung

I assure that this dissertation was written independently and without non-permissible help and that I used no sources than those specified in the dissertation. All quotations that have been extracted from published or unpublished sources have been marked as such. Third parties, except the named co-authors of chapter 4, 5, 6, 7 and 8, were not involved in the context-related or material generation of this dissertation; in particular I did not engage the service of a PhD consultant (Promotionsberater). No part of this work has been used in other PhD processes.

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