Utilization of alternative phosphorus resources by regenerative agriculture practice based on mycorrhizal fungi and biostimulants for restoration of Lake Chamo watershed, Ethiopia

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Abstract

Issues of degradation of soil and water resources, shrinking biodiversity and low agricultural output in Sub-Saharan Africa are accelerated by global climate change, worsening struggle for sustainment of livelihood of a rapidly growing population in Africa within the next decades to come. Additionally, declining resources of phosphate raw material in quantity and quality needed for fertilizer production may worsen the situation as modern agriculture is fundamentally dependent on fertilizer usage.

On a global scale, prioritizing SSA, restoration of degraded soil and water resources is inevitable, but must acknowledge the intensification of agricultural production in SSA in coming decades as a need of highest urgency. However, with lacking infrastructure and financial restrictions, usage of common fertilizer by small-scale subsistence farmers in rural areas of developing countries often is low. As soils in SSA are notoriously deficient in nutrients, and thus often show high P fixation properties, fertility and hence yields are low in general.

Thermal-treated bone char from recycled animal bones could serve as a low-cost, sustainable, novel P fertilizer in organic agriculture. Producible on-site from an untapped waste resource, bone char comprised of biological hydroxyl apatite can be utilized efficiently for enhanced plant P supply by mediation of mycorrhizal fungi and P solubilizing soil bacteria.

Mycorrhizal fungi serve as a biological soil infrastructure for the exchange of nutrients, water and information between host plants, strengthening soil biology by enhanced formation of stable SOM complexes. Consequently, mycorrhizal fungi improve host plant resistance to abiotic stress of drought, salinity, toxicity as well as biotic stress of pest and pathogen attack along with biocontrol against weeds, especially in cooperation with other plant growth promoting fungi of the order Sebacinales and soil bacteria. For their proliferation and facilitation of stable SOM formation in soils, bio char from woody waste materials is incorporated in the system as another soil amendment. The usage of biostimulants as an emerging technology for low-cost, rapid enhancement of plant growth can be utilized as well to ensure stimulation of soil and plant health and fostered biomass production and crop yields accordingly, to achieve increased productivity and profitability in short transition time.

All measures are integrated in a holistic approach based on interconnected principles of regenerative agriculture, agro-forestry, system of crop intensification and holistic planned grazing to constitute a multi-layered, agricultural management system for the productive restoration of degraded land and water resources, designed for tropical regions in developing countries, focusing on resource-poor small-scale subsistence farmers in rural areas.

In pot experiments conducted 2018/2019 in Arba Minch, Ethiopia, it was found, that bone char can serve as an alternative fertilizer material in tested soil and environmental conditions, as biomass production was increased by +100 % over untreated control of *Cajanus cajan*. Tested biostimulants however did not display positive or negative effects on biomass production and seem susceptible to abiotic stress conditions, especially when soil fertility and certain soil minerals may be lacking.

Concluding, a holistic agricultural system was designed in this conceptual study, enabling farmers to restore degraded land and water resources while intensifying their agricultural production. By activation of untapped waste resources, locally available and producible, bone char could serve as an alternative low-cost, kick-starter fertilizer material to be utilized in soils by enhanced soil life in general, and mycorrhizal activity specifically. Crop yields could be increased, while degraded soils are rebuilt in their fertility and value for services of water storage and climate change mitigation.

Author keywords: bone char; bio char; biostimulants; mycorrhizal fungi; regenerative agriculture; productive restoration.

Table of Contents

I. List of Figures

II. List of Tables

III. Abbreviations and Symbols

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
AEC	Anion exchange capacity
AM	Arbuscular mycorrhizal
APX	Ascorbate peroxidase
BioC	Biochar
BoneC	Bone char
CaP	Calcium phosphate
CAT	Catalase
CEC	Cation exchange capacity
CM	Cow manure
CMN	Common mycorrhizal network
DAS	Days after seeding
G3P	Glycerole-3-phosphate
GR	Glutathione reductase
HA	Hydroxyl apatite
HS	Humic substances
IAA	Indole acetic acid
MDA	Malondialdehyde
MLE	Moringa leaf extract
NP	Nanoparticles
OCP	Octacalcium phosphate
PAL	Phenylalanine ammonia-lyase
PGPB	Plant growth promoting bacteria
PGPF	Plant growth promoting fungi
PH	Protein hydrolysates
POD	Peroxidase
ROS	Reactive oxygen species
Rubisco	Ribulose-1,5-bisphosphat-carboxylase/-oxygenase
SA	Salicylic acid
SAR	Systemic acquired resistance
SSA	Sub-Saharan Africa
SOC	Soil organic carbon
SOD	Superoxide dismutase
SOM	Soil organic matter
SON	Soil organic nitrogen
SCI	System of crop intensification
SRI	System of rice intensification
TPS	Terra Preta Sanitation
TSP	Triple Superphosphate

Elements

Al	Aluminium
As	Arsenic
B	Boron
C	Carbon
Ca	Calcium
Cd	Cadmium
Cl	Chlorine
Cu	Copper
Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel

P	Phosphorus
Pb	Lead
S	Sulfur
Se	Selenium
Si	Silicon
Ti	Titanium
U	Uranium
Zn	Zinc

Chemical formula

NH_3	Ammonia
NH_4^+	Ammonium
CO_2	Carbon dioxide
$H_2PO_4^-$	Dihydrogen phosphate
N_2	Dinitrogen
H^+	Hydrogen
H_2O_2	Hydrogen peroxide
HPO_4^{2-}	Hydrogen phosphate
H_2S	Hydrogen sulfide
OH^-	Hydroxide
NO_3^-	Nitrate
NO	Nitric oxide
N_2O	Nitrous oxide
SiO^{2-}	Silicic acid
$NaCl$	Sodium chloride
$NaHS$	Sodium hydrosulfide
Na_2SO_4	Sodium sulphate
H_2SO_4	Sulfuric acid
TiO_2	Titanium dioxide

1 Introduction

1.1 Motivation of this work

In latest times, climate change became one of the most discussed issues for humankind. However, it is just one of major issues of global dimension to be faced by humankind in the next decades. Often unheard or barely communicated in the public, issues of ongoing soil degradation, shrinking biodiversity, decline of fossil phosphorus raw materials, low levels of agricultural production in Sub-Sahara Africa (SSA), coupled with tremendous population growth in the next decades, are likely to overlap – and to worsen – with changing environmental conditions such as climate change. Potential effects of this superposition could be – and partly already are – dramatic in their ultimate consequence. Already today, ethnic and national conflicts for land and water resources and following, displacement and migration process on the national, regional and global levels as well as political instability in many regions of the world, are on the rise, with fanatism, terror and war as just the most severe, visible consequences.

To once again turn tables around and to stop and reverse detrimental anthropogenic impact on the environment, senseful combination of new insights in various professions of environmental, agricultural and soil science could create strong synergistic effects, needed for the reversal of the contrasts of unproductive restoration and degrading agriculture. Instead, the creation of adapted, holistic agriculture systems for productive restoration of degraded land and water resources can be facilitated, benefitting people and the environment, again on the local, regional, national and global level. Here, the acknowledgment of the needs and constrains of foremost targeted, resource limited people in rural areas in developing countries is crucial to gain acceptance and willingness for any change, where it may be most needed. Such an exemplary holistic system is designed in this study.

Over the shorter term, producing a sufficient harvest is often a question of financial investment rather than specific agricultural knowledge, penalizing resource limited farmers. Facing environmental destruction from decade-long conventional agriculture, state of the art practices should be re-considered and evaluated for room for improvement, especially in low-yielding regions, struggling to bear sufficient harvests to nourish its population by quantity and quality.

With shrinking resources, changing environmental conditions and lacking production capacity of food and feed on hand, directly affected people should not be simply viewed as helpless victims, but as potential, self-sustaining executives of recently emerging approaches of environmental restoration, targeting the regeneration of our all very base of life: Fertile soil.

1.2 Organization of this work

In chapter 2, global issues of population growth, soil degradation, change of environmental conditions and declining mineral phosphate resources and their possible interconnected effects on an already fragile agricultural production in SSA are highlighted.

Chapter 3 reviews in depth the difficulty of phosphate for agricultural production with special focus on SSA and addresses bone char as a possible alternative, organic fertilizer material in small-scale subsistence farming in rural regions. Following, a set of various measures including plant growth promoting fungi and bacteria, biochar and trace elements as soil amendments and plant additives with biostimulatory effect on plant performance are discussed.

In chapter 4, all measures are integrated in a broader holistic approach for adapted agricultural management with the primary intent to increase efficiency of bone char as a feasible alternative P fertilizer material and soil P in general as well as to contribute to overall intensification of agricultural production and restoration of soil status, based on regenerative agricultural management. Here, also a step-by-step guideline for implementation of the proposed system is presented.

Chapter 5 deals with the experiments performed in this study about soil amendments of bone char and biochar to constitute alternative fertilizer materials as well as plant additives to serve as biostimulants for enhanced plant production in rural areas of Arba Minch region, Ethiopia.

Chapter 6 presents a case study of Lake Chamo region, Ethiopia, in which the proposed system is blueprinted for both small-scale subsistence farmers and commercial banana farmers in the Lake Chamo watershed.

Chapter 7 summarizes the findings and concepts of this study and is closed by further recommendations for future research on the topics discussed.

2 Global issues and aim of study

2.1 Demographic development and agricultural production levels

Global population is expected to rise from around 7.6 billion in 2018 to over 9.9 billion in 2050 (World Population Data Sheet, 2018). While global fertility rates are declining from 2.5 to 2.2 in the same time span, they will remain highest in Africa at 3.0 (decreased from 4.5 children per woman in 2015) (UNICEF, 2014). Accordingly, Africa will have the major share of increasing world population and will face a remarkable increase of inhabitants, more-than-doubling, from 1.284 billion in 2018 to almost 2.2 billion in 2050, with Nigeria (increase from 195.9 to 410.6 million inhabitants), Democratic Republic of Congo (increase from 84.3 to 215.9 million inhabitants) and Ethiopia (increase from 107.5 to 190.9 million inhabitants) with the greatest absolute numbers of births in Sub-Saharan Africa (SSA) (UNICEF, 2014; World Population Data Sheet, 2018). Consequently, also the total population density will double from 39 to 80 people per square kilometre between 2015 and 2050 (UNICEF, 2014). Further, as the greatest fertility rates are among the least wealthy quantiles of population of African countries in general, the major population growth can be expected in rural areas of the region (UNICEF, 2014). However, the share of urban population is expected to increase over time, to more than 56 % in 2050.

The changing relation between shares of urban and rural population could alter the already fragile balance between food producing and food consuming population, with imposing higher pressure on a shrinking share of rural people to “feed” a rising share of urban people, with less working force.

When only arable land is considered, population density for SSA countries in 2018 was already at 546 people per km^2 (World Population Data Sheet, 2018). Doubling population bears extreme and drastic consequences for food production and food security in SSA potentially, as total area of arable land is likely to diminish by soil degradation and changing environmental conditions, while overall agricultural output per area already is low.

Table 1: Change of crop productivity and average cereal yield in comparison of global regions between 2000 and 2016, after The World Bank Group (2017).

Region	Crop production index 2004 - 2006 = 100		Cereal yield kg/ha	
	2000	2016	2000	2016
World	87.8	128.1	3,089	3,967
East Asia & Pacific	85.4	133.1	3,998	5,071
Europe & Central Asia	93.2	112.3	2,891	3,785
Latin America & Carribean	82.2	134.7	2,852	4,178
Middle East & Northern Africa	80.4	110.0	1,818	2,626
North America	94.7	116.3	5,131	7,368
South Asia	91.5	141.1	2,376	3,132
Sub-Saharan Africa	83.2	135.4	1,182	1,400

Although crop productivity and average yields were improved over the last decades in SSA, total agricultural output per hectare remains low in global comparison (see Table 1). In the eye of rapid population growth in the next decades as well as the current low yields per area in SSA, there is strong urgency for intensification of agricultural production in order to establish and maintain food and water security.

2.2 Soil degradation

Soil degradation not only in Africa but globally is a constraining issue for humankind. Soil degradation can be seen as processes leading to deteriorating ability of the soil to bear harvest of food, feed, fuel or fibre and to serve for other ecosystem functions of water generation, storage of nutrients and carbon (C) and a climatic buffer. Those processes include wind and water erosion, decline in soil organic matter (SOM), decline in soil biology, nutrient imbalances, loss of biodiversity, salinization of soils, as well as contamination, acidification, compaction, sealing, crusting and waterlogging (Karlen and Rice, 2015). Currently, 24 % of the global land area are considered degraded (FAO and ITPS, 2015).

Ongoing soil degradation on a global scale finds expression indirectly by declining efficiency of nitrogen (N) fertilizer over the last decades. While in 1960 80 % of N fertilizer inputs were recovered in harvest output, this value decreased to below 30 % in 2000 (Tilman et al., 2002). Hence, lowered N fertilizer efficiencies decrease the profitability of agricultural operation and increases the risk of environmental pollution by excess N. Also, fertilizer consumption varies strongly between different world regions. While in some regions with almost saturated soils, only removed nutrients are replaced (especially western countries), in others, soils are charged with nutrients (especially P in Asia), while again in others, nutrient removal by crop harvest is far outperforming nutrient addition (especially in SSA; see Figure 1) (FAO, 2013; FAO and ITPS, 2015).

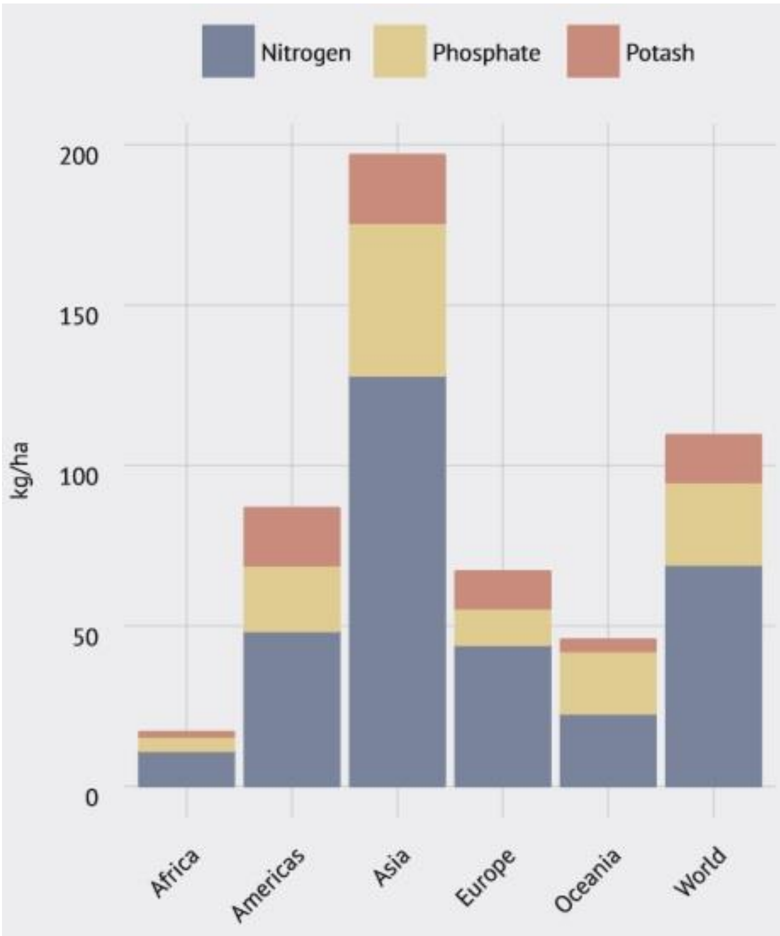

Figure 1: Fertilizer consumption in nutrients per ha of arable land in gobal comparison (2009), from FAO (2013), p. 16.

In SSA, the majority of people is still dependent on fuelwood or fuelwood derived materials like charcoal for primary energy consumption. This led to strong pressure on natural forest areas and gradual deforestation in most parts of SSA (Rudel, 2013), which contributed to a change in micro climate and substantial greenhouse gas (GHG) emissions (Pan et al., 2011).

Next for the gain of fuel wood or material for charcoal production, natural forest areas in SSA often are utilized for expansion of agricultural sites in order to cope with low agricultural yield potential of existing arable lands. Yet, lowered SOM content of agricultural soils and less dense or only temporally vegetative cover by agricultural operation leads to higher rates of erosion and loss of soil fertility, hence contributes to soil degradation processes (Bot and Benites, 2005).

Again, current rates of soil degradation and the diminishing capacity of degrading soils to bear sufficient harvests for nourishing a growing population demands massive response in terms of soil restoration approaches and adapted intensification of agriculture in SSA.

2.3 Changing environmental conditions

Global soil degradation contributes to changing environmental conditions like climate change, as GHG are emitted from mineralized SOM. Precipitation water cannot be retained or infiltrated and stored, but is drained of in destructive surface runoff, while wind velocities above ground cannot be reduced by natural vegetation, further increasing drying of the soil and the overall ecosystem, with negative impact on the micro climate (Kravcik et al., 2007; Liniger et al., 2011).

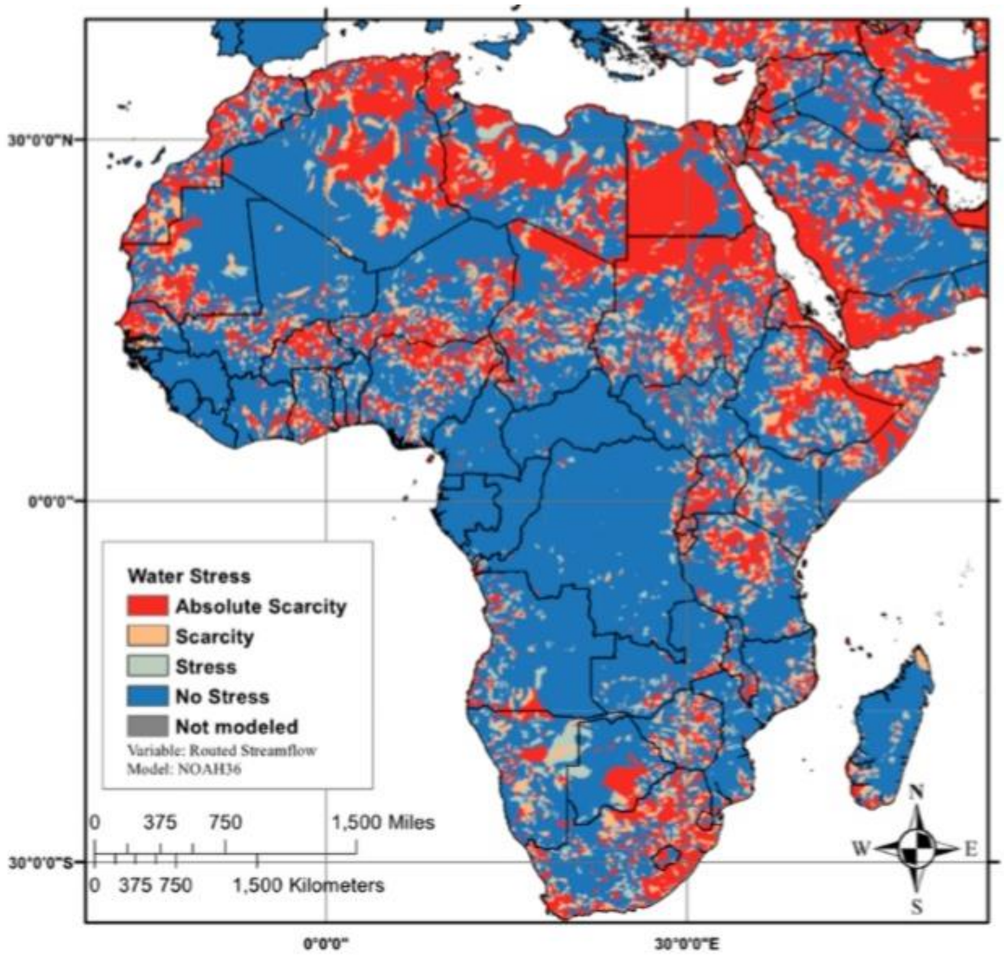

Figure 2: Acute water scarcity monitoring in Africa in January 2019 using Falkenmark Index categories (no stress = >1700; stress = 1000 – 1700; scarcity = 500 – 1000; absolute scarcity = <500 m³/year/capita), from McNally et al. (2019), p. 6.

According to recent acute water scarcity monitoring in Africa by McNally et al. (2019), large parts of northern, eastern and southern Africa are struck by severe water scarcity due to general population growth and declining annual mean streamflow (see Figure 2).

Following pronounced water scarcity and ongoing soil degradation, agricultural production is expected to decrease in most parts of Africa until 2050, when compared to current production levels (FAO, 2018; World Bank, 2010).

Only worsening the threat of food and water insecurity, issues of soil degradation and changing environmental conditions must be tackled by development of drought resistant, high-intensive agricultural production systems to intensify agricultural production in SSA, despite difficult soil conditions typical for the region. Simultaneously, any alternative agricultural system needs to cope with the strict need of resource-poor farmers to produce a sufficient first harvest within the first season of implementation or transition. As fertilizer consumption is chronically low in SSA (see Figure 1), as a starting point, improved soil nutrient content should be considered as a fundamental base for enhanced food production in SSA.

2.4 Phosphorus as an essential, but fossil resource for global food production

Phosphorus (P) as one of the main nutrients for plant growth, is of essential status for functioning vegetation and crop growth and in broader view, vital for any proliferation of living organisms (Amelung et al., 2018; Steen, 1998; Syers et al., 2008). Although P is naturally occurring in soils in various forms of inorganic and organic P, the concentration of plant available P in the soil solution is low due to its high reactivity with soil constituents. Replenishment of P by dissolution of inorganic P and mineralization of organic P and overall desorption of easily bound P, followed by diffusion processes for transportation towards the receiving roots in the rhizosphere of the plant, is slow and often not sufficient to meet the P demand of plants (Kirkby and Johnston, 2008; White and Hammond, 2008).

To enable high crop yields, in conventional agriculture the application of P containing fertilizer derived from acidified rock phosphate is common (Cordell et al., 2009; Steen, 1998). Triple Superphosphate (TSP) is a water-soluble fertilizer comprised of monocalcium phosphate, which can supply substantial amounts of immediate available P for plants (Smil, 2000). Anyhow, under strong acidic or alkaline soil conditions, solubilized P ions can easily adsorb to oxides of iron (Fe), aluminium (Al) or calcium (Ca) or even precipitate as phosphates accordingly, which again are only slightly soluble (Syers et al., 2008; Tiessen, 2008). Hence, the handiness and advantage of water-soluble fertilizer over soil P reserves may easily be diminished in a short time fraction after application in unfavourable soil conditions (Jalali, 2006; Mengel, 1997).

Over the last decades, in many areas of the world, the typical low fertilizer recovery rates by plant uptake on the one side and rapid P fixation on the other side led to a high P status of soils close to or above saturation (FAO, 2006; Römer, 2013; Tiessen, 2008). Predominantly this is the case for developed regions with intensive agriculture practice, as only around 10 to 25 % of added P is utilized by plants in the first season, while the majority is reversibly fixed in the soil matrix (Johnston and Poulton, 2019; Syers et al., 2008). In contrast, in developing countries, mostly in tropical regions, where infrastructure and purchasing power for agricultural goods is often not sufficient, usage of P fertilizer is much less common, while leaching and erosion of P is intensified by poor agricultural and environmental practice and intense rainfall characteristics (Smaling et al., 2006). Ultimately, agricultural soils in these regions often suffer from deficiency or availability of soil P, leading to low crop yields accordingly.

Exacerbating the situation, rock phosphate is a fossil, non-renewable resource, with deteriorating reserves in quantity and quality, estimated to last another 60 to 150 years (Smil, 2000; Steen, 1998; Vance et al., 2003). The remaining rock phosphates often is of lower quality, as contamination especially with cadmium (Cd) and uranium (U) is more and more common, leading to impure products with potentially toxic side effects on the environment (Kirkby and Johnston, 2008; Pinnekamp et al., 2011; Rothbaum et al., 1979; Smil, 2000).

Humankind will face more and more a decline in suitable P fertilizer supply, while the need to increase agricultural yields is rising. Alternative pathways for more efficient usage of fossil P resources, P recycling from waste water and agriculture wastes and enhanced utilization of P loaded soils by adapted soil and plant management seem inevitable (Cordell et al., 2011; Vassilev et al., 2013).

2.5 Aim of study

All things considered, inorganic P fertilizer can be seen as one of the main foundations for development of humankind in the 20th century, enabling economic and population growth to today's levels. Nevertheless, as high-grade reserves are declining, availability of fertilizers in needed quantities and quality will become more restricted in the coming decades, especially in developing countries with already struggling access to sufficient production resources. Yet, in these countries, most of the future increase of world population will take place (World Population Data Sheet, 2018). Accordingly, the necessity for increased agricultural production in these regions will be of high urgency. However, extension of agricultural fields often goes hand in hand with deforestation of already limited forest stocks and soil degradation, further aggravating the severe consequences of the changing local micro and the global macro climate and corresponding water cycles (Kravcik et al., 2007). As water resources are already scarce, drought events and the thereby expected stagnation or decline in agricultural yields in many of those regions will further exacerbate the struggle for survival (WWAP (United Nations World Water Assessment Programme)/UN-Water, 2018).

For proliferation of humankind in the decades to come, all those issues of declining resources, soil degradation, environmental changes and thereby conditioned yield decline need strong respondance from the world community, as social, cultural, economic and environmental consequences otherwise could be catastrophically – on region, national, continental, global levels.

Contributing to that cause, in this conceptual study, a holistic approach for productive restoration of degraded soil and water resources based on regenerative agricultural practice and the usage of locally available organic soil amendments is developed. In this approach, the decline of resources and deteriorating environmental conditions in Africa are to be acknowledged as well as the urgent need for fast transition and economical sound practice.

Concluding, the overall aim of the approach is the rapid transition towards regenerative, organic agriculture, intensified in its production capacity by quality and quantity, without creating further dependency on external, fossil production goods, while degraded land, water and biosphere resources are restored in their functionality, diversity and sustainability.

As a key starting point for transition, agronomic measures to increase P supply to plants from unavailable soil reserves and organic P fertilizer materials are reviewed and integrated into the holistic system. Further, other suiting soil and plant amendments as well as regenerative management practices are integrated to further increase efficiency of soil P utilization in general as well as to enable agricultural intensification and resilience to biotic and abiotic stress. Based on comprehensive literature review and pot experiments performed in 2018/2019 in Ethiopia, an adapted agricultural management system is designed with specific focus on practicability and realisation in the watershed of deteriorating Lake Chamo, Arba Minch region, Ethiopia, serving as a case study.

3 Literature review

It became apparent, that over the next decades, global intensification of agricultural crop production is a necessity for establishment and maintenance of food security for a growing world population. Albeit, approaches for intensification may vary for different world regions with different natural, geographical, climatic, historical and social conditions. Predominately, in places of highest population growth, namely SSA, immense efforts are needed, as fertile soils and natural ecosystems are degraded and partly already lost in an accelerating fashion (FAO and ITPS, 2015), resulting in low crop production efficiency per area of cultivated land in global comparison.

However, many limitations for pursuing development of the conventional agriculture sector in SSA as well on the global scale lay ahead, of which a decline of fossil P fertilizer base materials in quantity and quality is only one, even if often overlooked.

Certainly, agricultural food production immensely relays on sufficient soil nutrient supply. Therefore, to enable access to sufficient plant nutrition in regions so far struggling, new approaches of alternative P fertilizer materials from recycled organic wastes, or more in general, adapted agricultural management for utilization of soil nutrients including fixed P reserves, may be suitable approaches in Africa of nowadays, but also for other regions in the future to come.

Consequently, bone char as such an alternative, renewable P fertilizer is discussed in the following, including adaptations and additions of the agricultural system to meet the needs of resource-poor, small-scale subsistence farmers as a functional, economic and ecological sound fertilizer practice, enabling improved crop production and benefits to other ecosystem services over the short, middle and long-term.

3.1 Bone char as an alternative P fertilizer in rural small-scale farming

3.1.1 P general

P is an essential nutrient, vital for all living organisms, involved in many physiological and biochemical processes of plants (Steen, 1998; Syers et al., 2008).

P is abundantly present in soils in substantial quantities in various forms of inorganic and organic P in the range of 100 to 3,000 mg P*kg^{-1}, which translates roughly to about 200 to 6,000 kg P contained in 20 cm of topsoil per hectare (Amelung et al., 2018; Mengel, 1997; Smil, 2000; Tiessen, 2008). Yet, only a small fraction is solubilized in the soil solution and therefore plant available in the form of Dihydrogen phosphate ($H_2PO_4^-$) or Hydrogen phosphate (HPO_4^{2-}) ions. In fact, several constrains limit P availability to plants in soils for different reasons, eventually resulting in P deficiency and hence P limitation of plant growth.

3.1.2 P soil behaviour

P in soils is comprised of inorganic and organic fractions, differing in their chemical composition and extractability by plants. Inorganic P can be adsorbed to outer and inner surfaces of other soil constituents like clay minerals, organic matter and oxides of Fe and Al. Also, depending on the P sorption capacity of the soil, inorganic P can precipitate as phosphates in reaction with Fe and Al in acidic or with Ca in alkaline soils, respectively, or can be bound in primary minerals. Organic P is found in living soil microorganisms and plant and microbial residues, in which it is present as derivates of inositol hexaphosphate ($C_6H_{18}O_{24}P_6$) (Amelung et al., 2018; Kirkby and Johnston, 2008).

For inorganic P in the soil matrix to become plant available, P can be desorbed from the surfaces of adsorbent soil constituents by ligand exchange, in which adsorbed P are substituted by hydroxide (OH^-) anions, soil anions like silicic acid (SiO^{2-}) or organic acids including carboxylates of citrate, malate or oxalate, all competing for the same binding sites. Also, P can be mobilized by these carboxylates by chelation with metal cations (Fe or Al in acidic, Ca in alkaline soils) that formerly complexed with P, which then is released in the soil solution as plant available P. Accordingly, P mobilization is enhanced by increasing OH^- concentration in the soil solution, respectively by a higher pH value achievable by soil liming (Amelung et al., 2018; Syers et al., 2008).

Dissolution of inorganic P from minerals like apatite is slow in general and considerable only in acidic soil environments or under strong plant root, microbial or fungi mediated proton and acid release, as under the influence of high H^+, but low Ca^{2+} and OH^- concentration (Koele et al., 2014; Mengel, 1997).

Concentration of organic acids, including humic and fulvic acids, can be enhanced by decomposition of added organic matter to the soil and by the inoculation of P solubilizing microorganisms, producing organic acids (Alori et al., 2017; Kirkby and Johnston, 2008; Mengel, 1997; Parfitt et al., 1975; Schaller et al., 2019; Sharma et al., 2013).

Organic P contributes to the P ion concentration in soil solution through decomposition and mineralization. Hydrolysis of organic P is induced and mediated by the release of enzymatic phosphatase from plant roots, P solubilizing bacteria and arbuscular mycorrhizal (AM) fungi in response to P deficiency (Alori et al., 2017; Feng et al., 2002; Kirkby and Johnston, 2008; Richardson and Simpson, 2011; Vance et al., 2003) and can be essential for P supply in highly weathered, tropical soils deficient in Ca and Si, resulting in low pH and high P fixation characteristics (Barrow et al., 1977; Mengel, 1997).

When fertilizer rich in P is added to the soil, bacterial activity can be enhanced, depending on the C:P ratio of added fertilizer substrate and SOM content of the soil in general. As microorganisms take up P and utilize it in their metabolism, at least temporarily, considerable amounts of added P can become immobilized and therefore plant unavailable (Marschner, 2008). Only after decay of the microorganisms, P becomes available in the soil matrix again (Mengel, 1997).

The removal of solubilized P from soil solution by plant uptake in the rhizosphere creates a steep concentration gradient between the depleted root zone and the bulk soil, which drives the slow process of diffusion transporting soluble P along the gradient towards the lower concentration. This process is highly dependent on sufficient soil moisture and favourable, porous, non-compacted soil structure, thus can be very slow in dry or compacted soils, often not able to meet plant demand of P under these conditions (Bolan, 1991; Kirkby and Johnston, 2008; Mengel et al., 2001).

Due to its high reactivity in soil, once solubilized, P can easily again be re-adsorbed or re-fixed in the soil matrix (Syers et al., 2008; Tiessen, 2008), depending on the pH value, the P buffer capacity and the equilibria between the different P pools in soil, when diffusing through the soil matrix. Hence, many soils contain considerable amounts of total P, but are deficient in its plant available form, limiting plant growth.

The different P pools in soils can be distinguished by the plant availability of contained P fractions. Immediately available P in soil solution is considered as the first P pool. The second pool of soil P is constituted by readily available P, adsorbed on the surface of soil constituents (clays, SOM, soil aggregates), which is in equilibrium with the first pool. When the P concentration in the soil solution drops due to plant uptake, it can easily be transferred into solution. The P buffer capacity, and hence the critical “active” P supply ability of soils to keep P in solution by immediate replenishment of removed P, is determined by these first two P fractions and decisive for adequate plant growth over the short-term. Over time, adsorbed P can diffuse into internal structure of the adsorbent and becomes absorbed accordingly, while the vacant binding site on the outer surface can again bind additional P. This occlusion results in a much less plant extractability of absorbed P, comprising the third P pool fraction. The last P pool distinguished shows very little extractability of P and is mostly constituted of P precipitates of Al and Fe or Ca and mineral P. In calcareous soils with a higher pH, P can easily precipitate in reaction with Ca as Octacalcium phosphate (OCP). Similar, in acidic soils, quickly after application, P from water-soluble fertilizer can become fixed by Fe or Al (Parfitt et al., 1975). This P pool only can be accessed for P supply over long time periods or by corresponding effects of alteration of soil conditions and enhanced activity of soil biology in the rhizosphere of plants (Kirkby and Johnston, 2008; Syers et al., 2008) (see Figure 3).

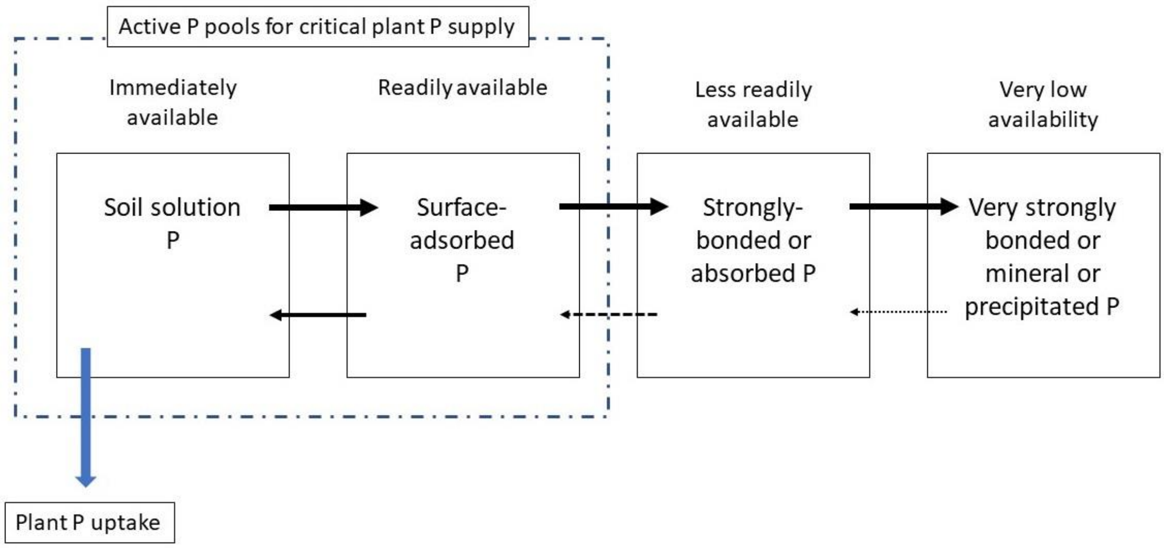

Figure 3: Conceptual diagram for soil P pools, categorized in terms of their P availability for plant P supply. Wibbing (2020), adapted from Johnston and Poulton (2019), p. 9.

As the P concentration in soil solution is low in general and quickly depleted in growing stage of plants, while only slowly replenished, water-soluble, inorganic P fertilizer are used in conventional agriculture in order to maintain critical P supply for crops.

3.1.3 Conventional P fertilizer

Inorganic fertilizers like TSP containing mostly water-soluble monocalcium phosphate are produced from sparingly soluble mineral or sedimentary rock phosphates by acidification with sulfuric or phosphoric acid, reaching a P content in the final fertilizer product of around 20 % (FAO, 2006). Average concentration of P in the source material is low, therefore high quantities are needed. This is coupled with the generation of phosphate gypsum as a substantial waste stream to be disposed, containing most of the toxic elements from the source material with potentially hazardous effects on the environment (Smil, 2000).

Applied to the soil, inorganic P fertilizers release plant available P ions into the soil solution in contact with water, which is taken up in the rhizosphere and utilized in plant growth and maintenance (White and Hammond, 2008).

In contrast, organic fertilizers are derived from residues of animal farming and slaughtering, from composting of crop residues or utility plants or from treatment of wastewater, all representing a renewable P source from waste streams. However, as only small fraction of organic fertilizer is directly plant available, first the organic matter needs to undergo decomposition and mineralization in the soil matrix. To enable these microbiological processes in the receiving soils, C status of soils should be managed by co-application of decomposable C sources and enhancement of C content in soils through stable SOM formation induced by adapted management in general (Amelung et al., 2018; Bot and Benites, 2005).

Today's agriculture is heavily dependent on fertilizer application (Cordell et al., 2009; Smil, 2000). In many acidified soils with little content of organic matter, fixation of P is strong, which makes only a minor fraction of the added total P plant available for a short time period after application. In long-term experiments, Johnston and Poulton (2019) found, that only around 13 % of added P fertilizer would be utilized in the first two P pools, in plant available form, thus contribute directly to crop production. The large majority of P was fixed in the plant unavailable P pools, significantly reducing the short-term efficiency of P fertilizer in non-saturated soils. However, in contrast to former common understanding, this fixation in the soil matrix is majorly reversible, increasing the P recovery efficiency of inorganic fertilizer up to 90 % over a lengthy time scale, when post-application season effects are considered (Barrow, 1983; Hernandez et al., 2005; Jalali, 2006; Kirkby and Johnston, 2008; McDowell and Sharpley, 2003; Smil, 2000; Syers et al., 2008). However, the recovery rate of applied P fertilizer is highly depending on soil conditions, activity of soil biology and plant management and selection and can remain low, especially in economic terms.

Nevertheless, this "underutilization" of applied P fertilizer led to accumulation of substantial P loads in most soils under intensive agriculture practice near to or above the saturation point (FAO, 2006; Römer, 2013; Tiessen, 2008) and is shifting the economic output from the costly input over many years into the future. In regions with highly limited purchasing power or adequate infrastructure (e.g. SSA), P fertilizer consumption therefore can be uneconomically and thus is low in general, which contributes to soils deficient in plant available P and low agricultural production (FAO and ITPS, 2015; Smaling et al., 2006; Steen, 1998). However, as demand is rising and high-quality resources are running low, a decrease in prices, and thus better availability in SSA, is not to be expected in the future.

Phosphate containing ore is a fossil, non-renewable resource, with limited reserves and resources mainly in Morocco, China, USA and Russia. Studies on the existing global P reserves vary, as global reserves (mineable with current technology and costs) are stated to last between 60 and 150 years under current extraction rates (Smil, 2000; Steen, 1998; Vance et al., 2003). Anyhow, it is clear, that P is a non-substitutional, fossil resource, highly needed for food production. As the world market is dominated by only a few producer countries, fertilizer prices also are under influence of political agendas and instability, market restrictions and trade disputes (Cordell et al., 2009). Furthermore, it is evident, that quality of rock phosphate reserves is declining, while costs for mining, processing and waste disposal are increasing, leading to increase in contamination and prices of fertilizer (Pinnekamp et al., 2011; Smil, 2000).

As reserves of high-grad rock phosphate are more and more depleted, declining fertilizer quality by increasing contamination with toxic heavy metals (e.g. Cd) and radionuclides (e.g. U) is reason for concern, thus limits of toxic elements contained in fertilizer were set (Smil, 2000). Although U is not likely to be taken up by plants, it can accumulate by long-term fertilizer application in the topsoil and be transported by erosion or leaching into corresponding water bodies with potential hazardous effects on aquatic life or drinking water (Kirkby and Johnston, 2008; Rothbaum et al., 1979). In the case of Cd, plant uptake mainly seems to be driven by soil pH. It was shown, that the Cd uptake of crops can indeed be enhanced, when inorganic P fertilizer containing Cd was applied over long-term to acidic soils (Nicholson et al., 1994).

Another environmental issue arising from intensive usage of inorganic P fertilizer is the increasing nutrient concentration of P in water bodies, contributed by the nutrient flow from overfertilized agricultural soils towards water bodies by erosion, causing eutrophication (Kirkby and Johnston, 2008).

Therefore, high soil P content and regulating framework limit the surplus application rates of P fertilizer in industrialised countries (Kratz et al., 2010), as often only light application of P fertilizer is needed to replace the P lost in harvest in P loaded soils (FAO, 2006). Nevertheless, global fertilizer demand is expected to increase, as global population continuously increases and diet habits are changing towards a more P consuming meat and dairy based diet in course of economic growth and wealth development in many parts of the world, e.g. urban areas of SSA, India and China (Cordell et al., 2009).

A decrease of P consumption could be achieved socially through change towards a less meat and dairy based diet and technically through more efficient fertilizer application, utilization of loaded P reserves in agricultural soils, change of agricultural system with plants adapted to P deficiency and activation of alternative, re-usable P sources from waste streams, such as struvite, humanure, sewage sludge or slaughterhouse wastes (Cordell et al., 2011, 2009; Szymanska et al., 2019; Vassilev et al., 2013).

Anyhow, as P is one of the most important resource for the existence of humankind, new approaches have to be developed to minimize the dependency on a fossil, declining resource. In accordance with needed efforts for improved recycling of P containing organic wastes, there is strong agreement, that enhanced utilization of soil P reserves can support sustainable P usage for secured global food production in the future (George and Richardson, 2013; Mengel, 1997; Vance et al., 2003).

3.1.4 Conventional P fertilizer in Sub-Saharan Africa

Soils in SSA are highly variable in their composition and properties. Soils can often be highly weathered, strongly acidic or alkaline and high in clay contents dominated by kaolinite. Generally, due to rapid mineralization or lack of biomass production, SOM fraction of most soils is low, which contributes to poor soil structure and low nutrient availability or content, especially of P and N (Bot and Benites, 2005; FAO and ITPS, 2015).

When mobile base anions of Ca, magnesium (Mg) and silicon (Si) are leached from those soils, acidity often is high, enhancing mobility of Fe and Al. In contrast, in calcareous soils, Ca is readily available. In both cases, overall P fixation capacity in plant unavailable form can be high and occurs rapidly after fertilizer application, reducing the short-term P use efficiency of fertilizer materials measured as recovered P in plant biomass from fertilized soils (Hernandez et al., 2005; Jalali, 2006; Smil, 2000; Syers et al., 2008). Therefore, in order to increase plant available P in soil solution, applied rates of P fertilizer must do both, gradually saturate P pools of soils over time and increase readily available P in the soil solution for direct plant P supply, simultaneously. Only after saturation of P fixation capacity of soil, needed P fertilizer quantities can be reduced without affecting crop yields negatively (Helfenstein et al., 2018). Accordingly, recommended P fertilizer rates for P deficient soils under intensive cultivation can be high, in the range of 150 to 170 kg P per hectare (Mengel, 1997).

Further, to fully utilize P fertilizer in their effect on plant growth, supply of N and K as well as micronutrients also needs adequate management (Amelung et al., 2018). However, due to ongoing soil degradation with shrinking SOM contents on a global scale (FAO and ITPS, 2015), N fertilizer use efficiency (measured as the ratio of output N in crops and input N in soils) is declining as well, from 80 % in 1960 to below 30 % in 2000 (Tilman et al., 2002). Hence, to obtain the same crop yield, higher quantities of fertilizer need to be added, as a larger (and increasing) fraction is lost by volatilization or leaching. The immediate P use efficiency may also decline due to higher rates of P fixation by shrinking organic matter content in most soils, as results from Johnston and Poulton (2019) indirectly indicated. Concluding, when the capacity of readily available P pools in soils cannot be maintained due to decline of SOM content (e.g. induced by conventional agricultural management), then again, more P fertilizer must be added for the same P supply to plants as the equilibrium of the soil matrix may be shifted towards P fixation. Other way around, if SOM content is gradually increased (as in regenerative agriculture systems), then the ability of soils to supply P to plants from hardly soluble P materials may be increased and may reach the critical P value for optimal plant growth, as the equilibrium can be shifted towards P solubilization. However, if not lost due to erosion processes, fixed P remains in soils in reversible form and can be gradually utilized over the long-term (Johnston and Poulton, 2019; Syers et al., 2008).

While industrialized countries with a history in intensive agricultural production loaded most of their soils at or above saturation point in the past decades (FAO, 2006; Römer, 2013; Tiessen, 2008), developing countries often lack the fertilizer capacities to balance nutrient removal by crop production, hence are mining their soil resources as soil nutrient balancing shows (FAO and ITPS, 2015).

These effects only add to the relative high costs for conventional fertilizers in regions of SSA, which were found to easily multiply by several folds, depending on the distance covered between place of exportation and final usage (FAO and ITPS, 2015; Sanchez, 2002). Here, fertilizer markets are underdeveloped, while infrastructure often is poor, especially in rural areas. Resource-poor subsistence farmers also often lack the financial capacity for purchase of inorganic fertilizer products. Considering the declining resources of P base materials and possible price increases in the world markets, without an immense financial investment by governmental bodies in the foreseeable future, conventional P fertilizer seem almost inaccessible for a majority of small-scale farmers in rural areas of developing (landlocked) countries.

3.1.5 Alternative P fertilizer materials

As the usage of conventional P fertilizer materials seems not likely for a major fraction of African farmers, alternative P fertilizer materials and adaptation of the agricultural system may be more suitable for needed agricultural intensification, while demand of local availability and feasible economics can be met, without costly and time-consuming investment neither by user nor governmental bodies.

In recent years, P recycling from waste water treatment became focus of study, with Struvite being a valid substitute for conventional P fertilizer, with similar properties as TSP, enabling enhancement of plant growth (Cabeza et al., 2011; Kratz et al., 2010; Römer, 2013). Also, sewage sludge from wastewater treatment is considered as a possible P recycling material, but can contain toxic loads of heavy metals and organic pollutants (Pinnekamp et al., 2011). As one of the main resources for recycled P, resource-oriented wastewater treatment is a crucial key stone for overcoming dependencies on fossil P resources in the future. Where infrastructure for centralized wastewater treatment is lacking, as in many urban and almost all rural areas in SSA, low-cost resource-oriented sanitation may rather contribute to P recycling from wastewater than high-tech solutions. Pit latrines and sewage tanks can be used for collection of compostable, nutrient rich excrement substrate. As a general, more convenient approach, Terra Preta Sanitation (TPS) offers decentralized, mobile sanitation devices on the household level, designed to safely collect and treat human excreta for following composting (De Gisi et al., 2014; Factura et al., 2010). Anyhow, progress of introduction and implementation is slow and often lacking acceptance among potential users as sanitation is often considered as a critical topic for cultural reasons.

To gain adequate amounts of fertilizers from resource-oriented wastewater treatment for substantial impact of nutrient supply, again large investments would be required from governmental bodies and policy makers.

Concluding, as “external” P materials are hardly available in rural areas, and “internal” P resources of wastewater are difficult to utilize over the short-term, bone char from animal slaughterhouse waste may represent a valuable, recyclable, so far untapped P resource, extending available quantities of cow manure (CM) as the common standard fertilizer in those areas, which is locally available and producible on-site by small-scale farmers with very little investment costs.

3.1.6 Bone char as a novel P fertilizer

Bone char (BoneC) is an artificial, alternative organic P fertilizer with biological hydroxyl apatite (HA) as the main P composite from recycled slaughterhouse waste, gaining attention in recent years (Leinweber et al., 2019; Vassilev et al., 2013). It is derived in the process of pyrolysis from animal bones, in which the bone biomass is decomposed into volatile, fluid and solid components of BoneC at a temperature range from 350 to 750 °C, in no or little oxygen conditions (Diekow, 2017), leaving the inert residue materials in a highly porous structure with a large inner surface area.

The quantity and quality of BoneC varies depending on the process variables of bone material constituents, age and species of animal, bone pre-treatment (rendered/unrendered), co-pyrolysis of biomass (e.g. woody material, crop residues, manure), process temperature, heating rate and residence time (Wu et al., 2003; Zwetsloot et al., 2014). Anyhow, in contrast to water soluble inorganic P fertilizer, BoneC represents an organic, multi-nutrient, slow-release P fertilizer with benefitting properties for soil conditions in regards of bulk density and porosity, water storage capacity and immobilization of toxic soil components such as Cd and U (Leinweber et al., 2019).

Available quantities of bones for significant impact on national P fertilizer supply were estimated to be substantial for the example of Ethiopia, and could substitute around 50 % of yearly supplies of conventional P fertilizer (Simons et al., 2014). BoneC, recycled on national, regional and local levels may be considered as an essential, indigenous material to cover the P gap in developing countries, supporting needed intensification of agriculture in the decades to come.

3.1.6.1 BoneC chemical composition

Bones contain biological HA, which in comparison to mineral HA in rock phosphate, shows slightly lower crystallinity, a higher carbonate concentration and a significant OH^- deficiency in the finished char product, which all results in a better solubility in soils (Cabeza et al., 2011; Saeid et al., 2014; Zimmer et al., 2018; Zwetsloot et al., 2014). According to findings of Zwetsloot et al. (2014), with rising temperature, organic P compounds in the raw material were cleaved during pyrolysis and utilized into the inorganic P fraction, adding to an increased total plant availability of P from high-temperature BoneC. Additionally, with increasing pyrolysis temperature, the fraction of OCP, a less crystalline phosphate hence better soluble in water (Mengel, 1997), seemed to decrease in pyrolysed BoneC, while the formation of HA was increased. This was true for rendered bones (OCP fit of 60°C to 750°C decreased from 83.4 to 6.2 %, while HA fit increased from 19.7 to 94.9 %; analysed with Phosphorus K-edge XANES spectroscopy), to a lesser extent also for bones with meat residues (OCP fit of 60°C to 750°C decreased from 71.9 to 33.2 %, while HA fit increased from 0 to 69.3 %), similar to rendered bones with co-pyrolysis of wood biomass (OCP fit of 220°C to 750°C decreased from 88.8 to 33.2 %, while HA fit increased from 11.3 to 68.5 %). Only when woody biomass (or crop residues of maize) were co-pyrolysed with unrendered bones still containing meat residues, the formation of calcium phosphate (CaP) crystals associated with high HA fraction seemed to be strongly hindered (OCP fit of 220°C to 750°C increased from 67.3 to 89.2 %, while HA fit increased from 0 to 11.6 % only).

A possible explanation was given as organic acids from the added biomass and meat residues may have been adsorbed on the surface of the crystals, blocking binding sites no longer able to serve as nuclei for continued crystal growth. Also, speculated by the authors, the presence of strong metal ions acting as inhibitors of CaP crystallization, such as ions of copper (Cu), zinc (Zn) and Mg may have reduced crystallisation, although substantial amounts were not detected (Zwetsloot et al., 2014). Similar, Cabeza et al. (2011) reported higher P availability in soils from added Sinter-P fertilizer in comparison to regular meat-and-bone ash. The former was produced from a mixture of meat-and-bone material, sodium carbonate and silica in a rotary furnace at over 1000 °C, while the latter was simply mono-incinerated at the same temperature range. As Sinter-P is composed of more amorphous calcium sodium phosphates due to the additive materials than regular meat-and-bone ash, full crystallisation could be hindered by the same reasons cited in Zwetsloot et al. (2014). Hence, Sinter-P showed a higher relative fertilizer efficiency as regular meat-and-bone meal, almost as high as TSP under acidic soil conditions (Cabeza et al., 2011), which again could give hint on better solubility of amorphous OCP over crystalline HA, as stated by Olsen et al. (1977) and Sposito (1989) cited in Mengel (1997).

This resembles in the findings of a ten-fold higher water extractability of P from high temperature BoneC with high OCP fraction fit, in comparison to BoneC with a high HA fraction fit. Total P available for plant growth measured as P extractable by formic acid increased with higher process temperature, regardless of OCP or HA fraction fit. Although high-temperature BoneC from rendered bones reached a P mass concentration of 15.2 %, which is comparable to P concentration of inorganic P fertilizer, its solubility in water is much less with under 1 % directly water-soluble (Zwetsloot et al., 2014).

Concluding, BoneC in general may be a mixture of majorly biological crystalline HA and minor amorphous OCP with a P content of up to 15 %, a high pH of around 10 and a very low water extractability, but high total P availability measured in formic acid extraction, all depending mainly on pyrolysis conditions (Zwetsloot et al., 2014).

3.1.6.2 BoneC Parameters

Additional benefits of BoneC emerge from the increased inner surface area, found to be between 42 and 114 m^2/g (Leinweber et al., 2019), which can serve as a protected habitat for soil microorganism and easily exchangeable adsorption sites for soil nutrients, possibly increasing the cation exchange capacity (CEC) of soils, just like wood biochar (Jin, 2010; Joseph et al., 2010; Postma et al., 2013). Also, the bulk density and porous structure of a BoneC amended soil can be improved, resulting in improved water storage capacity (Leinweber (2017) cited in Leinweber et al. (2019)). It was further found, that BoneC can immobilize toxic heavy metals like Cd in the soil by adsorption and overall increase in soil pH (Morshedizad et al., 2016; Nicholson et al., 1994; Siebers and Leinweber, 2013; Zimmer et al., 2019), while the content of heavy metals or radionuclides in BoneC itself is almost non-existent in contrast to inorganic P fertilizer from rock phosphate (Morshedizad and Leinweber, 2017; Pinnekamp et al., 2011; Rothbaum et al., 1979; Siebers and Leinweber, 2013). Thus, Cd or U contamination from former, intensive application of inorganic P fertilizer could be countered by usage of BoneC for simultaneously soil remediation and P fertilization.

3.1.6.3 BoneC Food safety

Bones from diseased cow can contain the transmittable pathogen bovine spongiform encephalopathy. To prevent spreading, bone and meat meal from slaughterhouse wastes are banned for cattle feeding by European Union (European Commission, 1994), but nevertheless, products from safe animals are allowed in organic farming as soil fertilizer (Cascarosa et al., 2012). Anyhow, bones are heat sterilized during pyrolysis, as already temperatures as low as 200 °C for 1 hour were shown to inactivate the pathogen (Taylor, 1998). Therefore, BoneC can be considered free of bovine spongiform encephalopathy, when proper pyrolysis temperatures and duration times are guaranteed.

Also, when glyphosate contaminated feed or plants are consumed, glyphosate was found to accumulate majorly in the bone structure of animals (Samsel and Seneff, 2015). However, glyphosate is easily volatilized by temperatures above 230 °C (Narimani and da Silva, 2019), so it can be assumed, that BoneC is a glyphosate free material, even from contaminated animal bones, suitable in organic production.

3.1.6.4 Conclusion: BoneC

P is a valuable resource, on which all agriculture production relays fundamentally. Challenging soil conditions, declining resources in quantity and quality of fossil P and changing environmental conditions affect agricultural practice and hence crop yields globally. Alternatives of organic fertilizer like BoneC from recycling waste streams possess interesting properties and could lead the way to a more closed, sustainable P cycle in agriculture, especially in low-input practices in rural areas of developing countries, with limited resources and restricting infrastructure.

BoneC can serve as a safe, renewable, multi-purpose soil amendment for enhanced nutrient supply of P but also Ca and Mg with additional benefits on soil fertility, namely soil life activity, water storage capacity and immobilization of toxic heavy metals. The slow release characteristic of P may be more suitable for meeting P demand of plants in a prolonged growing phase, typical for grasses, grains or perennials (Leinweber et al., 2019; Siebers and Leinweber, 2013) and prevention of P losses by leaching and erosion. In acidic soils, BoneC is more soluble due to high H^+ concentration, which again increases OH^- concentration in the soil solution in this process (Mengel, 1997). Following, this may contribute to soil pH increase and hence improved P status of the soil by desorption of fixed P. In alkaline soils, BoneC is less soluble, hence may be less efficient in supplying P for crop uptake.

Anyhow, fertilization practice needs to meet functional, ecological and economical demands in intensive annual crop production and acceptance of farmers. A short-term enhancement of yields seems crucial for introduction of any alternative agriculture system, especially in regions with limited financial resources and thus, high pressure to secure the daily livelihood. Therefore, plain application of BoneC without adaption of the agricultural system seems not sufficient due to slow release of P.

BoneC is not fully recommended as a general P fertilizer for conventional agriculture system (Zimmer et al., 2019), but may be useful as an effective alternative to inorganic P fertilizer after pre-treatment or under certain soil and environmental conditions, occurring naturally or being facilitated by adapted agricultural management as shown in the following chapters.

3.1.7 Enhanced P availability in soils by agronomic measures

In soils with low levels of readily available P, plants acquired a diverse set of mechanism and strategies to acquire sufficient nutrition. Certain agronomic measures may support plant in their efforts of P solubilization, depending on soil and plant properties (Horst et al., 2001).

In general, P availability in soils can be enhanced by the addition of organic matter, e.g. by addition of farmyard manure or crop residues. Firstly, contained P can be mineralized and used for plant growth. Secondly, by release of organic anions during decomposition of organic matter, P can be desorbed from binding site of soil constitutes and made available in the soil solution (Guppy et al., 2005; Kirkby and Johnston, 2008).

Diffusion processes of P in the soil solution are dependent on stable soil moisture content and a well-structured, non-compacted soil (Bolan, 1991; Kirkby and Johnston, 2008). Favouring conditions for high mobility of P can be achieved by minimal- or no-tillage operation and maintenance of a soil cover by vegetation or mulch (Syers et al., 2008). Further, enhanced levels of SOM showed general improvement of soil condition and fertility, such as increased soil water storage capacity, lower bulk soil density with larger porous space, more active soil life and higher buffer capacity for cations and anions such as P in soil solution, all resulting in elevated P levels and mobility in soils (Bot and Benites, 2005).

Root growth is favoured by these conditions, which again increases plant P acquisition by improved soil exploration (Kirkby and Johnston, 2008). Elevated NH_4^+ supply to plants either by application of fertilizer, organic matter or cultivation of N_2 fixing legumes can also lead to increased P solubility by acidification of soils due to proton release from nitrification of NH_4^+ or balancing of charge following plant cation uptake (Amelung et al., 2018). Regardless, this management practice can only be considered supportive in alkaline soils, as otherwise pH can drop too low and again immobilize P and other trace elements, while Al can be freed in toxic levels. The other way around, when the soil pH is increased by liming, adsorbed P can be freed and thus becomes better available by plants. A pH range of 6.5 to 7.5 is considered ideal for optimal P supply (White and Hammond, 2008).

The adaption of the planting and cropping system towards P deficient soil condition seems to be an important factor for increasing P availability, as was shown for *Cajanus cajan* (Ae and Okada, 1993) and *Lupinus albus* L. (Horst and Waschkies, 1987), which both in comparison to non-adapted plants were able to gather considerable amounts of P from soils deficient in plant available P by adapted root mechanisms.

When experiencing deficient P supply, it was found that plants increase the root biomass by enhanced transport of photosynthesis products to the root system in order to more effectively scavenge the soil for P. In this course, increased growth of adventitious, lateral roots in the topsoil was observed, as well as a higher density and length of root hairs in P rich areas. Under P stress, roots tend to be finer and less in diameter to access finer soil pores and to reduce the C cost for exploring distant soil areas (Lynch and Brown, 2008; White and Hammond, 2008).

Furthermore, fixed P can be solubilized by a variety of plant and microbial induced processes in the rhizosphere. Firstly, plants and microorganisms can excrete protons to dissolute inorganic P (White and Hammond, 2008). Secondly, plant roots can excrete C compounds to increase microbial activity of P solubilizing soil bacteria and AM fungi in direct proximity to P containing soil constituents (Morgan et al., 2005), although microbial biomass itself again can take up considerable amounts of P in competition to plants (Marschner, 2008). Further, roots (Marschner, 2008), specific bacteria (Alori et al., 2017; Richardson and Simpson, 2011; Sharma et al., 2013) and AM fungi (Feng et al., 2002; Jansa et al., 2011), all can secrete the enzyme phosphatase, which mediates the hydrolysis of organic P. Finally, organic anions including carboxylates of citrate, malate or oxalate can be secreted by plants, competing for binding site with adsorbed P or acting as chelators by fixing adsorbent metal ions in the soil, which also releases bound P (Mengel, 1997).

When a P source was explored and made available by processes described above, additionally, plants can upregulate P uptake by producing high affinity P transporters in the root epidermis, so a higher ratio of P is taken up from the soil solution (Liu et al., 1998).

All those processes and mechanisms can help to increase P uptake of plants, but the most important factor in increasing P supply in P limiting conditions may be the activity of various soil microorganisms, namely plant growth promoting bacteria (PGPB) and AM fungi with P solubilization properties, both which will be discussed in chapter 3.2.

3.1.8 Enhanced P availability of BoneC

Although P availability from BoneC is low due to chemical P characteristics, it still can enhance plant growth of crops with longer vegetation period substantially, e.g. grasses and grain crops, which tend to form an intensive rooting system (Leinweber et al., 2019), more suitable for P acquisition from slow-release fertilizers. P availability of soils fertilized with BoneC may also benefit from specific plant selection, crop rotation and intercropping systems (Horst et al., 2001).

In any case, water extractable P may not be the most useful parameter to evaluate the effectiveness of organic P fertilizer in practice. In highly fixing, acidic soil conditions, Zwetsloot et al. (2016) found, that P uptake of plants supplied with TSP and BoneC (rendered bones pyrolysed at 750 °C for 45 minutes) differed only significantly, when plants were not colonized by AM fungi. When plants were in symbiosis with AM fungi, P uptake from water-soluble TSP source and insoluble BoneC was in the same range, although water extractability of P was 260.5 mg*g^{-1} for TSP and only 0.5 mg*g^{-1} for BoneC (see Figure 4). These findings may indicate, that when water solubilized P as from TSP is re-fixated rapidly in challenging soil conditions, then overall P supply to the plant may be majorly depending on mycorrhizal activity, able to supply P from scarcely soluble fertilizer like BoneC or fixed soil P. Here, it is hinted, that fertilizer recovery effectiveness can be influenced drastically by activity of soil microorganisms and soil and plant conditions, not solely by its water solubility.

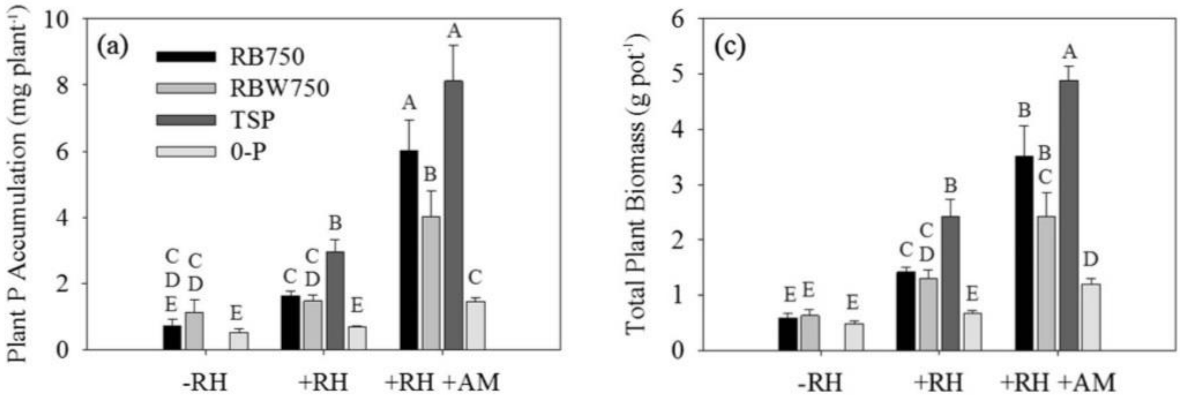

Figure 4: Biomass and nutrient uptake of Z. mays after 5 weeks of growth in a highly P fixing soil fertilized with bone char produced at 750 °C (RB750), bone char and wood biochar co-pyrolyzed at 750 °C (RBW750), Triple Superphosphate (TSP), and no P additions (O-P). Means and standard error are given (n = 5 except for -RH with n = 2-3): (a) plant P uptake, (c) total plant biomass. On the x-axis, foraging strategy of maize is altered through the absence of root hairs (-RH), the presence of root hairs (+RH) and the presence of root hairs and addition of AM inoculants (+RH + AM). Different capital letters indicate significant differences ($p<0.05$) between treatments as determined by student's t-test, from Zwetsloot et al. (2016), p. 101.

Further, certain soil bacteria with P solubilizing abilities can be inoculated to soils to increase P availability from insoluble P sources (Alori et al., 2017; Saeid et al., 2014; Sharma et al., 2013), while a BoneC particle size between 0.5 and 1 mm was found to maximize release of P, according to findings of Morshedizad and Leinweber (2017). Therefore, P availability of BoneC in the rhizosphere may be increased by specific plant selection and management, enhanced soil life activity of beneficial soil microorganisms and fine granulation to levels similar of inorganic, water-soluble P fertilizer. This could enable a competitive, sufficient P supply from contaminant free, renewable, locally available P fertilizer with an adapted soil and plant management, designed for enhanced utilization of BoneC and residual soil P in general.

Moreover, to overcome dissolution of P, especially in neutral or alkaline soils due to high OH^- concentrations, BoneC can be enriched with sulfur (S), e.g. by using BoneC as a biogas desulfurization agent before application as fertilizer to soils (Morshedizad and Leinweber, 2017; Zimmer et al., 2019, 2018). In this pre-treatment, hydrogen sulfide (H_2S) from the biogas stream is bound towards the BoneC surface and thus can be oxidized in the soil by various microorganisms. This oxidation leads to the formation of sulfuric acid (H_2SO_4), causing a decrease in pH in close proximity to the BoneC particle in the soil, which again increases P solubility of BoneC (Zimmer et al., 2018). A higher P solubility from S enriched BoneC compared to untreated BoneC was confirmed in recent studies (Morshedizad et al., 2016; Morshedizad and Leinweber, 2017), which may result in an elevated apparent nutrient recovery efficiency of 10 to 15 % for S enriched BoneC, almost as effective as TSP (> 18 %) and significantly higher as untreated BoneC (< 3 %) (Zimmer et al., 2019). Hence, as the authors concluded, pre-treated BoneC by S loading can serve as a multi-element fertilizer containing P, Ca and S, almost as effective in P availability as TSP, but from a renewable resource, not contaminating the soil.

3.1.9 Conclusion: Adaption of agricultural system for utilization of BoneC and fixed soil P

Although plants developed a set of various measures to counteract P deficiency, in intensive agricultural practices, the application of inorganic P fertilizer from fossil reserves to increase P concentration in soils is still an essential practice to secure global food production. Yet, due declining reserves of P fertilizer and its uneconomical, non-efficient and wasteful management practice in many parts of the world, a revision of agricultural practice seems urgent.

An alternative agricultural system, designed to facilitate P uptake from hardly soluble external materials as well as plant unavailable, fixed soil P by a diverse set of methods could be a suitable foundation. Methods of enhanced P supply in strongly fixing soil conditions could include nurturing of P solubilizing AM fungi and bacteria (see chapter 3.2), enhancement of stable SOM levels in soils and liming by biochar (see chapter 3.3) and a regenerative agricultural management system in general (see chapter 4.1).

In the case of SSA, where fertilizer consumption is on a global low for various reasons, great potential may lay in the utilization of locally recycled P materials like BoneC, which in combination with AM fungi could supply sufficient quantities of P to plants, even in non-saturated soil conditions of high P fixation capacity as shown by Zwetsloot et al. (2016). Hence, high costs for conventional fertilizers could be avoided, if accessible at all. This way, resource-poor small-scale farmers would be able to improve the P availability in their operation and could intensify their production.

Nevertheless, this process should not be restricted to developing countries only. At some point in time, also in industrialised countries with high soil P levels, these P resources will have to be mined through adapted agronomic measures, of which nurturing of AM fungi may be one of the most important.

3.2 Plant growth promoting fungi and bacteria

A broad range of diverse soil microorganisms bear capabilities of plant growth promotion, including properties of enhanced P solubilization and supply. In the following, the most important soil microorganisms in this regard are discussed.

3.2.1 Mycorrhizal fungi

3.2.1.1 Basics: Mycorrhiza

Mycorrhizae are biotrophic fungi present in most natural soils, obligately engaging in symbiosis with host plants (Fitter et al., 2011; Smith and Read, 2008). The symbionts normally form a mutualistic relationship through fungal colonisation of the plant roots. In exchange of mined nutrients and water resources from the soil, AM fungi are rewarded with carbohydrates from photosynthesis of the host plant. A benefit for both partners can be created, but depending on bidirectional adaptability of fungi and plant species and their specific cost-to-benefit ratio, the symbiosis can also become one-sided, hence parasitic (Smith and Read, 2008).

Belonging to the phylum of *Glomeromycota*, mycorrhizal fungi types can be distinguished by their specific morphology (Redecker and Raab, 2006). The most essential types in terms of agricultural cultivation are ectomycorrhiza, commonly hosted by woody perennials, and endomycorrhiza, which can colonize a large majority of global plant species. Further, the most well-received member of the latter type is the arbuscular mycorrhizal (AM) fungus, which is exclusively discussed in this study due to its widespread occurrence in diverse ecosystems around the world and ability to colonise a broad set of plant species, annuals as well as perennials (Smith and Read, 2008).

In many studies, deeper knowledge about AM fungi symbiosis was gained. Remarkably and often seen as the fundamental terms of symbiosis, AM fungi can enable sufficient nutrient supply (especially P) in challenging soil conditions of low-nutrient availability (Fitter et al., 2011; Smith and Read, 2008) or abiotic stress conditions, such as salinity (Evelin et al., 2012; Rabie and Almadini, 2005), drought (Neumann et al., 2009) or toxic soil constituents (Alori and Fawole, 2012). In such conditions, the widespread, spacious mycelium of AM fungi can represent a substantial extension of the actual root system, activating a much larger soil volume for sufficient nutrient and water supply (Jansa et al., 2011; Smith et al., 2011).

3.2.1.2 AM fungi morphology and development

Host plants can be colonised by AM fungi through asymbiotic or symbiotic infection. In the former case, a new symbiosis is established through infection by spore germination or fragments of mycelium or detached mycorrhizal roots, all serving as infectious propagules (Smith and Read, 2008). In the latter case, mycelium of an existing symbiosis is expanding in the soil matrix and infects neighbouring plants, thus the symbiosis is extended and a common mycorrhizal network (CMN) is formed, interlinking multiple plants of the same or different species, of the same or different age and growth status (Heijden and Horton, 2009).

Germination of spores is triggered by the excretion of plant signals including strigolactones (Akiyama and Hayashi, 2006; Smith et al., 2011). The same root exudates support the pre-symbiotic growth of the fungal propagules towards the hosting roots, so time and distance are shortened until symbiosis can be engaged (Sbrana and Giovannetti, 2005). In general, mycorrhizal colonisation of plant roots can be rapid, with as little as 3 to 6 days after seedling emergence (Read et al., 1976). However, if no host plant is present in the proximity of mycelium propagules, hyphal growth is paused and only continued, when roots or other compatible hyphae can be tracked. In this state, propagules stay infective for up to 30 days (Goltapeh et al., 2008).

As pre-symbiotic hyphae run on the surface of the root, they swell and form the appressorium, serving as the entrance point into the root. From here, depending on the AM type, hyphae colonize intercellular root structures to form arbuscules and vesicles inside the cortical cells (Arum type), while hyphae of Paris types only colonize the intracellular space and form predominantly hyphal coils (Smith and Read, 2008) (see Figure 5). Only after forming these structures serving as the exchange matrix for symbiotic products, the fungi can gather carbohydrates from the host for extraradical hyphal extension into the surrounding soil volume. Before, it is solely dependent on the limited resources of its propagule (Smith and Read, 2008).

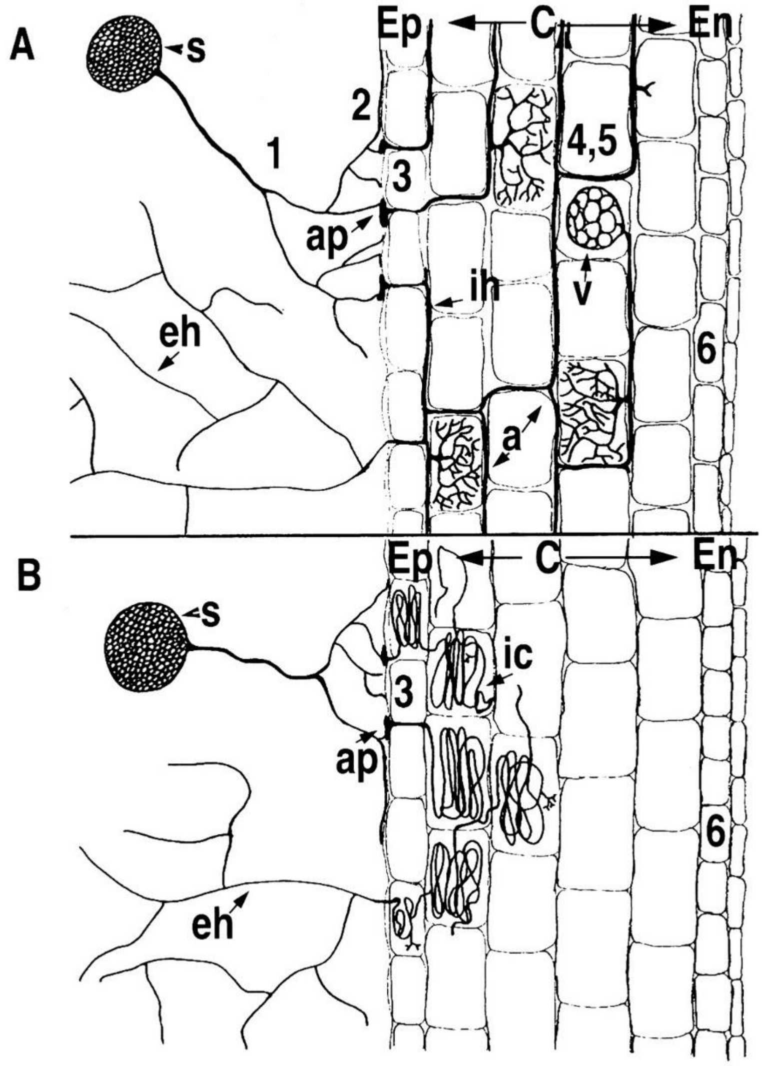

Figure 5: Schematic diagram of root colonization of (A) Arum and (B) Paris type arbuscular mycorrhizal fungi, from Barker et al. (1998), p. 1203.

Following, the fungus extends its extra-radical mycelium far into the soil, more than 20 cm in distance of the roots (Jansa et al., 2003; Mikkelsen et al., 2008), far beyond the actual rhizosphere and depletion zone for nutrients and water acquisition by fine root hairs (see Figure 6). With a diameter of 2 to 20 µm, hyphae can access much finer soil pores than root hair (Smith et al., 2010), again increasing the active, utilised volume of the hyphosphere manifold, while nutrients and water can rapidly be transferred to roots via the mycelium, bypassing challenging soil conditions (Smith et al., 2011). Also, excess nutrients can be stored inside the fungal compartments for later exchange, depending on the offered C quantities (Bolan, 1991; Callow et al., 1978).

Figure 6: Soil exploration by extraradical hyphae of fungal mycelium after establishment of plant-fungi symbiosis, from Professor Emeritus David Read, University of Sheffield, UK.

3.2.1.3 Carbon costs for mycorrhiza symbiosis

Allocated photosynthesis derivates from the host plant are utilized by the fungal partner for proliferation and maintenance of the mycelium. AM fungi may receive 5 to 20 percent of the total C fixed by the plant in the form of hexose, exchanged at the apoplastic interface of the colonised root system (Smith and Read, 2008).

Interestingly, high accumulation rates of surplus C in above-ground biomass though can downregulate photosynthesis (Pego et al., 2000). Accordingly, when plant growth was nutrient limited, Fredeen et al. (1989) and Qiu and Israel (1992) found enhanced accumulation of C in root tissue of plants, as high C concentration in above-ground biomass was primarily detrimental for maintenance of photosynthesis levels (Fitter, 1991). Yet again, the allocation of “stored” surplus C from vegetal to fungal biomass may represent a strong C sink for the plant, affecting photosynthesis rates positively (Fitter, 1991). So, if surplus C is utilized in symbiotic trade and “discarded” to fungal biomass in the soil, photosynthesis rates of mycorrhizal plants under nutrient limitation may be less likely to be downregulated. This mechanism of maintaining a continuous photosynthesis may increase overall C assimilation of the plant, hence an increased photosynthesis performance could be achieved (Miller et al., 2002; Wright et al., 1998). Accordingly, the net C cost for host plants engaging in mycorrhizal symbiosis could become negligible, when only surplus C is utilized anyway (Fitter, 1991). Benefits in terms of nutrient and water supply would be at neutral costs for the plant.

Furthermore, by virtue of the improved nutrient supply and other factors induced by the fungal partner, this initial loss of C may be additionally compensated by enhanced plant productivity including larger specific leaf area, higher chlorophyll content of leaves, improved water status of the plant, improved stomata regulation, concluding, a further improved photosynthesis capacity (Aroca et al., 2007; Miller et al., 2002; Sheng et al., 2008; Slabu et al., 2009). Overall biomass production of plants due to mycorrhizal symbiosis could be enhanced.

3.2.1.4 Soil formation by AM fungi

Large soil deposition of C from above- to below-ground biomass, induced by AM fungi, can enhance the soil organic C content with various effects on overall soil fertility and soil life activity (Bot and Benites, 2005; Rillig et al., 2001).

AM fungi activity can improve soil formation processes, leading to higher rates of infiltration and water storage capacity as well as promotion of root growth and gas exchange. Firstly, a high hyphae density in the soil matrix, creating a skeletal structure to bind mineral and organic soil constitutes together, was found to correlate with properties of water-stable aggregates (Miller and Jastrow, 1990; Rasse et al., 2005; Rillig and Mummey, 2006). In this formation phase, soil particles are bound together physically by hyphal connections (see Figure 7), which then are stabilized by exudated polymers and deposited necromass from microbial activity, including fungal glomalin, in a following bio-chemical stabilization phase of soil formation (Jastrow et al., 1998; Rasse et al., 2005).

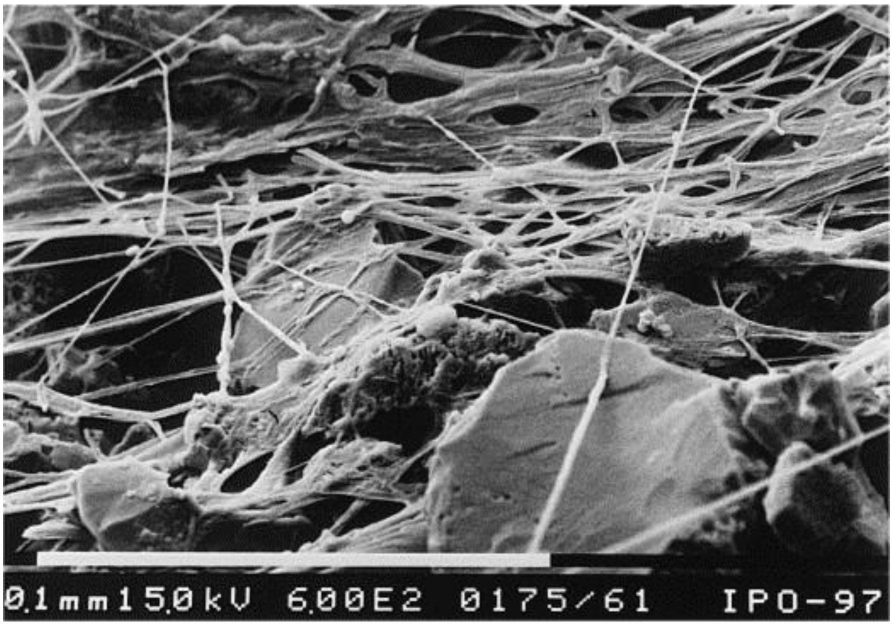

Figure 7: Scanning electron micrograph of fungal hyphae as a soil structuring medium, from Breemen et al. (2000), p. 167.

The glycoprotein glomalin fundamentally contributes to the improved soil formation process. The glue-like substance from hyphal decay and exudation was found to accumulate in mycorrhizal soils (Rillig et al., 2001). It can act as a glue between soil particles, forming water-stable macro aggregates with a coating layer on the surface, improving the resistance against mechanical destruction by swelling-shrinking processes (Rillig and Mummey, 2006; Wright et al., 1999). Also, inbound organic matter is better protected from microbial decomposition, resulting in increasing content of stable SOM over time (Nichols, 2008). Hence, the bulk density of stabilized, well aggregated soils may be improved, resulting in enhanced infiltration, water stability and water storage properties (Fahad et al., 1982). This all can lead to a more favourable soil status with higher aggregate stability (Wright and Anderson, 2000), indirectly increasing plant productivity. Improved soil structure through AM fungi activity in soils further facilitates the superior nutrient supply of colonized host plants.

3.2.1.5 N acquisition by AM fungi

The essential nutrient N is present in soil in many different complex organic and inorganic forms. Anyhow, plants are dependent on inorganic NH_4^+ or NO_3^- available for uptake, essential in the process of photosynthesis (Amelung et al., 2018). Due to its high mobility in most soils, formation of N depletion zones is not considered to be limiting factor, but low concentration and high demand of N by plants are (Smith and Read, 2008). This often results in N being a major limiting factor in plant productivity (Bücking and Kafle, 2015; Perez-Tienda et al., 2012).

Under impact of drought or salinity, N availability in soils though can be drastically reduced by diminishing diffusion rates or competition from other soil nutrients like chlorine (Cl) or sodium (Na) for root uptake (Frechilla et al., 2001). Anyhow, plants can benefit from AM fungi mediated N transfer from soil volume of the hyphosphere (Hodge and Fitter, 2010; Leigh et al., 2009).

Although AM fungi itself, without saprotrophic abilities, cannot decompose organic N sources such as organic matter, AM fungi still can manage N availability in soils. Fungal hyphae explore the soil for nutrients, growing into nutrient rich patches in greater distance to the plant (Bücking and Kafle, 2015; Hodge and Fitter, 2010). By relocation and exudation of received C into the soil, AM fungi actively navigate and stimulate benefitting microbial communities for decomposition and mineralization of N containing organic matter (Atul-Nayyar et al., 2009; Bücking and Kafle, 2015; Herman et al., 2012). N uptake and transfer to the host plant is achieved efficiently, as was reported by Perez-Tienda et al. (2012). The authors concluded, that the AM fungi uptake and transport system for NH_4^+ shows higher affinity than the vegetal system, indicating a higher ability to extract and transfer N even under low concentration, potentially non-extractable for plants itself. This is in accordance with findings of Burleigh (2001), whose observation indicate a possible downregulation of the plant N uptake system, while the fungal system can be upregulated (Koegel et al., 2013), enhancing N transfer to the host plant. Accordingly, other studies report AM fungi responsible N accumulation in maize to reach up to 75 % of total gathered N (Tanaka and Yano, 2005).

Biological N_2 fixation by *Rhizobium* spp. bacteria living in symbiosis with leguminous crops is well studied over the last decades (Denison, 2015). This major N supply system can be seen as a key element in any organic farming practice, as N becomes easily accessible, without the possible detrimental effects on the soil environment or investment costs of synthetic N fertilizer (Byrnes, 1990). Anyhow, stimulating effects of AM fungi colonization on enhanced N_2 fixation based on the improved P of the host plant was reported in many studies (Barea et al., 1987; Bulgarelli et al., 2017; Júnior et al., 2017; Rabie and Almadini, 2005; Wani et al., 2007; Zaidi and Khan, 2007). Hence, a symbiotic relationship between the partners host plant, AM fungi and N_2 fixing bacteria may be engaged.

Concluding, these findings indicate a possible benefit of AM fungi partnering for the host plant in terms of N acquisition, both by AM fungi improved, symbiotic N_2 fixation and increased N availability and uptake from soils, especially viable under abiotic stress conditions.

3.2.1.6 P acquisition by AM fungi

P acquisition of plants can be limited in challenging soil conditions with low diffusion rates (Bolan, 1991; Kirkby and Johnston, 2008), high P fixation capacity (Syers et al., 2008), low P solubility (Mengel, 1997; Parfitt et al., 1975), or general low P content. Anyhow, plant P supply can be fundamentally influenced by symbiotic AM fungi, with expanded soil exploration and increased P solubilization, improved uptake and transfer as the major mechanisms responsible (Jansa et al., 2011).

In general, P uptake of plants is facilitated by two major pathways, predominantly mediated by soil conditions and the species of symbiotic partners involved (Nagy et al., 2009; Smith et al., 2011). Direct P uptake is performed by root hairs and root epidermis within the rhizosphere of the plant. P is absorbed into the roots and transported by specific P transporters, which are upregulated by corresponding gene expression. The effectiveness of this uptake pathway is dependent on the P concentration in soils. Once depleted due to low solubility and slow diffusion rates of P in most soils, this P uptake pathway is gradually shut down, P transporter gene expression is decreased (Javot et al., 2007; Schnepf et al., 2008). In the same extent, AM fungi symbiosis and its induced mycorrhizal P uptake pathway becomes vital, exploring the soil by hyphal branching, absorbing nutrients and water far beyond the depletion zone of the roots within the hyphosphere, rapidly contributing to the plant P supply (Bolan, 1991; Nagy et al., 2009; Schnepf et al., 2008; Smith et al., 2011). Respectively, under these conditions, mycorrhiza induced gene expression of fungal-specific P transporter genes was shown to be upregulated (Javot et al., 2007). Under certain conditions the fungal pathway can even supply exclusively all P accumulated by the plant (Smith et al., 2004), especially when the mycelium network is fully established after some days of growth and the rhizosphere is depleted in P (Schnepf et al., 2008). The mycorrhizal contribution to P supply may be viable even when rates of root colonisation are low or AM fungi plants may not accumulate more P than non-mycorrhizal plants (Nagy et al., 2009; Smith et al., 2004). The other way around, when soils were saturated with P and growth was not P limited, AM fungi root colonization of host plants were found to be decreased and plant-specific P transporter gene expression indicated an active direct P uptake mediated by the plant (Jansa et al., 2011; Javot et al., 2007).

As P concentration in plant available form is low in almost all soils, P allocation, P solubility and respectively P dissolution capacity are important factors in plant nutrient supply.

Fungal hyphae have a small diameter of 2 - 20 µm and therefore they are able to grow into much smaller pores than root hairs, constituting a larger P absorption surface area (Bolan, 1991; Schnepf et al., 2008; Smith et al., 2010). Also, the mycelium can explore soil areas far beyond the actual narrow depletion zone of root hairs, extending more than 20 cm into the soil with more than 2.5 mm of tip movement per day were reported (Jansa et al., 2003; Mikkelsen et al., 2008; Schnepf et al., 2007; Smith et al., 2004). Formerly unavailable nutrient sources in distal soil locations, non-accessible or too far away for plant uptake, can be utilized by AM fungi.

Additionally, by AM fungi induced mechanisms, insoluble, organic P sources can become plant available. Firstly, hyphae grow vigorously into nutrient rich patches of organic matter and facilitate decomposition and mineralization, freeing contained nutrients (Hodge and Fitter, 2010; Leigh et al., 2009). They can alter microbial activity and composition in their hyphosphere by stimulating exudation of carbohydrates and water into soil (Herman et al., 2012; Jones et al., 2004; Toljander et al., 2007). In this favourable environment for decomposer microbes and microbe-hypha interactions, also P solubilizing PGPB were observed to interact synergistically with AM fungi (Ordoñez et al., 2016; Zaidi and Khan, 2007). Anyhow, also AM fungi themselves were found able to excrete enzymatic phosphatases, which supports the hydrolysis of organic P (Feng et al., 2002; Wu et al., 2011). Concluding, by intentionally proliferation into patches of organic P, exudation of carbohydrates and water, the microbial community is altered and activated by AM fungi to facilitate P availability.

Next to organic P fractions, also the availability of sparingly soluble, inorganic P sources in soil like phosphates of Fe-, Al- or Ca or mineral P in HA can be enhanced by fungal mediation of P supply. AM fungi were observed to exudate H^+ and organic acids acting as chelators, freeing P (Bolan, 1991; Koele et al., 2014), which led to the speculation, that fungal glomalin production may be a fundamental mechanism to break down stabile Fe-P, freeing Fe as a substantial component of glomalin, while P is freed as a "by-product" (Cardoso and Kuyper, 2006). Zwetsloot et al. (2016) even found comparable rates of P supply from a mycorrhizal, P fixing soil fertilized with insoluble BoneC, when compared to the same soil fertilized with water-soluble TSP.

Furthermore, when the C supply of the host plant is low, e.g. due to shading, AM fungi can buffer accumulated P in fungal compartments for later transfer. This storage mechanism ensures continuous P supply to the host and adequate C revenue to the fungi (Bolan, 1991; Callow et al., 1978).

All things considered, substantial amounts of P from organic and inorganic sources can be allocated by fungal activity in soils, even in great distance from the final receiving plant, formerly unavailable for plant uptake distance- and solubility-wise (Schnepf et al., 2008, 2007; Smith and Read, 2008). In contrast to P from inorganic fertilizer application, the location and time of solubilization and uptake of P may not be separated, when mediated by AM fungi, which may offer certain benefits in terms of P behaviour in soils. Fungal mediated solubilization and uptake of P occurs simultaneously, in close proximity, concurrent and mutually conditional through fungal activity. Thus, uptake and translocation in the conduit hyphae through the soil towards the roots of the host plants is expected to be rapid and overcome slow diffusion (Bolan, 1991; Smith et al., 2011). By this fungal mechanism, P fixing soil conditions and low moisture content may be bypassed, which otherwise would diminish P diffusion rates and hence plant P supply. This way, growth-limiting detrimental soil conditions of high P fixation capacity may be neutralized, as the P acquisition pathway towards plants is managed by the fungal partner. AM fungi can consequently enhance P supply of plants in P-limited soil (Zwetsloot et al., 2016). This may be a strong advantage and a key element for utilization of sparingly-soluble P sources from alternative fertilizers or fixed soil P in general.

Anyhow, the effectiveness of P transfer is highly dependent on the symbiotic partners. Differences between AM fungi species and even isolates of the same species coupled with host specific differences, influenced by soil conditions and vegetal management, all determine the outcome of the symbiosis (Heijden, 2004). Therefore, further evaluation of cooperative behaviour for crops, fungi and management in different regions of the world should be targeted in future research.

3.2.1.7 K acquisition by AM fungi

Potassium (K), next to N and P, is one of the major nutrients for plant growth, required in substantial amounts, for many vital processes such as photosynthesis or stomata operation. Although present in large quantities in soil (from 3.000 to 100.000 kg*ha^{-1}), it is often lacking in tropical, weathered soils, as K is leached out after the formation of K free kaolinite or Al- and Fe-oxides in the process of pedogenesis (Amelung et al., 2018).

Similar to P, K is present in soils as soluble K, ready for plant uptake in ionic form. Further, exchangeable K can be adsorbed at outer surfaces of clay minerals or humic matter or fixed in clay minerals. Structural K, included in the crystal structure of silicates, is the least soluble form of K in soils (Amelung et al., 2018).

Although K is highly abundant in soils, only a minor fraction is directly plant available. Yet, replenishing of depleted K soil concentration from desorption or dissolution of other K pools is usually considered sufficient for adequate plant growth (Amelung et al., 2018). Anyhow, when grown with AM fungi symbiotic partner, plants showed increased K uptake in their root system to some extent (Marschner and Dell, 1994).

Interestingly, K may serve as the main counter ion of accumulated P for fungal homeostasis, therefore it is taken up by AM fungi, especially when vegetal C supply is limited and stored P in fungal mycelium requires charge balancing (Bücking and Heyser, 1999; Garcia and Zimmermann, 2014).

Anyhow, the major advantage from AM fungi related K uptake becomes viable under stress conditions like contaminated soil, drought or high soil salinity. It was shown in many studies, that stress related struggle of plants can be alleviated by AM fungi symbiosis, mainly by improved selective supply of nutrients including N, P and K, discussed in the following.

3.2.1.8 AM fungi alleviation of salinity

Salinity is a fundamental problem in today's agriculture as it affects plant growth and productivity of many crops worldwide, especially in areas of high evaporation potentials (Hammer et al., 2011; Lambers, 2003). Without a change of management, affected soils may become more and more incapable to bear crops and may be entirely lost for human activities. Warning examples of degraded regions are plenty, with the degradation of the Aral Sea basin as one of the most famous (Qadir et al., 2009).

In saline soils high in sodium chloride (NaCl) or sodium sulphate ($\mathrm{Na_2SO_4}$), excessive $\mathrm{Na^+}$ concentration are present, while due to increased ionic strength, P solubility from already sparingly soluble sources is further reduced (Grattan and Grieve, 1992). Accordingly, it was found, that P levels in plants and phosphatases enzyme activity in soils were reduced under saline conditions (Evelin et al., 2012; Rabie and Almadini, 2005). Furthermore, N uptake of both plant available forms of N are in competition with chloride ($\mathrm{Cl^-}$) replacing nitrate ($\mathrm{NO_3^-}$) and sodium ($\mathrm{Na^+}$) replacing ammonium ($\mathrm{NH_4^+}$) (Frechilla et al., 2001). Further, salinity decreases the ability of $\mathrm{N_2}$ fixation by plants due to nutrient deficiency of mainly P and Ca (Evelin et al., 2012). Accordingly, decreasing N concentration in examined plants with increasing NaCl concentration in soil was found by Evelin et al. (2012) and Rabie and Almadini (2005).

Further, under saline conditions, $\mathrm{Na^+}$ is in competition to $\mathrm{K^+}$ for plant uptake due chemical similarities. Excess levels of $\mathrm{Na^+}$ can be observed in salinity stressed plants, increasing proportionally with higher NaCl proportion in the soil as was shown by Evelin et al. (2012), while $\mathrm{K^+}$ and $\mathrm{Ca^{2+}}$ often is deficient, represented by a high $\mathrm{Na^+/K^+}$ and $\mathrm{Na^+/Ca^{2+}}$ ratio (Estrada et al., 2013; Evelin et al., 2012; Rabie and Almadini, 2005). Also, ionic deficiency under salt stress can be observed for trace elements like $\mathrm{Cu^{2+}}$, $\mathrm{Fe^{2+}}$and $\mathrm{Zn^{2+}}$ (Evelin et al., 2012; Giri et al., 2007). Concluding, while important plant nutrients only can be acquired in minor concentrations, $\mathrm{Na^+}$ is accumulated easily in toxic concentration (Fortmeier and Schubert, 1995; Sheng et al., 2008). This nutrient imbalance leads to various effects, detrimental for plant performance. Hence, plant growth can be significantly reduced under saline conditions (Evelin et al., 2012; Fortmeier and Schubert, 1995; Slabu et al., 2009).

AM fungi were found capable to alleviate effects induced by salt stress and contribute to improved plant performance, when compared to non-mycorrhizal plants in saline soil conditions (Evelin et al., 2012). The increase of salt tolerance of plants associated with a fungal partner is attributed mainly to improved acquisition of limited soil nutrients, especially P (Rabie and Almadini, 2005), but also balanced uptake of other major soil nutrients like $\mathrm{K^+}$, $\mathrm{NO_3^-}$, $\mathrm{NH_4^+}$ and $\mathrm{Ca^{2+}}$ as well as trace elements (Evelin et al., 2012; Giri et al., 2007).

Enhanced nutrient supply in challenging saline soil conditions by AM fungi is realized by the enlarged active soil volume scavenged for resources and the efficient uptake and transport of ions in hyphae, when compared to soil transport mechanism (Bolan, 1991; Smith et al., 2011). The reported reduction of $\mathrm{N_2}$ fixation by legumes in saline soil was also alleviated by AM fungi due to improved P and water supply for $\mathrm{N_2}$ fixing PGPB (Rabie and Almadini, 2005). Further, ionic uptake by AM fungi is selective, discriminating against Na^{+} when present in excess, but favouring K^{+}, Ca^{2+} and Mg^{2+} (Evelin et al., 2012; Rabie and Almadini, 2005). Anyhow, AM fungi can supply the vegetal partner with Na^{+} in saline soils, when Na^{+} concentration is within acceptable levels, but may decreases uptake, when salt levels in soil become excessive, representing a Na^{+} buffer (Giri et al., 2007; Hammer et al., 2011; Ruiz-Lozano et al., 2012). Thus, fungal symbiosis contributes fundamentally to improved nutrient balancing under saline conditions.

AM fungi protect the plant from Na^{+} toxicity not only by excluded uptake in saline conditions, but also by regulatory prevention of Na^{+} translocation from root to shoot and leave biomass, while K^{+} uptake is increased. By percentage, more Na^{+} was stored in roots than was transported to leaves in mycorrhizal plants, when compared to non-mycorrhizal plants under saline conditions, as was shown by Evelin et al. (2012).

The mediation of high K^{+} and low Na^{+} concentration in leave biomass is another key element in AM fungi enabled salt stress tolerance of plants, as for enzymatic activities in the cytoplasm, photosynthesis and osmotic adjustment under salt stress, high K^{+} concentrations are needed (Estrada et al., 2013; Giri et al., 2007; Slabu et al., 2009). When K^{+} is substituted by Na^{+} due to high Na^{+} accumulation in leaves, the K^{+} regulated stomata system of the leaves is not operative and becomes dysfunctional, leading to uncontrollable transpiration (Slabu et al., 2009), turning into necrosis of the leave (Fortmeier and Schubert, 1995).

The other way around, with improved K^{+}/Na^{+} ratio, mycorrhizal plants under salt stress exhibit improved water status (Aroca et al., 2007). The authors observed improved leaf water content of plants after AM fungi infection due to sustained balance of transpiration stream (reduced) and vegetal sap flow (increased) in saline conditions, which is in accordance with findings of Sheng et al. (2008). Improved rates of photosynthesis accompanied by enhanced chlorophyll content among other beneficial effects such as improved water use efficiency was reported by Sheng et al. (2008) for mycorrhizal maize plants in saline conditions.

3.2.1.9 AM fungi alleviation of drought stress

Drought stress bears similar consequences like high salinity, as soil nutrients become less available in soils and nutrient imbalance disturbs the transpiration system. Therefore, as was discussed for salinity, also drought stress tolerance of plants can be alleviated by AM fungi activity, mainly due to improved access to soil nutrients like P and K and water resources from deeper soil layers or fine pores and regulation of the transpiration system.

Overall, AM fungi affect resistance to high salinity and drought induced stress both directly and indirectly. Indirectly, the water status is improved by beneficial soil formation, benefitting soil fertility and water storage capacity, as increased properties of soil water storage were observed after harbouring mycorrhizal plant roots (Augé, 2001). This observation may relate to AM fungi mediated formation of water-stable aggregates, mainly due to exudation of glomalin, and the highly branched hyphal network connecting different soil particles (Wright et al., 1999).

Directly, in terms of acquisition of water, AM fungi increase the access to soil volume for water and nutrient scavenging in greater distance to the plant root. The active soil volume to gather nutrients and water is enlarged. Due to their fineness, fungal hyphae can explore soil pores inaccessible for thicker root hairs, which may contain bound water. This way, water resources below the wilting point in a high osmotic potential environment may become available (Dakessian et al., 1986). Also, absorbed water can be efficiently transported by conduit hyphae of the mycelium towards the host plant (Ruiz-Lozano et al., 2012; Ruiz-Lozano and Azcón, 1995; Ruth et al., 2011).

Moreover, AM fungi also can have a beneficial impact on the root hydraulic properties of salt or drought stressed plants, which suffer a physiological drought as high salt content or simply lack of water in the soil lowers the osmotic potential. To again shift the water gradient in favour of water flow from the soil inside the roots, the osmotic potential is decreased by the enhanced accumulation of inorganic ions like K^+ or solutes (e.g. proline) in the root structure (Ruiz-Lozano et al., 2012). This AM fungi induced osmotic adjustment is indicated by findings of Aroca et al. (2007), who found improved root hydraulic properties in mycorrhizal plants, leading to an increased uptake of water from soils under drought or saline stress.

A changing climate and non-adapted agriculture practice, including deforestation, over-usage of fertilizer and pesticides and excessive irrigation, partly even with saline water, already caused the degradation of vast areas of arable land. This trend is likely to continue (Porcel et al., 2012), as pressure for enhanced yields and with it the need for agricultural intensification grows stronger. As was shown, AM fungi symbiosis offers mechanisms for improved stress tolerance of plants grown under detrimental conditions of salinity and drought. Hence, under such abiotic stress, activity of AM fungi should be considered as a key element in a holistic approach, maintaining influx of soil nutrients, while improving the water status of plants.

3.2.1.10 AM fungi alleviation of toxic soil conditions

AM fungi can support the plant supply with low mobility micronutrients, when uptake otherwise is limited due to low concentration or low solubility (Jansa et al., 2003). However in contaminated soils due to geological or anthropogenic pollution (e.g. landfills, long-term usage of contaminated fertilizer) (Nicholson et al., 1994; Rothbaum et al., 1979), micronutrients can act as toxic heavy metals when present in excessive concentration, potentially lethal to plants and soil microorganisms (Wang et al., 2006). Here, AM fungi can have a redeeming effect between necessary plant supply on the one hand and restrain of excessive exposure and alleviation of toxicity on the other hand (Ferrol et al., 2016).

AM fungi can contribute to decreased availability of Al and manganese (Mn) in soils, when excessively present, as found by Alori and Fawole (2012). They reported a decline in Al and Mn concentration, both in soil and plant shoot biomass, when plants grown in contaminated soil were colonized with AM fungi. Here, also it becomes visible, that AM fungi not only restricted uptake and transfer of elements in toxic concentrations to the vegetal partner, but also actively immobilize certain heavy metals by adsorption. These findings are in accordance with Joner et al. (2000), who found a strong AM fungi metal sorption capacity of Cd in contaminated soil. Inferentially, the accumulated Cd can be removed from the active soil and immobilized by the fungal compartments as transfer to the host plant is restricted (Joner and Leyval, 1997). Similar, other heavy metals can be accumulated and immobilized selectively in fungal cells when present in the soil in excessive concentrations, as was shown for Fe, nickel (Ni), lead (Pb), Zn and arsenic (As), depending on soil conditions and the active AM strain or isolate and its adaptivity to the contamination conditions (Kaldorf et al., 1999).

It also was speculated, that through AM fungi improved P status of plants in otherwise challenging conditions of heavy metal contamination, the higher biomass yield and increased uptake of nutrients “dilute” the concentration of accumulated heavy metals below non-toxic levels, hence plant growth is less or non-affected (Christophersen et al., 2009; Fitter, 1991).

Indirectly, by hyphal exudation of glomalin into the soil, AM fungi further restrict the availability of toxic heavy metals for plants. Glomalin was found to strongly sequester Cu, Cd, Pb and Mn by adsorption and complex formation in soils into sparingly soluble forms, thus it can contribute to a reduction of availability for plants when present in excess, enabling improved plant growth in contaminated sites (González-Chávez et al., 2004).

As is a non-essential, toxic element, present in contaminated soils from geological and anthropogenic pollution in similar chemical constitution as P ions (Christophersen et al., 2009). Therefore, it was found to compete with P for plant uptake and can be transferred to the plant by the same vegetal uptake system of P (Asher and Reay, 1979).

This vegetal P uptake system can be downregulated in favour of the fungal uptake system as discussed before in this chapter. Accordingly, in AM fungi colonized plants, substantially less P may be derived via the direct pathway, which shows low selectivity for detrimental, concurrent As uptake. In contrast, through upregulation of fungal P uptake and complete mediation of P supply, mycorrhizal plants may accumulate As to a much less extent, if any, which is almost entirely excluded from uptake and transfer by the fungal partner (Christophersen et al., 2009). Accordingly, As uptake from a contaminated soil was significantly lower from mycorrhizal plants than from uncolonized plants, shown by the same authors.

As a conclusion, AM fungi can be considered a key element of remediation of heavy metal contaminated soils. By the mechanisms of microbial biosorption, selective facilitation or restriction of transfer within the plant and immobilization within fungal compartments, heavy metal availability for plants can be drastically reduced, while nutrient supply in otherwise challenging soil conditions is enhanced. Polluted soils could be restored and recultivated, toxic conditions imposed by mismanagement such as long-term usage of polluted fertilizer could be remediated.

3.2.1.11 AM fungi alleviation of pest and pathogen attack

Many soil borne pest and pathogens like root feeding nematodes or biotrophic fungi diminish plant productivity by feeding on living root tissue and nutrient absorption, causing severe root damage to infested plants and loss of vigor (Veresoglou and Rillig, 2012). Anyhow, AM fungi were found to substantially contribute to bioprotection of colonized host plants against pest and pathogens by indirect mechanisms (Harrier and Watson, 2004).

AM fungi mediate the nutrient supply for host plants, even in low-fertile soil conditions or under abiotic stress situation, as discussed before. The improved, better balanced nutrition status of the host plant may already strengthen the general plant defense mechanisms, reducing the susceptibility to pathogen infection. Further, the improved nutrient status of the plant may contribute to compensation in the pest and pathogen aftermath (Harrier and Watson, 2004).

Morphological changes of the root system of colonized host plants were observed, possibly contributing to bioprotection of plant mediated by AM fungi. Dehne et al. (1978) cited in Akthar and Siddiqui (2008) found increased lignification of cell walls of mycorrhizal tomato and cucumber, which were less susceptible of *Fusarium* wilt. The strengthening of the root cell wall and jointly, the competition for space and infections sites by high colonisation rates of AM fungi (Smith (1988) cited in Harrier and Watson (2004)), may decrease the infection capacity of soil borne pathogens, when AM fungi already engaged in host plant symbiosis, "occupying" the root surface. Further, the permeability of root membranes was decreased after AM fungi induced P supply of a deficient plant, as was shown by Graham et al. (1981). Hence, root colonisation by pathogens or nematodes of mycorrhizal plants may be aggravated due to presence of AM fungi. Contributing, Wick and Moore (1984) found mycorrhizal plants to be able to produce wound barriers post attack, at faster rates than non-mycorrhizal plants, indicating enhanced plant defense due to AM fungi activity. Moreover, after finalized AM fungi colonisation, the exudation of photosynthates in the rhizosphere by the host plant generally is lowered, possibly reducing the stimulus effect needed for hatching of nematodes (Baker and Cook (1982) cited in Akthar and Siddiqui, 2008).

Concluding, by a broad set of various mechanisms, AM fungi can indirectly alter the susceptibility of plants to common pest and pathogen attack and alleviate and compensate damage in the aftermath by enhanced biomass production. Therefore, in organic agricultural system, AM fungi can be considered a bioprotection agent with plant growth promoting properties. Prevention of abiotic stress helps to combat biotic stress, as weakened plants are more likely to be attacked by pest and pathogens. Therefore, to prevent diseases and pathogen attack in the first place, the creation of healthy, fertile soil conditions mediated by AM fungi should be prioritized in a holistic approach.

3.2.1.12 AM fungi alleviation of *Striga* weed

Weed infestation is widespread in many parts of the world, ecologically and economically especially severe in SSA. Here, large areas under cultivation of small-scale subsistence farmers with low purchasing power and lack of access to agricultural production goods struggle to bear a substantial harvest, free from pest damage. Sincerely severe is the infestation of cereal crops like maize, wheat and sorghum with weeds of *Striga* species, particularly *Striga hermonthica*, affecting roughly two thirds of cultivated land (Bouwmeester et al., 2003). Yield losses due to the parasitic weed was estimated to average 68 % in a Nigerian trial (Kim et al., 2002), which represents a huge burden for achieving food security in affected regions. Hence, infestation prevention strategies by a set of approaches were developed, including alternative agronomic practices and the utilization of AM fungi again as possible key element of a parasitic weed control strategy (López-Ráez et al., 2012).

The phytohormone strigolactones is secreted by plants acting as a chemical signal for host plant recognition, but also attracts parasitic weeds. It equally induces seed and spore germination of *Striga* and AM fungi in the soil (Akiyama and Hayashi, 2006; Lendzemo et al., 2007). Once germinated from the seed, *Striga* attaches to the root of the host plant, drawing water, nutrients and metabolites from it (Othira et al., 2012). Accordingly, the host plant struggles and may experience total yield loss.

The potential of AM fungi mediated *Striga* suppression lays in the downregulation of strigolactones exudation once the mycorrhizal colonization is fully completed. Thus, with lowered exudation of Strigolactones, *Striga* is less likely to infest mycorrhizal plant roots. Hence, a rapid root colonisation of the host plant would inhibit *Striga* seed germination and growth, may cancelling out damage by *Striga*, while productivity of the host plant could be increased, as was shown by several studies (Lendzemo et al., 2007; López-Ráez et al., 2012; Othira et al., 2012). This major effect of AM fungi towards *Striga* control could constitute a key element in low-input farming practice, but needs further assessment, especially how to secure rapid fungal colonisation of crops and corresponding response of reducing strigolactones exudation (Lendzemo et al., 2007).

3.2.1.13 Conclusion: AM fungi

All things considered, AM fungi can be seen as one of the most important microorganisms in soils and a key element in a holistic approach for organic, low-input farming practice to be developed in this study, not destroying but restoring arable soil. These multi-purpose, symbiotic fungi bears feature directly or indirectly beneficial for soil fertility and plant vitality.

Firstly, the fungal extension of the actual root zone far beyond the rhizosphere increases the active soil volume for nutrient and water gathering. Secondly, the alteration of microbial communities and exudation of solubility agents increase nutrient availability, especially of P and trace elements. Improved soil formation and aggregate stabilization benefits rates of infiltration and water storage of mycorrhizal soil, thus erosion processes are reduced, thirdly. Furthermore, the fungal mediation of nutrient and water uptake and transfer through the soil towards the host plants bypasses slow diffusion rates and nutrient fixing processes, hence improves the nutrient and water status of the host. All these effects become especially viable, concluding, in the presence of challenging soil and environment conditions of degraded state, low fertility and abiotic and biotic stress, which all can be lessened by fungal activity. Despite all these major benefits, the total net C costs for the host plant remain low or even become nil, as they may be compensated by enhanced plant productivity or allocation of surplus C. The symbiosis, under certain conditions, could be cost-neutral but benefit-positive for the host plant.

Nevertheless, many of the effects are highly dependent on the soil and environment conditions as well as the specific AM fungi and host plant species and their individual interaction, adaptability and cost-to-benefit ratio, and may be positive, neutral or negative in each individual case. Further research is needed to determine suiting fungi-plant combinations, adapted to local soil and environment conditions and intended agriculture management and practice.

Many of the AM fungi effects may even be strengthened and only come into full play, when not only colonising one single plant, but many plants of the same and different species, of the same and different growth status. Potentially, AM fungi organised in such CMN could serve as a biological soil infrastructure and the interlink of a cooperative community of plants.

3.2.1.14 Common mycorrhizal network

Present in almost all terrestrial ecosystems, AM fungi can form spacious CMN, colonising not just one single but multiple individual plants concurrently (Fellbaum et al., 2014; Heijden and Horton, 2009; Kiers et al., 2011), by fusing or extension of below-ground mycelium (Croll et al., 2009; Giovannetti et al., 2004; Mikkelsen et al., 2008). An interconnection for sharing nutrients, carbohydrates, water and information between the plant partners can be created under fungal mediation (Heijden, 2004).

As AM fungi show little host specificity (Smith and Read, 2008), fungal linkage of plants can be intraspecific, between plants of the same species, as well as interspecific, between plants of different species (Heijden and Horton, 2009; Moora and Zobel, 2010). Also, plants of different growth status (seedlings/adults) and in general, dominant and subordinate plants (canopy/understory) can be joined in the same network (Grime et al., 1987; Heijden and Horton, 2009; Pietikäinen and Kytöviita, 2007). Individual terms-of-trade between the multiple plant and fungal partners are highly complex and may be dependent on specific cost-to-benefit ratios, identities of plants, identities of AM fungi, plant species combination and soil and environmental conditions (Heijden and Horton, 2009).

Anyhow, multiple ecological benefits in terms of productivity (Walder et al., 2012), water status (Egerton-Warburton et al., 2007) and resistance to biotic stress (Song et al., 2013) for interconnected plants may arise from the formation of spacious, interconnected CMN, predestined for promotion in organic agriculture management as proposed in this study.

3.2.1.15 Formation of CMN

CMN can be formed by one single AM fungi colonising plants by continuous extension into the soil, “including” plant after plant into the network during its soil exploration. However, more likely as AM fungi are typically abundant in natural soils, CMN can form by extension and overlapping of extra-radical hyphae of multiple AM fungi in the soil (Mikkelsen et al., 2008). When in contact, in the process of anastomoses, compatible hyphae fuse into one consistent mycelium with cytoplasmic continuity, interconnecting the respective host plants into one community (Croll et al., 2009; Giovannetti et al., 2004; Mikkelsen et al., 2008). Hyphae of the same fungus or another fungus of the same species or isolate show high compatibility for anastomoses, while hyphae of fungi from different species are discriminated against (Mikkelsen et al., 2008). Thus, in most natural soils, multiple individual CMN may co-exist and compete for the same transferable soil resources as well as for carbohydrates from manifold colonised, shared host plants, each underlying its specific cost-to-benefit interaction (Heijden and Horton, 2009).

3.2.1.16 Nutrient exchange and carbon utilization in CMN

Properties of nutrient and C exchange between vegetal and fungal partners interconnected in a CMN are highly diverse and seem to differ for various plant-fungi combinations as well as for different cropping systems.

In symmetric monoculture systems (plants express the same C source strength), plants share the C costs for their shared CMN and the resulting nutrient gain in equal terms. However, in asymmetric monoculture systems (shaded/non-shaded, defoliated/non-defoliated or juvenile/adult plants with differing C source strength), where not all plants contribute equally to establishment and maintenance of the CMN, the nutrient allocation as the reward for C supply is shifted reciprocal to the dominant vegetal partner (Fellbaum et al., 2014; Kiers et al., 2011).

Nevertheless, in such an asymmetric monoculture system, the obtained C from the donor plant is partly utilized by AM fungi to fully maintain the root colonisation of the weaker plant, which still is supplied with a considerable amount of nutrients, although not met by its low C supply. Hence, this donor-receiver relationship is C strength-decisive in intraspecies systems, as C supply of hosts triggers the majority of nutrient transfer (Fellbaum et al., 2014).

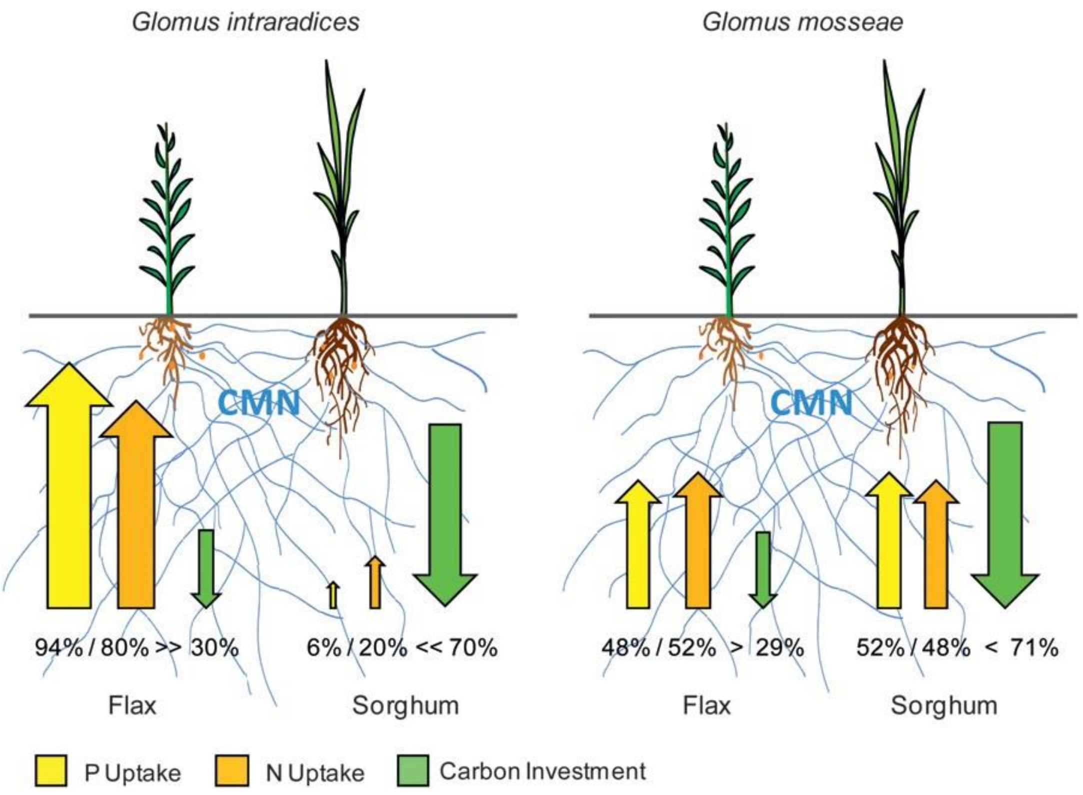

Figure 8: CMN in poly-culture of Sorghum (as C main donor) and flax (as nutrient main receiver), from Walder et al. (2012), p. 794.

In contrast, in a polyculture with interspecies plants linked by CMN, the specific terms-of-trade of each individual plant of the network towards the fungal partner seem primarily decisive for the rate of C supply rewarded with nutrients (see Figure 8). As plants of different species seem to differ in their mycorrhizal compatibility, cost-to-benefit ratios may not be equal for individual host plants (Walder et al., 2012).

Walder et al. (2012) observed the formation of a mycorrhizal interspecies donor-receiver system, in which the strong C source plant sorghum (*Sorghum bicolor*) acted as the main C donor for the CMN of AM fungi *G. intraradices*, while receiving only a minor nutrient inflow (sorghum share of total C supplied to AM fungi: 70 %; sorghum share of total nutrient supplied to plant pair: P 6 %, N 20 %). In contrast, the receiver plant flax (*Linum usitatissimum*) gained the bulk of nutrients (flax share of total nutrients supplied to plant pair: P 94 %, N 80 %) with only little C costs (flax share of total C supplied to AM fungi: C 30 %) (see Figure 8). Flax was much more efficient in nutrient gain from the CMN, at much lower C costs. Interestingly, while the receiving flax was able to increase its biomass by 46 %, the donor plant sorghum showed only a negligible growth depression of 7 %, resulting in enhancement of total net biomass, when compared to the respective mycorrhizal monoculture control groups flax-flax and sorghum-sorghum (see Figure 9).

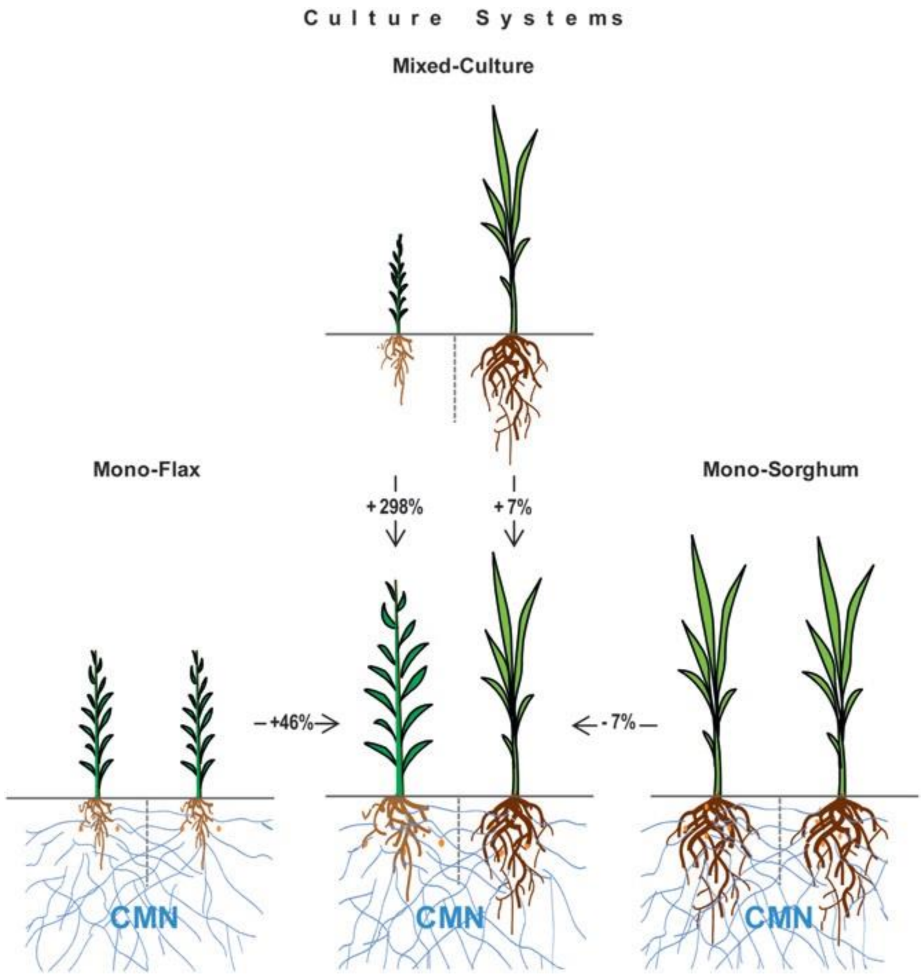

Figure 9: Comparison of total biomass production in mycorrhizal or non-mycorrhizal mono- or poly-culture systems of Sorghum and/or Flax, from Walder et al. (2012), p. 791.

With the bulk of C supplied and no significant reduction in biomass productivity, it was speculated by the authors, that sorghum primarily allocated surplus C to the fungal partner. Hence, the C costs of the main C donor plant for CMN establishment and maintenance may become negligible, when met by allocation of surplus C anyways (Fitter, 1991; Miller et al., 2002; Wright et al., 1998). Concluding, while the symbiosis is almost free of charge for the receiver plant, the main C donor plant may easily bear the bulk of C costs. Hence, the establishment and maintenance of the CMN may come without substantial negative expenditures, while the total net biomass production of the interspecies system could be enhanced (Walder et al., 2012).

This effect may become especially viable in asymmetric, interspecies systems of juvenile and adult plants (e.g. intercropping of perennial and annual crops). While the spacious CMN is established and maintained mainly by the mature, strong, perennial C donors at negligible costs, emerging seedlings of the annual crops may directly connect to an established network, much larger they could create quickly themselves and gain access to nutrients and water from distal soil locations immediately, with almost no investment costs. Simultaneously, by the fast engagement in mycorrhizal symbiosis, *Striga* infestation of seedlings may be reduced, as exudation of signal molecule strigolactones would diminish rapidly. Seedling establishment could be drastically improved, but again, seems dependent on the specific compatibility of donor, receiver and prevalent AM fungi, as was reported by Grime et al. (1987). Concurrent with findings of Fellbaum et al. (2014), for seedlings of the same species as the mature plants maintaining the CMN, no disproportionally access to nutrients was observed (Grime et al., 1987; Heijden, 2004; Moora and Zobel, 1998; Pietikäinen and Kytöviita, 2007), indicating that AM fungi may contribute indirectly to the prevention of invasive plant growth, but support enhancement of biodiversity and coexistence.

In a second experiment by Walder et al. (2012) with the same plant pair but AM fungi *G. mosseae* forming the CMN, the C costs still were shared asymmetric in favour of receiver plant flax, but nutrient share was almost equal for sorghum and flax. Here, for the same amount of C supplied, sorghum gained considerably more P by *G. mosseae* than by *G. intraradices*, thus the functional compatibility of the sorghum and the fungal partner was more efficient (see Figure 8).

All in all, CMN exhibit properties of a biological soil infrastructure for share of nutrients and water and could contribute to enhanced performance of organic agriculture systems. Nevertheless, more research is needed to identify beneficial interspecies donor-receiver-fungi combinations for respective soil, environmental and agricultural conditions. Also, agricultural systems should be developed, in which perennial plants can establish and maintain CMNs at negligible C costs, to which annual plants easily can connect and draw nutrients and water rapidly after germination, so overall biomass production of the system can be increased.

3.2.1.17 Water allocation and distribution by CMN

Next to enhanced acquisition and transfer of water resources from the hyphosphere, AM fungi can also alleviate salinity and drought induced water stress in their partnering host plants by a set of mechanisms, as previously discussed. However, CMN may add another effect for improved water status of the ecosystem.

Under drought stress and severely dry top soil conditions, the nocturnal translocation of water from relatively unstressed, deep rooting plants to fungi by the process of hydraulic redistribution (HR), was reported by Querejeta et al. (2003). Hydraulically lifted water was directly supplied from host roots to connected hyphae in severely dry soils (water potential as low as -20 MPa in 0 to 8 cm depth of top soil), maintaining the integrity and even proliferation of mycelium into the soil, in close proximity as well as great distance to plant roots (up to 14 cm were observed). Even after prolonged duration of drought (more than 80 days), water transfer from plant to fungi was still active, although decreased. Transfer only occurred nocturnal and to associated, symbiotic partners, as parasitic fungi were discriminated against with no water acquired.

These findings indicate a highly adapted strategy for drought stressed ecosystems and true symbiosis between host plant and AM fungi, in which the same resource is acquired and transferred by both partners, in both directions, depending on temporal and spatial availability for each partner (Querejeta et al., 2003). Moreover, otherwise inaccessible resources due to environmental conditions (fixed, immobile nutrients in dry soils with limited microbial activity) or location in the soil (water in groundwater layers), each can only be acquired in substantial amounts by unique activity of one symbiont and accordingly utilized by both symbionts only, when actively shared. A highly mutualistic symbiosis is formed, with total dependency on the respective symbiotic partner.

Furthermore, water received from an uplifting plant can be distributed through the mycelium to other drought stressed plants, interconnected in the same network. Substantial amounts of water (up to one third of the daily evapotranspiration of receiving plants) may be allocated. In affected plants, effects of drought stress were decreased, respectively (Egerton-Warburton et al., 2007). This donor-receiver system between deep rooting plants acting as a water donor and shallow rooting plants acting as the receiver, all mediated by CMN, could potentially improve resistance to drought stress of all the ecosystems affected, with benefits of enhanced nutrient availability for the donor plant.

Concluding, these effects of a biological irrigation system could potentially enhance the water status and resilience of a drought stressed, diverse polyculture, where suiting deep and shallow rooting plants are interlinked by CMN, without any harmful consequences of conventional irrigation practices in areas with high evaporation or saline groundwater.

3.2.1.18 Enhanced pest control by CMN

Additional to general AM fungi inducible mechanisms of pest, pathogen and weed control discussed before, the organization and interlinkage in a CMN may further enhance the resistance of colonised plants against biotic attack.

Utilizing the spacious network as a conduit, plant defense related chemicals as warning signals can be transferred by the mycelium, serving as a communication device for interlinked plants, concluded by Song et al. (2013). They observed the herbivore induced upregulation of defence activities in infested plants, leading to the emission of warning signals into the mycelium, transferred to interlinked plants. Accordingly, plant defense mechanisms in not-yet infested plants were upregulated before the actual infestation and plant damage of warned plants was decreased in comparison to non-mycorrhizal plants not receiving the warning by mycorrhizal transmission.

Concluding, with an intact CMN serving as a biological soil infrastructure for the exchange of nutrients, water and information between the symbiosis partners, significant benefits for the ecosystem could be created, including increased resistance to pest and pathogen attack, without detrimental effects of conventional pesticide application.

3.2.1.19 Conclusion: CMN

These results indicate, that with the suiting combination of host plants and AM fungi species, beneficial CMN can be formed, established and maintained by a strong donor plant at negligible costs, when surplus C is utilized (Walder et al., 2012). The CMN can act as a biological soil infrastructure for nutrient, water and information transfer between plants of the network, potentially supporting juvenile plants with immediate symbiosis almost free of cost, supplying the bulk of nutrients and water needed for establishment (Heijden, 2004; Heijden and Horton, 2009), while resistance to drought (Egerton-Warburton et al., 2007) and pest attack (Song et al., 2013) may be enhanced, as well as overall net biomass productivity of the ecosystem (Walder et al., 2012).

3.2.1.20 Impact of agriculture management on AM fungi and CMN structures

Imposed by intensive agriculture, inappropriate practices altering the biotic and abiotic soil conditions can strongly interfere with activity and proliferation of AM fungi. Although abundantly present in natural soils, AM fungi are found less in quantity and diversity in soils under conventional practice (Banerjee et al., 2019). Several factors can diminish AM fungi activity.

Tillage operation turning the soil upside down not only can create a less permeable plough pan in the depth of the soil, it also leads to decreasing AM fungi spore numbers and hyphal length density in the top soil layer, as fungal compartments are relocated in deeper soil layers (Boddington and Dodd J.C., 2000). Moreover, established AM fungi networks are fragmented and disturbed, reducing the ability of infection (Jasper et al., 1989). Stabilized soil aggregates are disbanded, while infiltration conductivity of topsoil and deeper soil layers diminish. Also, decreased levels of glomalin concentration were reported induced by tillage operation (Wright et al., 1999). When the soil is left barren, without any vegetative cover, surface runoff may enhance erosion processes, with the loss of fertile top soil, in which commonly most AM spores are present (Oehl et al., 2005). Soil compaction induced by intensive trampling or traffic can increase the soil bulk density, which again reduces the ability of hyphae to extend into the soil (Li et al., 1997). The active soil volume scavenged by AM fungi is reduced, so is the amount of accessible nutrients and water.

High input levels of inorganic fertilizer can decrease fungal activity as well, as the plant may sufficiently gather needed P from water-soluble fertilizer sources by root uptake. The need for symbiosis is lowered, root colonisation is reduced accordingly (Gryndler et al., 2001). Therefore, also non-nutrient related benefits of AM symbiosis may become inactive. Also, usage of pesticides including fungicides diminish AM fungi activity. Over time, the abundance and diversity of AM fungi species perish in conventional agricultural soils (Smith and Read, 2008).

Furthermore, the fungal species richness is reduced by continuous monocropping and cultivation of non-mycorrhizal plants as well as extended fallow period (Oehl et al., 2003). AM survival is drastically reduced, spore count in the soils diminished by a non-adapted, inappropriate plant selection and soil management.

All these techniques are performed commonly in conventional farming. Accordingly, AM fungi activity and proliferation and species richness may decrease (Banerjee et al., 2019; Müller, 2013), with possible downside on yield quantity- and quality-wise as well as on the overall ecosystems in terms of fertility and stability of soil over the long term.

In stark contrast to conventional practice, organic farming was observed not to diminish but to favour AM fungi activity and proliferation (Banerjee et al., 2019). Higher numbers of spores and total hyphal length density was found after the addition of organic manure (Gryndler et al., 2001). Application of insoluble, long-lasting organic fertilizer may be optimal for a strong AM fungi activity in soils (Treseder and Vitousek (2001) in Jansa et al. (2006)).

Furthermore, no-tillage or reduced tillage operation, in which soil is loosened but not turned, showed high rates of AM root colonisation, even after fragmenting the mycelium (Müller, 2013). Due to anastomoses, the hyphal compartments can easily re-establish the fungal network and supply nutrients and water (Croll et al., 2009).

In an adapted crop rotation system, Müller (2013) showed the ability of a former established CMN to extend its symbiosis with the follow-up crop after the previous host plant decayed, giving evidence for a multiple season activity of CMN in undisturbed soils, when mycotrophic host plants are continuously available. This also indicates, that plants may benefit especially from early access to an established fungal network, supplying nutrients and water at low C costs as the fungal structures are already established (Heijden, 2004; Heijden and Horton, 2009).

Also, reported by Müller (2013), AM fungi can utilize nutrient sources from decaying host plants and transfer them to an interconnected receiver plant of the same network. Hence, more adapted cropping systems could be considered appropriate for support of AM fungi activity. Examples were given by Salami and Osonubi (2003), who found a tremendous yield increase of Cassava, when grown in an agroforestry with inoculation of AM fungi and regular pruning of trees supplying nutrient rich mulching material.

All things considered, in order to utilize beneficial AM fungi activities in agricultural soils, organic farming practices should be considered for implementation, focusing on poly-cropping with intercropping of legumes and perennials, mulching and application of suiting organic soil amendments, like BoneC and biochar.

3.2.2 Sebacinales

Of the basidiomycetous order, root endophytic fungi Sebacinales can form various mycorrhizal symbiosis types with host plants and are ubiquitous in almost all terrestrial ecosystems, in almost all soils, colonizing a large majority of plants (Weiß et al., 2011). Sebacinales, next to the colonization of important crops like *Triticum* and *Zea mays*, were found able to establish a symbiotic relationship also with plants of *Brassicaceae* (*Arabidopsis thaliana* (Riess et al., 2014; Weiß et al., 2011) and *Brassica campestris* L. ssp. Chinensis (Sun et al., 2010)), which are considered not compatible with endomycorrhizal fungi (Smith and Read, 2008).

In contrast to endomycorrhizal fungi, some strains of endophytic Sebacinales show the ability to shift their metabolism in a continuum between saprotrophism and biotrophism, depending on the environmental conditions and their life stage.

In a first phase after germination from spores, Sebacinales colonize plant roots extracellular on the root surface. This initial stage of the life cycle is followed by a biotrophic growth phase, in which the root epidermis is penetrated by hyphae, colonizing living root hairs and cortical cells by intracellular growth. Colonization often is restricted to the maturation zone of the root, while the meristematic and elongation zones of the root tip is not colonized (Deshmukh et al., 2006; Jacobs et al., 2011).

In the following cell death associated colonization phase, fungi induced die-off of colonized cells occurs. Dead cells are heavily infested with hyphae for intracellular sporulation (Deshmukh et al., 2006). In this saprotrophic phase, endophytes may gain a head start above other saprotrophic microbes by established colonization in living roots, inducing cell death and concurrent starting decomposition of died plant matter for reproduction (Porras-Alfaro and Bayman, 2011).

For successful colonization, the innate root immunity against microbial infection is impaired by fungi induced manipulation of plant immune signalling, leading to the non-recognition of the infestation by the plant. Accordingly, typical defense response (e.g. oxidative burst) was suppressed in colonized roots, allowing root colonization. This overall mechanism was suggested as mainly responsible for the broad host range of endophytes (Jacobs et al., 2011; Qiang et al., 2012; Zuccaro et al., 2011).

After established colonization, some strains of endophytes may elicit plant growth response or priming of the plant defense system against abiotic or biotic stress.

In research of Waller et al. (2005) evidence emerged, that Sebacinales strains of *Serendipita indica* can significantly enhance plant growth of barley (*Hordeum vulgare* L.), both in presence and absence of salt stress. Without salt stress, infection with *S. indica* increased shoot fresh weight of barley by 65 %, while salt stress of plants and following decrease in biomass production was significantly alleviated by the fungus, when compared to the uninfected, stressed control. Further, root colonization did also elicit a priming effect at the plant defence system, as the antioxidant system was upregulated, evident by higher levels of ascorbate following colonization of plants. Coherent, infected plants were more resistant to diseases of the root by *Fusarium culmorum* and also the leaves by powdery mildew (*Blumeria graminis* f. sp. *hordei*) and biomass reduction by pathogenic infestation was significantly diminished by *S. indica*, accordingly. Here, a systemic induced disease resistance becomes evident, as a higher antioxidative capacity in the leaves as well as the roots was found, elicited by the infection with root endophytic fungi (Waller et al., 2005).

Similar results of plant growth promotion were observed for plants under water stressed (Hosseini et al., 2017). The authors reported elevated plant growth (increase in root volume and chlorophyll content) for water stressed wheat (*Triticum aestivum* L. cv. Chamran) colonized by *S. indica*.

Sun et al. (2010) reported positive effects of endophyte *S. indica* on the growth performance of Chinese cabbage (*Brassica campestris* L. ssp. Chinensis). Plant growth as well as resistance to abiotic stress of drought was improved (root and leave fresh weight: +38 % and +46 %, increased chlorophyll content, upregulation of antioxidant system measured as increased content of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) with decreasing content of malondialdehyde (MDA)).

Similar effects of root endophytes were found for *Arabidopsis thaliana* associated with *S. herbamans* co-cultivated with *P. indica* and *P. annua* by Riess et al. (2014), indicating a broad range of so far overlooked Sebacinales strains inducing plant growth and defence mechanisms in crops.

Although above-ground biomass of *Nicotiana attenuate* inoculated with *S. indica* and *Sebacina vermifera* was increased by +28 %, direct nutritional benefits from endophytic symbiosis were suggested unlikely due to a lack of extra-radical mycelium for scavenging water and nutrients for the host plants (Barazani et al., 2005). However, nutrient efficiency could be improved by Sebacinales, as elevated nitrate reductase activity and improved P transportation within colonized plants was found (Sherameti et al., 2005; Yadav et al., 2010). Thus, Sebacinales may affect nutrient status of plants indirectly.

Interestingly, Sebacinales were found abundantly in plant roots of Sudan grass, which was simultaneously colonized by other endophytes and also AM fungi (Venneman et al., 2017). It seems, that AM fungi and Sebacinales can co-exist and may even exhibit a close association and possible interconnectedness (Venneman et al., 2017), as the colonization habit and symbiosis paradigm of those two symbiotic fungi differ strongly.

Further, endophytes may also survive in absence of any host plant by decomposition of organic matter, as shown by Qiang et al. (2012) and Zuccaro et al. (2011), who cultured endophytes on synthetic media. Therefore, inoculum from Sebacinales could be produced more easily than from AM fungi, as no trap host plants may be needed in the process.

Concluding, root endophytic Sebacinales, with their broad host range and colonization ability, could be considered as a potential biostimulant and biocontrol agent in organic, sustainable agriculture practice, with great agronomical potential for a broad spectrum of crop cultivation in stressed and non-stressed conditions, majorly independent on plant selection and management system (Waller et al., 2005; Weiß et al., 2016, 2011). However, with an adapted management system, additional benefits of Sebacinales with AM fungi and PGPB may be activated. Once inoculated, Sebacinales may proliferate in agricultural fields after crop harvest by decomposing dead plant roots and other organic matter, until new emerging roots can be colonized, which could be a tremendous benefit in conventional agricultural practice, struggling to maintain a continuous vegetation cover. Future research should determine ways of efficient on-farm inoculum production of Sebacinales, in high concentration and quantity needed for the reported effects, to harness the benefits of this exciting biostimulant.

3.2.3 Plant Growth Promoting Bacteria

3.2.3.1 Biological N_2 fixation

Leguminous plants typically cover their N demand by engaging in a mutualistic symbiosis with PGPB of *Rhizobium* spp. For engaging symbiosis, rhizobacteria colonize and infect the root structure of the leguminous plant by formation of nodules serving as a protective habitat. N_2 from the atmosphere is fixed into ammonia (NH_3) and exchanged for carbohydrates (Whitehead and Day, 1997). In N deficient soils or soil that show only very little N mobility due to drought, maintaining N supply by biological fixation could be considered as a major advantage for enhanced plant productivity. Further, other non-symbiotic, associative diazotrophic microbes can fix atmospheric N_2, benefitting also non-leguminous plants, presumably in anoxic, reduced soil conditions as often found in stabilized soil aggregates. However, their contribution may be small (Boring et al., 1988), but recent findings indicate a possible underestimation of their role, as many microbes were found to have diazotrophic abilities (Dos Santos et al., 2012).

Interestingly, multiple studies report the stimulating effects on nodule formation and N_2 fixation in general, resulting in higher plant performance, when N_2 fixing bacteria are co-inoculated with other PGPB, e.g. P solubilizing bacteria, and AM fungi (Barea et al., 1987; Bulgarelli et al., 2017; Júnior et al., 2017; Rabie and Almadini, 2005; Wani et al., 2007; Zaidi and Khan, 2007).

3.2.3.2 P solubilization

As discussed earlier, large reserves of P are present in most soils, yet their plant availability is low due to strong adsorption or fixation in most soils. Accessing these P reserves by microbial activity of P solubilizing bacteria thus could change agricultural practice fundamentally and enhance crop yields, especially in developing countries without sufficient access to agricultural production goods.

Soil bacteria of the family *Pseudomonas* spp., *Agrobacterium* spp. and *Bacillus* spp., amongst others, were found to exhibit P solubilizing capacity of both, inorganic and organic P fractions in the soil (Alori et al., 2017; Pandey et al., 2006; Sharma et al., 2013). A common mechanism behind P solubilization is the exudation of organic acids in the process of organic matter decomposition and excretion of H^+ while NH_4^+ assimilation, lowering soil pH. Further, organic anions excreted by PGPB acting as chelators (e.g. citrate, malate or oxalate) can de-complex phosphates, freeing P and compete for P binding sites on adsorbing surfaces of soil constituents. All these mechanisms make inorganic P plant available, while the microbial excretion of enzymatic phosphatases supports the hydrolysis of organic P (Alori et al., 2017; Richardson and Simpson, 2011).

As the refixation of solubilized P is lowering availability in strongly fixing soils, AM fungi with their ability of rapid P uptake and transfer to the host plant, bypassing detrimental soil conditions, show synergistic, co-operative effects when co-inoculated with PGPB (Ordoñez et al., 2016; Zaidi and Khan, 2007).

3.2.3.3 Phytohormones and ethylene reduction

A major effect of beneficial PGPB is the reduction in plant ethylene synthesis when facing biotic and abiotic stress, such as flooding, extreme temperatures, presence of toxicants, phytopathogens, drought or salinity (Glick, 2004). Ethylene, if accumulated in elevated concentration in plant tissue, causes growth inhibition, cell senescence and necrosis and is majorly responsible for diminishing plant growth in such situation.

When colonizing the root surfaces, vegetal exudates of tryptophan trigger the synthesis of indole acetic acid (IAA) by PGPB. The phytohormone IAA is taken up again by the plant root and can stimulate plant cell proliferation and elongation, just as other phytohormones of gibberellin, cytokinin and abscisic acid (ABA) induced by PGPB activity (Karadeniz et al., 2006). However, IAA also can induce the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) synthase, which supports the formation of ACC, a precursor of ethylene. Due to increasing concentration, ACC is then exudated into the rhizosphere, where it is transformed by ACC deaminase producing PGPB into ammonium and metabolized, hence removed from the plant tissue, diminishing ethylene synthesis (Glick, 2004). Accordingly, lowered levels of ethylene and corresponding improved plant growth in abiotic stress conditions were reported induced by PGPB, producing ACC-deaminase (Mayak et al., 2004).

Interestingly, the formation of nodules in legume plants by *Rhizobium* spp. was observed to be decreased by high ethylene concentration (Drennan and Norton, 1972). Accordingly, the presence of ACC deaminase producing PGPB in challenging soils can elevate the effectiveness of rhizobacteria to induce nodulation of legume host plants (Glick, 2004). The authors further speculated, that the same effects may also benefit the establishment of mycorrhiza symbiosis of AM fungi and their host plant, as ethylene levels caused by the infection process could be reduced, resulting in less plant damage, which is in accordance with findings of Guinel and Geil (2002).

3.2.3.4 Properties of biological pest control

Some PGPB also exhibit properties of biocontrol against diseases through the synthesis and exudation of anti-fungal compounds, such as chitinase, salicylic acid, siderophores and hydrogen cyanide, known to activate systemic resistance of plants (Bashan and De-Bashan, 2005; Pandey et al., 2006). These compounds may be active in the inhibition of phytopathogenic fungi by various mechanisms, including the exclusion from Fe supply. As microbial produced siderophores exhibit a higher Fe affinity than fungal siderophores, available Fe is sequestered out of reach of pathogens (Bashan and De-Bashan, 2005). This competition for soil nutrients is supported by competition for living space on the surface of host plants and blocking of entry points into plant cells, contributing to biocontrol of PGPB (Bashan and De-Bashan, 2005).

3.2.3.5 Mycorrhizae helper bacteria

Also, PGPB can directly influence the ability of other soil microorganisms, such as the activity of mycorrhizae. Xie et al. (1995) found *Rhizobium* sp. and *Bradyrhizobium* sp. to act indirectly as mycorrhizae helper bacteria, since they would induce legume roots to increase production of flavonoids. Those chemical signal molecules can improve nod formation for N_2 fixation, but also seem to be able to stimulate AM root colonization, concurrently. Further, *Paenibacillus* sp. bacteria may improve hyphae growth (Hildebrandt et al., 2002), which all in all could contribute to the establishment of a tripartite symbiotic associations between legumes, PGPB and AM fungi in soils (Xie et al., 1995).

3.2.3.6 Conclusion: PGPB

By all these effects exhibited by PGPB, plant vitality and productivity can be enhanced, as was shown in several studies. Although not all PGPB show each of the mentioned properties, many of them bring a broad spectrum of abilities to the table, which together can significantly improve plant performance (Mayak et al., 2004; Ordoñez et al., 2016; Pandey et al., 2006; Wani et al., 2007) and may also exhibit synergistic, co-operative behaviour, further increasing their beneficial impacts, when co-inoculated with AM fungi (Barea et al., 1987; Bulgarelli et al., 2017; Júnior et al., 2017; Rabie and Almadini, 2005; Wani et al., 2007; Zaidi and Khan, 2007). Therefore, it seems important to elevate and protect activity of natural occurring, adapted PGPB in soils, so their effects on plant performance can be utilized efficiently.

3.2.4 Conclusion: Soil microorganisms for promotion of plant growth

Plant growth promoting fungi (PGPF) like AM fungi exhibit tremendous ability to increase nutrient and water supply in challenging soil conditions of low fertility, salinity, water stress or toxic contamination.

AM fungi improve resistance to biotic stress like pest and pathogen attack as well as weed infestation and can stabilize soils endangered by erosion processes and degradation. Organised in a spacious CMN, AM fungi interlink various plants of the same and different species, forming a strong, co-operative plant-fungi-community with additional ecological benefits.

Root colonizing fungal endophyte Sebacinales in contrast to AM fungi do not increase nutrient supply of the colonized host plant. Instead, Sebacinales induce enhanced plant fitness by alleviation of abiotic and biotic stress at low or no additional C costs as dead host cells are utilized for metabolism, which can result in improved plant growth.

PGPB, living freely in the rhizosphere or attached to root material, were shown capable to affect plants positively by enhanced N_2 fixation, P solubilization and synthesis of phytohormones or biocontrol agents. Among others, *Rhizobium* spp., *Pseudomonas* spp. and *Bacillu*s spp. show multiple properties of the mentioned above and could be considered as microbial biostimulants, enhancing beneficial rhizosphere activities, potentially resulting in enhanced plant performance.

Concluding, bearing the ability of P solubilization, among others, the inoculation of PGPB strains and AM fungi propagules joined by integration of Sebacinales in agricultural fields with adapted management practice and plant selection could improve the effectiveness of BoneC fertilizer and the availability of soil P reserves to needed levels for intensive agriculture production, while representing a practical, economically feasible, environmentally sound commodity, especially in developing countries. Therefore, this approach may represent an alternative way of P fertilization, as a competitive alternative for intensive usage of inorganic fertilizer and synthetic pesticides. Thus, by better understanding of the relationship of plants, AM fungi and soil bacteria and their effect on availability of nutrient sources in soil, a most beneficial system could be designed for plant growth promotion in organic agriculture, even under biotic and abiotic stress conditions.

3.2.5 Conclusion: BoneC as a competitive fertilizer in mycorrhizal soils

Slow release of P from BoneC constitutes the major downside of this organic fertilizer for intensive farming practice, as discussed before (Zimmer et al., 2019). In this chapter, the ability of AM fungi and certain soil bacteria to solubilize P was discussed. Concluding, there is evidence by Zwetsloot et al. (2016), that in challenging soil conditions of strong P fixation capacity, by nurturing of referred soil microorganisms in an adapted holistic agricultural system, the P availability of BoneC can be increased drastically, to competitive levels of inorganic fertilizer like TSP in short-term. If this level of fertilizer recovery can be established under field conditions, BoneC could become a true alternative for inorganic fertilizer, especially in SSA, where fertilizer availability is low, very costly and soils are P deficient often. Furthermore, plant unavailable P materials either from soil reserves or water-insoluble fertilizer material could be utilized efficiently by this approach, in general.

A series of mechanisms induced by AM fungi activity may increase the P supply from BoneC and fixed soil P to interconnected host plants. Firstly, most obvious, the enhanced soil exploration of hyphae in larger distance to plant roots and also into smaller pores of the increased active soil volume makes nutrients available, formerly unavailable by position in the soil matrix (Bolan, 1991).

Secondly, hyphae exhibit a faster uptake rate of P than plant roots (Cress et al., 1979; Sanders and Tinker, 1973), as they display a more direct, close contact to P sources, when they grow directly onto the surface (Sanders and Tinker, 1973) or even into crystalline structures of P containing compounds such as BoneC (Leinweber et al., 2019). Also, hyphae possess a larger surface area (Sanders and Tinker, 1973) and higher affinity (Cress et al., 1979) for uptake and transport of P. This leads to a lower threshold concentration for P uptake of AM fungi than of most plants, especially in high P adsorbing soils (Bolan, 1991; Bolan and Robson, 1983; Mosse et al., 1973).

A lowered threshold in turn may improve diffusion rates, thirdly, since the concentration gradient between P in soil solution and fixed P may be steepened (Bolan, 1991). In combination with high uptake rates and P storage ability of AM fungi inside its mycelium (Bolan, 1991), total net P desorption may be enhanced, which could be explained by a continuous rapid removal of P from the soil solution and a shifted P equilibrium in favour of P desorption (Morshedizad and Leinweber, 2017).

Furthermore, finally, the distance for diffusion of P until uptake could be shortened by extending hyphae, growing towards the P source (Bolan, 1991; Sanders and Tinker, 1973). The direct uptake of P from insoluble sources in the very place of solubilization by activity of fungal mycelium may represent an efficient pathway for P acquisition of otherwise unavailable nutrient sources (Mikkelsen et al., 2008). The controlled "solubilization on demand" by punctual exudation of protons, organic acids, enzymes and C compounds (inducing in turn activity of PGPB, supporting P solubilization) by AM fungi directly at the corresponding surface of the inorganic or organic P compound, in direct proximity of roots or hyphae, followed by straightway spatial and chronological uptake of P, may decrease the refixation of solubilized P along the diffusion pathway in soils with high P fixation capacity. No or only little amounts of P may enter the soil solution at all, which would be in accordance with findings and conclusion of Newman (1988), Newman et al. (1992) and Müller (2013), who all reported immediate uptake and transfer of nutrients by AM fungi from a decaying host plant towards the living receiver plant.

This discrepancy between chemical solubility and total plant P uptake of organic P fertilizer like BoneC also found by Zwetsloot et al. (2016) may indicate the importance for mediation of P solubilization and uptake by soil microorganisms. This way, the need for diffusion processes and the "risk" of adsorption and refixation of P along the diffusion pathways in soil towards the plant root may be reduced or completely avoided in mycorrhizal soils, when compared to non-mycorrhizal soils. Thus, this mechanism may not find display in enhanced concentration of plant extractable Olsen-P in the soil solution as this transport step could be bypassed. Hence, solubility data for fertilizer or Olsen-P in soil solution may not fully reflect the P availability of sparsely-soluble, organic fertilizer in mycorrhizal soils (Zimmer et al., 2019).

Further, this mechanism may be supported by findings of Thibaud et al. (1988), who found a major P uptake from decomposed organic sources, above that from inorganic fertilizer, when both were added to the soil simultaneously. The authors reported, that when plants were supplied with both, TSP and plant aerial biomass with low C:N ratio, plants acquired more P from the organic source, than from the inorganic source, as solubilized P from inorganic fertilizer was refixed on the way towards the rhizosphere, while organic P was solubilized and taken up only by direct activity of roots and coherently, solubilizing microorganisms. This could give hint for a more direct pathway of P uptake from organic matter, depending on active vegetal or fungal nutrient allocation, dissolution, uptake and transport, with less interference of potentially disadvantageous soil conditions.

As water-soluble P fertilizer like TSP can show a rapid P fixation after application in P fixing soils (Barrow et al., 1977; Bolan, 1991; Jalali, 2006; Mengel, 1997) and a low fertilizer recovery in the first year anyway (Johnston and Poulton, 2019; Syers et al., 2008), BoneC as an alternative to TSP may become feasible, when AM fungi and P solubilizing PGPB are concurrent nurtured by an adapted agricultural system. The necessary competitiveness of BoneC to TSP in mycorrhizal soils with high P adsorption capacity was shown by Zwetsloot et al. (2016). Despite an advantageous water solubility of TSP over BoneC, the authors found BoneC to be in the same range of effectiveness for P fertilization in a highly P fixing, mycorrhizal soil compared to commercial TSP fertilizer (see Figure 4). Therefore, it is speculated here, that under strong P fixing and water stressed soil conditions (Bolan, 1991), a less soluble fertilizer like BoneC may be equally effective as TSP for P supply in presence of AM fungi, since it becomes only available after extraction through vegetal or fungal activity, without the need to "pass" the P fixing, diffusion-limited soil environment.

Concluding, several studies showed profound effects of AM fungi and PGPB on the solubility of insoluble soil P fractions in general (Koele et al., 2014), and of BoneC specifically (Zwetsloot et al., 2016). In this alternative P uptake process, under certain unfavourable soil conditions, water solubility of P fertilizer, diffusion and mobility of P by soil transport mechanisms may become of minor importance, in terms of total net P supply to plants and fertilizer recovery rate, while the opposite seems true for the level of AM fungi activity. Causal to that, negative effects on P availability by disadvantageous dissolution properties of insoluble P fertilizer like BoneC or declining water content of soils, e.g. caused by drought events, could be lessened. Coherently, the P acquisition capability of plants would be sustained and mediated primarily by activity of soil microorganisms. Accordingly, all soil P pools could be used as a source of nutrients, from readily available P in soil solution or adsorbed on surfaces of soil particles to precipitated or mineral P. Unavailable P sources of soils could become plant available by microbial activity (see Figure 10).

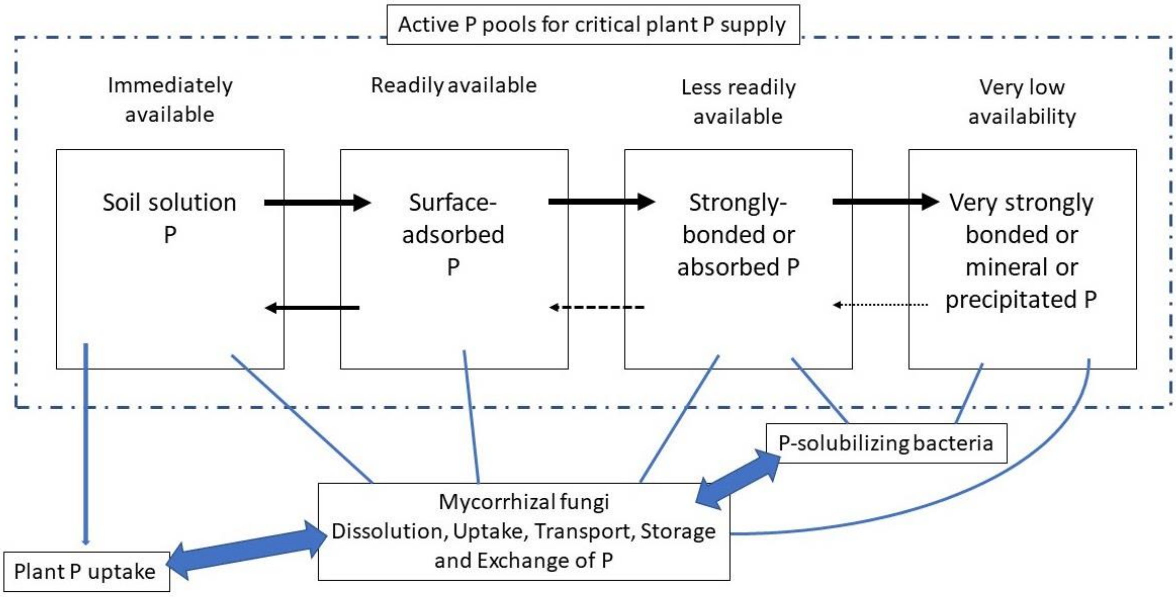

Figure 10: Conceptual diagram of P mobilisation from soil P pools by mediation of mycorrhizal fungi and P solubilizing soil bacteria through dissolution, uptake, transport, storage and exchange of P of all soil P sources with host plants. Wibbing (2020).

High rates of fertilizer recovery and immediate access to supplied P over the short-term and in the consequence high P uptake of plants may be the most important aspects of fertilizer usage in agriculture. BoneC in combination with AM fungi displayed those features in P fixing soils, on comparable levels as TSP (Zwetsloot et al., 2016).

Hence, all things considered, when BoneC in co-operation with AM fungi and P solubilizing PGPB can sustain the rates of P supply to plants, equal in quantity and timing to TSP, they could represent a valid alternative for conventional P fertilizer practice and a keystones to sustain P supply and availability in agriculture production in the coming decades, challenged by declining P reserves, a changing environment and unfavourable soil conditions. Maybe more importantly, BoneC could already today serve as a suitable, alternative P fertilizer material for small-scale subsistence farmers in rural areas of developing countries, at low costs and locally producible from a so far un-tapped waste resource.

To harness P supply from BoneC and from fixed P soil reserves in general, the promotion and nurture of AM fungi and their accompanying beneficial soil biology should be the first priority of an adapted agricultural system. Accordingly, additional soil and plant amendments, complementary to AM fungi proliferation and establishment and maintenance of beneficial soil biology, are included in the holistic system, as described in the following chapters.

3.3 Biochar as an additional soil amendment

3.3.1 BioC General

Black carbon is the charred residue of organic biomass, incompletely combusted during occurrence of natural fires (Atkinson et al., 2010; Cheng et al., 2008; Preston and Schmidt, 2006). In soils, due to its relative stability and recalcitrant properties, microbial decomposition of black carbon is low. Hence, it can represent a considerable fraction of total soil organic carbon (SOC) content, strongly supporting the formation of stable SOM, which is often to referred as humus (Glaser and Birk, 2012; Liang et al., 2010; Ponomarenko and Anderson, 2011). Therefore, black carbon is an important, natural occurring factor for long-term fertility and C sequestration in natural soils (Atkinson et al., 2010; Preston and Schmidt, 2006).

Similar, in anthropogenic "Terra preta de Indio" soils in the Amazonian region, a distinctive fraction of biochar (BioC) and BioC residues can be found, along other fractions of ashes, human and animal manure and kitchen and animal wastes such as bones, shells and feathers (De Gisi et al., 2014; Glaser and Birk, 2012). After centuries and millennia since creation, fertility of those man-made soils is still remarkably high in comparison to adjacent tropical soils, which led to research interest in mimicking the processes of Terra Preta creation to improve stable SOM pools and soil fertility in agricultural soils (Atkinson et al., 2010; Glaser and Birk, 2012).

3.3.2 Production and properties of BioC

BioC is a solid, carbonaceous material for soil amendment with polycyclic, aromatic hydrocarbons, produced in the process of pyrolysis by thermal degradation of organic materials at a temperature range of 350 to 750 °C in partly or complete absence of oxygen (Diekow, 2017; Joseph et al., 2010). The carbonization process leaves BioC compounds with high C content (up to 90 %; Cheng et al. (2008)), depending on feedstock composition and properties, with highly porous structures and great inner surface area, resulting in a low bulk density (Major et al., 2010a). BioC properties of pore characteristics (distribution, size and volume), as well as total surface area, fractional content of recalcitrant C, labile C and ash, resulting pH, electrical conductivity and nutrient content and availability, all depend mainly on the original biomass feedstock and pyrolysis conditions (Chan and Xu, 2009; Downie et al., 2009; Gundale and DeLuca, 2006; Keiluweit et al., 2010).

BoneC, discussed in chapter 3.1, is also a BioC, but pyrolysed from animal bone wastes and not from woody or agricultural waste sources high in C. Thereby, its C content is significantly lower in comparison to BioC, but contains high amounts of P, Ca and Mg. Therefore, both BioC and BoneC, have their unique functions for regenerative agricultural practice and soil restoration and both are considered as valuable components of the holistic approach developed in this study. However, in this chapter, non-bone BioC is discussed exclusively.

In general, high temperature BioC tends to be more chemically stable due to its aromatic, thermally altered structure and diminish proportion of functional groups, fostered by increasing pyrolysis temperature (Keiluweit et al., 2010; Preston and Schmidt, 2006).

With increasing temperature in the pyrolysis process, volatiles are more and more removed from the final BioC, which increases the porosity and volume of nano, micro and macro pores (Downie et al., 2009). Hence, BioC specific surface area from high temperature pyrolysis were found to exceed 400 g/m^2 (Chu et al., 2018; Downie et al., 2009), benefitting adsorption capacity for nutrients and organic C compounds. Further, it was found, that BioC releases humic and fulvic acids at low rates from thermal decomposed lignin into amended soils, which may contribute intensively to observed effects of stable

SOM formation in BioC amended soils over time (De Melo Benites et al., 2005; Hiemstra et al., 2013; Mao et al., 2013; Ponomarenko and Anderson, 2011; Trompowsky et al., 2005).

In manifold ways, the properties of BioC may strongly improve soil fertility and thus crop production by alteration of soil physical and chemical properties and soil life activity, when applied to soils as amendment. Therefore, BioC may be a keystone element for restoration, conservation, resistance and intensification of degraded agricultural land in challenging environmental conditions.

3.3.3 BioC as a soil amendment

Depending on the characteristics of the receiving soil, low BioC dosage between 1 to 20 $t*ha^{-1}$ may alter soil conditions profoundly (Glaser et al., 2002; Liu et al., 2012; Major et al., 2010b; van Zwieten et al., 2010a).

3.3.3.1 BioC effects on soil properties

Bulk density and water status of soils can be improved by BioC amendment. In clayey soils, the lowered bulk density by enhanced porous space from applied BioC contributes to a better penetration and exploration of soil volume by roots and fungal hyphae due to reduced tensile strength (Abel et al., 2013; Chan et al., 2007; Lehmann et al., 2011).

The water holding capacity of soils can be improved by BioC, as well as permeability and infiltration rate (Abel et al., 2013; Kammann et al., 2011; Liu et al., 2012). Soil water can be retained in macro and micro pores of BioC, and held especially in small pores against a strong osmotic potential of drying soils due to BioC pore morphology (Thies and Rillig, 2009). Improved availability of osmotic regulators such as K^+ in soil solution and hence in plants, induced by BioC, can improve water uptake and stomata regulation, resulting in improved drought resistance of plants grown in BioC amended soils (Kammann et al., 2011).

Interestingly, it was found, that a small proportion of applied BioC would be transferred to subsoil layers over time by bioturbation and percolation processes, which may contribute to enhanced nutrient and water uptake from those soil layers due to reduced bulk density and increased pore volume (Major et al., 2010a). The same study came to the conclusion, that BioC due to its initial hydrophobia, its low density and small particle size, is easily eroded in slope terrain during rain events. Accordingly, BioC should be used in a holistic approach of soil management, preventing surface runoff, but favouring water retention and infiltration, when incorporated into the soil matrix.

In BioC amended soils, P availability for plant uptake can be enhanced due to several effects. In acidic soils, high temperature BioC can increase the soil pH by liming effects (Atkinson et al., 2010; Glaser et al., 2002; van Zwieten et al., 2010a, 2010b; Yamato et al., 2006) due to its high content of ash from ongoing volatilization of C compounds (Keiluweit et al., 2010).

During thermal degradation, with increasing temperature, minerals of the feedstock also vaporize during BioC production. With N being mostly present in organic molecules of low thermal stability, N content in general is decreased already in low temperature BioC, while P, K, Ca, Mg along with trace elements of Zn, Fe and Mn, among others, are more stable in high temperature pyrolysis (Amonette and Joseph, 2009; Gundale and DeLuca, 2006). Hence, the ash content of BioC increases as well as the content of nutrients relative to the total mass (Keiluweit et al., 2010). Nevertheless, in comparison to inorganic or organic fertilizers like BoneC or TSP, fertilizing capacity of BioC by nutrient content may be of negligible order (Gundale and DeLuca, 2006; Thies and Rillig, 2009), as content and availability of nutrients especially of P is low, whit only some trace elements present in effective quantities (Naeem et al., 2014; see Table 2).

Table 2: Nutrient content and availability of wheat and rice wheat straw BioC (pyrolysed at 300 and 500 °C for 20 min at peak temperature), BoneC (from rendered or unrendered bones with or without corn or wood biomass, pyrolysed at 750 °C for 45 min) and Triple Superphosphate, adapted and modified from Naeem et al. (2014) and Zwetsloot et al. (2014)). (-) = not analysed, * = calculated as percentage of AB-DTPA extractable nutrient content to total content of biochar (adapted from (Naeem et al., 2014)), ** = calculated as percentage of water soluble nutrient content to total content of material (adapted from (Zwetsloot et al., 2014)).

Material	Pyrolysis temp. (°C)	Total nutrient content and percentage of plant available nutrients to total content							
		N	P	K	Ca	Mg	Fe	Zn	Mn
		g/kg of material					mg/kg of material		
Wheat straw BioC	300	13.8 (-)	2.6 (4.3%)*	30 (43.3%)*	6.3 (17.0%)*	4.5 (6.7%)*	158 (46.2%)*	47 (14.3%)*	106 (1.3%)*
Wheat straw BioC	500	8.5 (-)	3.4 (1.9%)*	36 (27.8%)*	8.7 (8.7%)*	6.9 (3.2%)*	422 (13.5%)*	70 (4.3%)*	163 (2.6%)*
Rice straw BioC	300	11.5 (-)	1.1 (4.1%)*	36 (50.0%)*	9.1 (6.2%)*	8.1 (19.1%)*	195 (16.7%)	67 (20.4%)*	396 (21.7%)*
Rice straw BioC	500	8.5 (-)	1.4 (1.6%)*	48 (22.9%)*	13.3 (3.1%)*	11.3 (9.7%)*	521 (4.1%)*	98 (5.7%)*	649 (19.5%)*
BoneC from unrendered bones	750	-	109.5 (3%)**	-	204.1 (-)	-	-	-	-
BoneC from unrendered bones and corn biomass	750	-	58.1 (4.3%)**	-	110.1 (-)	-	-	-	-
BoneC from rendered bones	750	-	153.2 (0.3%)**	-	337.1 (-)	-	-	-	-
BoneC from rendered bones and wood biomass	750	-	116.6 (0.4%)**	-	258.3 (-)	-	-	-	-
TSP	-	-	205.3 (10.0%)**	-	161 (-)	-	-	-	-

However, as content of oxides of Ca, K and Mg is enhanced during high temperature pyrolysis, formation of hydroxides is promoted, which again can form carbonates with soil liming properties in reaction with soil water and CO_2 (Joseph et al., 2010).

With increasing pH of soils, solubility of P and trace elements can be improved (Atkinson et al., 2010), while the mobility of potentially toxic heavy metal compounds like Al, Zn, Mn and Cu is reduced (Hua et al., 2009; Lehmann et al., 2003; Pandit et al., 2018; van Zwieten et al., 2010a; Yamato et al., 2006). Hence, P precipitation with those metals may be lowered, improving access to available P in the soil solution for plant roots and soil microorganism (Atkinson et al., 2010).

Availability of P for plant uptake is also improved by high adsorption capacity of amended BioC. Fresh, unoxidized BioC possesses great positive surface charge responsible for high anion exchange capacity (AEC) (Cheng et al., 2008). Solubilized P can be adsorbed to BioC surfaces in a labile state, increasing the plant available P pool in soils. It was further found by Cheng et al. (2008), that the displayed AEC of fresh BioC is transient and altered with increasing soil pH and temperature, as oxidized, aged BioC gains negative over positive surface charge, contributing fundamentally to increased cation CEC of BioC amended soils over time.

Coherently, the availability of other soil nutrients and trace elements such as Ca, K, Mg (Glaser et al., 2002; Pandit et al., 2018), and also boron (B) and molybdenum (Mo) (Rondon et al., 2007) were found to be enhanced in BioC rich soils. Bases contained in the ash fraction of BioC can enhance base saturation of soils (Glaser et al., 2002; Yamato et al., 2006). Thus, decline of pH by acidifying soil processes (e.g. from charge balancing by plant uptake of NH_4^+) can be lowered when BioC is amended, which may prevent substantial re-precipitation of solubilized P.

In coherence, Hammer et al. (2014) reported fertile microsites on BioC in soils with high P concentration, which could be out of reach from P fixing Fe, Al and Ca oxides. Interestingly, in accordance with recent findings of Takaya et al. (2016), they further reported higher P adsorption by ash-rich, high temperature BioC than ash-free BioC from low-temperature processes.

Yet, due to the high nutrient adsorption potential of fresh, untreated BioC, soil amendment may facilitate nutrient binding to BioC surfaces, when the external nutrient concentration in the soil solution is higher than the internal concentration at BioC surfaces, which can outcompete plant nutrient uptake. Hence, fresh BioC without post-treatment after pyrolysis may act as a competitor for soil nutrient uptake, whit observed negative effects on agricultural production (Joseph et al., 2018).

To accomplish increased nutrient availability in soils, BioC should be activated prior to soil application. In recent studies, co-composting of BioC as post-treatment with organic material such as agricultural wastes, kitchen wastes or animal and human manure was studied to induce nutrient loading of BioC with positive effects on crop growth, as nutrient availability for plant uptake was again increased (De Gisi et al., 2014; Hagemann et al., 2017; Joseph et al., 2018; Taghizadeh-Toosi et al., 2012a).

By co-composting of BioC with animal manure and green waste or straw respectively, both Hagemann et al. (2017) and Joseph et al. (2018) reported the formation of nutrient rich, highly porous, heterogenous, hydrophilic organomineral layers on outer and inner surfaces of BioC particles, consistent of porous micro aggregates, which were formed from BioC nanoparticles, clay-mineral complexes and humic-like substances from organic matter. This coating of BioC surfaces enhanced porosity, nutrient adsorption, water retention and redox activity of the BioC induced soil aggregates, while excessive oxidation was prevented. It contained drastically enhanced nutrient concentration (also of anionic N and P) and trace elements and were profoundly colonized by fungal hyphae.

When placed in nutrient deficient soils, the pre-treated BioC may exhibit nutrient leaching effects towards the soil solution, as the equilibrium will be in direction of nutrient desorption, thus nutrient availability for plants could be enhanced.

3.3.3.2 BioC effects on soil microorganisms

With high density of labile nutrients adsorbed on BioC surfaces, BioC particles in soil may become attractive, habitable “hotspots” for nutrient mining by roots, bacteria and fungi (Hammer et al., 2014; Warnock et al., 2007).

Hammer et al. (2014) observed growth of AM mycelium on and into pores of both ash-poor wood BioC and ash-rich, high pH BioC from chicken manure (see Figure 11). Here, AM fungi were able to directly utilize surface adsorbed P, which was considered not root available due to small pore size, but exclusively available to soil microorganisms. The authors found close fungal attachment to the BioC surface by secretion of exudates, which enabled direct nutrient uptake of P ions and transfer to plant roots, while diffusion into the soil solution could be effectively prevented.

Further Hammer et al. (2014) reported, that AM fungal hyphae also showed ability of biosensing by discriminating against nutrient-poor BioC, while favouring growth on and into nutrient rich BioC pores,

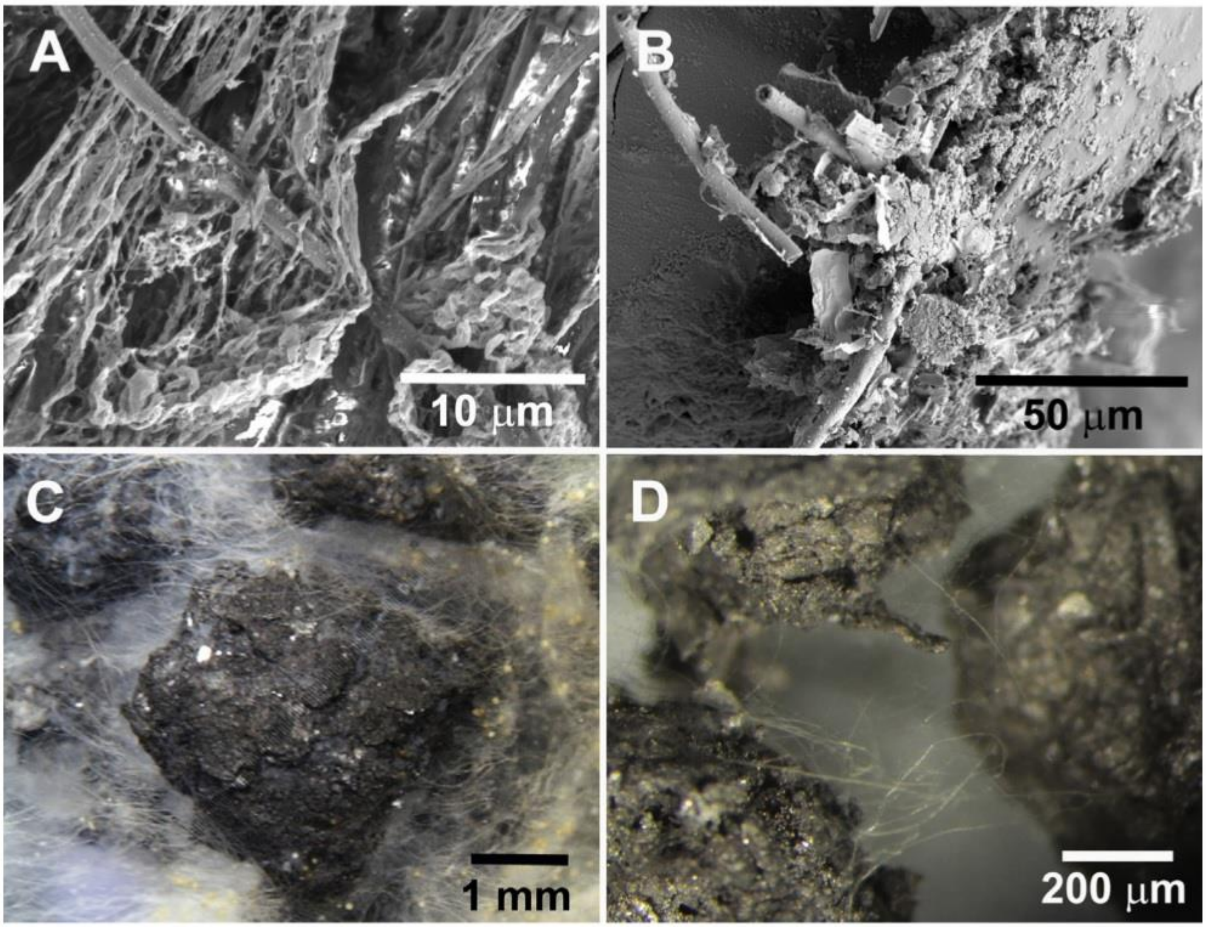

Figure 11: Microscopy images show the interaction of AM fungal hyphae and particles of chicken manure BioC. Outer surfaces of BioC are colonized by hyphae (A), as well as inner parts of BioC (B), as shown by cryoSEN. BioC can be completely covered by hyphae in M-medium (C), which can establish distinct hyphal connection of BioC particles in MilliQ water (D), from Hammer et al. (2014), p. 257.

which also increased AM fungi colonization of BioC (Hammer et al., 2014), similar to discussed fungal growth into organic patches in the soil matrix (see chapter 3.2.1.5).

Jin (2010), Steiner et al. (2008a) and (2007a) all reported enhanced abundance of microbes, when BioC was applied to soils. The small labile C fraction constituting 2 to 3 % of total BioC was rapidly metabolised, most of it over the course of one year (Major et al., 2010a). Yet, in low temperature BioC, labile C fraction is larger and therefore contributes to higher activity of soil microbes in the first weeks of application (Whitman et al., 2016). Anyhow, Steiner et al. (2008a) further reported, that pyroligneous acids from condensated pyrolysis exhaust gases and other pyrolysis condensate residues can spike microbial activity and total biomass alone, more profoundly than single BioC application. Thus, it was indicated, that soil bacteria can utilize pyrolysis residues along with the labile C fraction of BioC for metabolism, although the consumable fraction remains low in comparison to recalcitrant fraction.

As the abundance of microbes can be enhanced, also their community composition can be altered by BioC amendment (Graber et al., 2010; Steiner et al., 2007a). In accordance to findings of Graber et al. (2010), Zheng et al. (2018) reported a shift of bacterial community towards a more beneficial taxa of plant growth promoting *Pseudomonas* spp. and *Bacillus* spp., which may further improve the availability of key nutrients in soils like P from enhanced solubilization. These findings may be supported by Jin (2010), Jindo et al. (2012) and Yoo and Kang (2012), who all found increased levels of enzymatic alkaline phosphatases in BioC amended soils, which indicates enhanced utilization of soil P through microbial activity.

In this regard, BioC not only can activate microbial activity and thereby induces production of nutrient solving exoenzymes in soil solution, but may also serve as a reservoir for adsorption and protective retention of said enzymes in soils, preventing grazing and leaching, while their activity remains, according to findings of Sanchez-Hernandez (2018).

Among others, *Rhizobium* spp. activity and abundance were found to be enhanced by BioC induced availability of trace elements, such as B and Mo (Rondon et al., 2007). Several mechanisms may come into play in this regard: Firstly, adsorption of NH_4^+ on BioC surface decreases plant available N in soils (Steiner et al., 2008b), which may induce enhanced biological N_2 fixation by legume plants, as was shown by Rondon et al. (2007) with common bean (*Phaseolus vulgaris*). Secondly, BioC can enhance availability of P and trace elements, securing nutrition demands of N_2 fixing bacteria (DeLuca et al., 2009; Rondon et al., 2007), which is fundamental for functioning of N_2 fixation itself (Rabie and Almadini, 2005). Finally, BioC may act as a reservoir for chemical signalling molecules (e.g. flavonoids inducing nod formation in legume roots). Hence, adsorbed to BioC surface, these molecules are protected from leaching or predators, remaining active after desorption (Thies and Rillig, 2009). Concludingly, N_2 fixation was observed to improve in presence of BioC, with corresponding enhanced N_2 fixation rates and plant N status (Rondon et al., 2007; Thies and Rillig, 2009).

Interestingly, Xie et al. (1995) found the very same chemical signals responsible for formation of nodules in roots of legume plants to also affect the root colonizing activity of AM fungi. The production of chemical signals in legume roots was induced by *Rhizobium* sp. and *Bradyrhizobium* sp., which lead to increased rates of AM fungi root colonization. Further, according to Yoo and Kang (2012), BioC can also induce activity of *Paenibacillus* species, which were reported by Hildebrandt et al. (2002) to enhance fungal mycelium growth. Therefore, by enhancing activity of mycorrhiza helper bacteria (e.g. *Rhizobium* spp.) through BioC soil amendment, AM fungi symbiosis with host plants may be strengthened. In combination with reduced tensile strength of black carbon soils, AM fungi hyphae proliferation could be improved, which may be in accordance to increasing AM fungi root colonization rates of plants in BioC soils (Yamato et al., 2006).

In general, chemical signals needed for AM root colonization and spore germination (e.g. strigolactones) can be adsorbed on BioC surface and remain activity. Thus, BioC serving as a protective “reservoir” for AM signal molecules, may contribute to establishment of AM symbiosis (Akiyama et al., 2005; Warnock et al., 2007), which ultimately, can again increase access to soil nutrients and water. Therefore, a co-operative, beneficial tripartite relationship between N_2 fixing bacteria, AM fungi and legume plants may be established and induced by BioC in soils (DeLuca et al., 2009; Warnock et al., 2007; Xie et al., 1995).

There is strong indication, that BioC pores act as a beneficial, colonizable soil habitat and growth substrate matrix for soil microorganisms (Hammer et al., 2014; Jin, 2010; Joseph et al., 2010; Postma et al., 2013), with enhanced moisture levels even below the permanent wilting point of surrounding soil (Kammann et al., 2011; Lehmann et al., 2011) and presence of adsorbed organic C compounds and nutrients for metabolism (Liang et al., 2006; Thies and Rillig, 2009). Also, by alteration of oxidative state of BioC, changing adsorption conditions are created, with altered oxygen concentrations, high at the outer BioC surface, while decreasing towards the inner core (Joseph et al., 2010; Liang et al., 2006; Thies and Rillig, 2009), possibly benefitting a wide array of microbes, e.g. associative diazotrophs for additional N_2 fixation.

AM hyphae may also benefit from establishment of a protective space in soil by BioC pores, rich in nutrients of N and P (Thies and Rillig, 2009). It is speculated here, that BioC pores may actually serve as a last available habitat and consequently "meeting point" for diverse soil microorganisms in severely water stressed soils. BioC pores (or soil aggregates in general) may be the last resort for nutrient and water acquisition by fungal hyphae in these soil conditions. When fungal hyphae would directly gather nutrients inside the BioC pores from nutrient solubilizing bacterial partners as indicated by Hammer et al. (2014), once again limiting diffusion processes and lack of soil moisture would not cause total shutdown of plant nutrient supply (Jin, 2010; Thies and Rillig, 2009). Then again, when BioC actually is processed from P rich animal bones as discussed in chapter 3.1 and serves as a microbial-protective habitat with a diverse set of environmental conditions and concurrently, as nutrient rich substrate for P solubilization, fundamental effects on P supply in dry or P fixing soils could occur, as indicated by Zwetsloot et al. (2016).

3.3.3.3 BioC effects on soil processes

Following BioC induced alteration of soil properties and activation of beneficial soil life, also soil processes can be profoundly improved by BioC soil amendment, benefitting nutrient availability and usage efficiency as well as overall soil status.

Gundale and DeLuca (2006) and Nelissen et al. (2012) showed, that BioC can amplify net N ammonification as well as nitrification rates, hence N turnover in BioC amended soils, through stimulation of microbial activity. In accordance to that, Jin (2010) found enhanced levels of enzymatic leucine aminopeptidase, which facilitates the turnover of organic N fractions in BioC amended soils.

Yet again, by higher AEC and CEC, NO_3^- and NH_4^+ may be adsorbed at BioC surfaces, buffering plant available N (Nelissen et al., 2012; Steiner et al., 2008b; van Zwieten et al., 2010b). Further, this may prevent N losses by leaching, especially of highly-soluble NO_3^- after nitrification (Güereña et al., 2013; Steiner et al., 2007, 2008) or enhanced volatilization (of nitrous oxide (N_2O) from NO_3^- or ammonia (NH_3) from NH_4^+; Kammann et al., 2012). Hence, by BioC induced activity of soil microorganisms, nutrient availability in soils may be improved, benefitting plant growth.

The process of leaching can be reduced by BioC especially in sandy and highly weathered, acidic, clayey soils of the humid tropics (Atkinson et al., 2010; Steiner et al., 2008b; Yamato et al., 2006), as nutrients are retained by total surface sorption area and CEC of BioC, despite a higher nutrient content of soils (Liang et al., 2006). In accordance, several studies reported significant interaction of BioC and fertilizer, resulting in higher N fertilizer efficiency and responding yield increase, when N fertilizer was applied along with BioC due to retention of nutrients (Chan et al., 2007; Güereña et al., 2013; Lehmann et al., 2003; Steiner et al., 2008b; van Zwieten et al., 2010a; Yamato et al., 2006).

However, the volatilization of N as NH_3 becomes especially viable in alkaline soils treated with urea fertilizer or urine. Taghizadeh-Toosi et al. (2012) showed, that in BioC amended soils, NH_3 volatilization from NH_4^+ contained in cow urine was reduced by 45 % in comparison to unamended soils, while the plant uptake of N was increased. Therefore, the adsorption of NH_3 and NH_4^+ on BioC surfaces may reduce N losses.

Furthermore, in line with results of Van Zwieten et al. (2010), Kammann et al. (2011), (2012) and (2015) found reduced greenhouse gas (GHG) emissions in BioC amended soils, as N_2O formation was decreased in comparison to unamended soils, despite adopted practices stimulating N_2O emissions (soil water saturation, excessive N fertilization). BioC can improve aeration of soils, decrease denitrification by adsorption of NO_3^- (van Zwieten et al., 2010b), prevent excessive degradation of labile SOM for denitrification by rapid inclusion into stable soil aggregates (Liang et al., 2010; Wang et al., 2017) and increase N uptake by enhanced plant growth (Steiner et al., 2008b). All those effects were suggested as possible mechanisms for reduction of N losses and GHG emission by BioC amendment of soils.

Anyhow, next to nutrients also organic C compounds can be adsorbed onto the surface of BioC in soils and further may contribute to high surface charge density, which translates into a higher CEC (Liang et al., 2006). Van Zwieten et al. (2010) found uptake of heavy metals inside BioC macro pores, contributing to phytoremediation effects of BioC in contaminated soils.

Additionally, BioC was found to display high affinity to adsorb potentially toxic organic molecules such as residual herbicides, fungicides and pesticides (Hilber et al., 2009; Smernik et al. (2010) in Joseph et al., 2010) as well as growth inhibiting phenols and allelochemicals (Bonanomi et al., 2015; Gundale and DeLuca, 2006). Consistently, BioC may contribute to detoxification of soils, which toxicity otherwise may diminish or even prohibit root or microbial growth (Bonanomi et al., 2015; Wallstedt et al., 2002).

3.3.3.4 BioC effects on stable SOM formation

BioC can clearly facilitate soil phytoremediation and lowers abiotic stress factors for plant growth. However, former soil status is not simply restored, but soil structure and fertility can be drastically improved by BioC induced effects of enhanced formation of stabile SOM.

BioC application may contribute to the restoration and conservation of fertile soils over the long-term by fostering aggregation and formation of stabilized, organic-mineral macro aggregates with high content of organic matter and humic substances (HS), both of pyrogenic and non-pyrogenic C origin.

The formation of stable SOM aggregates with high CEC, due to slow, but steady mineralization of BioC, may be facilitated by release of humic acids and humic-like substances into the soil matrix (Hiemstra et al., 2013; Mao et al., 2013; Ponomarenko and Anderson, 2011; Trompowsky et al., 2005). De Melo Benites et al. (2005) found high aromaticity of humic acids from black carbon soils, which were highly stable and reactive, contributing fundamentally to soil aggregation in kaolinite soils.

In finer textured soils, BioC particles were shown to enhance the rapid formation of water-stable organo-mineral complexes (Liang et al., 2010; Wang et al., 2017). HS from slowly mineralizing BioC (Trompowsky et al., 2005) and large surface areas of BioC itself may serve as nucleation sites for aggregate forming processes with clay particles, supported by BioC induced activity of glomalin-producing mycorrhizal hyphae (Wang et al., 2017).

Due to the hydrophobic properties of glomalin and aromatic C structures of BioC itself, SOM included in the formed aggregates is well protected against microbial decomposition and mineralization, as physical disturbance is reduced by improved resistance to wetting-drying cycles (Rillig and Mummey, 2006; Wang et al., 2017).

Furthermore, several studies found alteration of soil enzyme activity in BioC amended soils by shift of microbial abundance and community composition. It was found by Jin (2010), that while activity of alkaline phosphatase (hydrolysing organic P) and leucine aminopeptidase (decomposing organic N) were increased drastically by BioC amendment of soil, enzymes for C mineralization of cellulose (ß-D-cellobiase) and of glucose (ß-D-glucosidase) were significantly lowered.

Highest activity of nutrient solubilizing enzymes was observed on BioC surfaces. Hence, necessity for diffusion of enzymes through the soil solution towards nutrient substrate may be avoided, when both, production of enzymes as well as following induced hydrolysis of organic nutrients is performed directly and in close proximity on and into the BioC porous structure. The author concluded, that P and N use efficiency was enhanced and corresponding C consumption by microbes was lowered by BioC, which decreased rate of C mineralization in BioC amended soils (Jin, 2010).

BioC amendment of soil led to a more efficient nutrient acquirement efficiency and thus decreased C utilization for nutrient supply, which again protected SOM levels in soil (Jin, 2010). Likewise, Yoo and Kang (2012) also reported enhanced activity of phosphatase, but diminishing ß-D-glucosidase activity in BioC soils. Conclusively, C mineralization of labile SOM may be strongly suppressed in BioC amended soils by enzymatic regulation, adsorption and inactivation of C mineralizing enzymes by BioC and rapid inclusion of labile SOM in protective macro aggregates, although the exact reasons remain unknown (Jin, 2010; Liang et al., 2010).

In clay free soils though, despite supporting water status and nutrient retention (Liang et al., 2006), BioC was found to enhance mineralization of SOM (Fang et al., 2015; Wang et al., 2017).

However, in finer textures soils, C losses may be reduced substantially. Overall SOM levels of pyrogenic and non-pyrogenic origin can be enhanced in BioC soils, when compared to BioC free controls (Glaser et al., 2002; Liang et al., 2010; Major et al., 2010a; Steiner et al., 2007b; Wang et al., 2017; Whitman et al., 2016).

These processes, all induced by BioC soil amendment, may contribute fundamentally to the formation of stable SOM along with the remarkable high levels of reactive HS found in Terra Preta soils (De Gisi et al., 2014; De Melo Benites et al., 2005; Glaser and Birk, 2012; Jin, 2010; Liang et al., 2010). Despite challenging environmental conditions of often acidic, highly weathered, clayey soils in high temperature tropical regions, highly fertile soil could be created by addition of suitable BioC, while C can be sequestered long-term over centuries to millennia, depending on BioC and soil characteristics (Fang et al., 2015; Kuzyakov et al., 2009).

3.3.3.5 BioC effects on plant growth

High fraction of stable SOM can increase water status and fertility of soils substantially. Next to alteration of abiotic stress factors, BioC may also improve biotic stress resistance of plants, as different mechanisms of BioC induced suppression of diseases were proposed by Bonanomi et al. (2015).

Firstly, BioC may induce systemic resistance in host plants. Mehari et al. (2015) were able to induce systemic resistance of tomato plants by soil amendment with greenhouse waste BioC (pyrolysed at 450 °C). As a result, tomato plants grown in BioC soils were primed for enhanced resistance, prior to infestation with pathogenic *Botrytis cinereal*, as their oxidative defense system measured as increased H_2O_2 content was upregulated. Consequently, severity of pathogenic attack was reduced substantially. Similar results were reported by Elad et al. (2010) for protection of pepper and tomato plants with BioC soil amendment against powdery mildew.

Secondly, microbial activity in the rhizosphere including AM fungi can enhance disease resistance by improved nutrient supply to plants, competition for infections sites and competition for soil nutrients (Harrier and Watson (2004)). Moreover, Postma et al. (2013) showed, that beneficial bacteria can colonize the surface of BioC particles and fundamentally contribute to resistance to plant pathogens. BioC induced increase of AM fungi root colonization of asparagus resulted in higher root biomass, but also in less root lesions from infestation with pathogenic fungi of *Fusarium* spp. (Elmer and Pignatello, 2011). Similar, Matsubara et al. (2002) reported strong depression of *Fusarium* wilt in mycorrhizal asparagus plants, when the growing substrate was amended with BioC.

Further, BioC may display fungitoxic effects in the soil to some extent (Bonanomi et al., 2015) as certain volatile organic compounds may be produced during pyrolysis and were found to be sorbed on BioC surfaces (Spokas et al., 2011).

Undecomposed or decomposing fresh organic materials can release phytotoxic compounds in the soil, especially in anaerobic conditions, which may increase pathogenic fungal growth and hence, the susceptibility to soil pathogens (Bonanomi et al., 2011). Therefore, finally, allelochemicals and phytotoxic compounds can be adsorbed at BioC surfaces, preventing possible root damage, which again decreases susceptibility for infestation (Bonanomi et al., 2015).

All things considered, BioC particles may serve as artificial soil aggregates, stabilizing and retaining organic C and solubilized nutrients by adsorption and prevention of leaching, volatilization or re-precipitation (Kammann et al., 2012; Steiner et al., 2008b; Yamato et al., 2006), containing water against high osmotic potential and harbour diverse soil microorganisms with beneficial properties for soil fertility and plant growth inside their aggregate matrix, protected from degradation, predators and unfavourable soil conditions. As BioC properties may affect soil constituents and microorganisms the most in close proximity to BioC particles, diverse habitats for proliferation of diverse microbial communities can be created (Joseph et al., 2010; Thies and Rillig, 2009).

Accordingly, in conjunction with a higher shoot-to-root ratio of plants grown in BioC amended soils and improved root growth in general (Kammann et al., 2011; Lehmann et al., 2011), the improvement of crop yield by BioC soil amendment were reported for a variety of plants and ecosystems (Lehmann et al., 2003; van Zwieten et al., 2010a; Yamato et al., 2006).

Lehmann et al. (2003) reported enhanced growth performance of cowpea (*Vigna unguiculata* (L.) Welp) of +43 %, when Ferralsol soil was amended with BioC produced from secondary forest biomass, applied at 10 % weight basis. BioC from unspecified wood at a single application of 20 $t*ha^{-1}$ enhanced nutrient availability in a Colombian savanna Oxisol, which resulted in increased maize yield of +28, +30 and +140 % in the second, third and fourth year after application, in comparison to the control (Major et al., 2010b). Moreover, Yamato et al. (2006) also showed improve yields of peanuts (*Arachis hypogaea* L.), maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* [L.] Walp) by BioC addition (BioC from *Acacia mangium* bark at 10 $l*m^{-2}$, fertilized with 50 $g*m^{-2}$ NPK). Especially maize benefitted from BioC addition, as yields almost doubles in comparison to single NPK application. Finally, positive effects for wheat yields by slow pyrolysed papermill waste BioC amendment (10 $t*ha^{-1}$) of a Ferralsol were reported, when applied along a chemical fertilizer (up to +250 % yield increase of wheat (*Triticum aestivum*), soybean (*Glycine* spp.) and radish (*Raphanus sativus*) in comparison to unamended control), while results of the same application of a Calcarosol varied between crop species. Hence, agronomic benefits of BioC application to soil may be primarily dependent on soil types and conditions (van Zwieten et al., 2010a).

3.3.4 Activation of BioC: nutrient loading by composting or Terra Preta Sanitation

According to findings of Hagemann et al. (2017) and Joseph et al. (2018), pre-treatment of fresh BioC by co-composting should be considered in practice, prior to soil application, as its effects on soil fertility and plant growth in receiving soil can be fundamentally improved.

For better closure of nutrient cycles, inspired by creation of anthropogenic Terra Preta soils in Amazonian regions, resource-oriented TPS became interest of scientific research in the last decade (De Gisi et al., 2014; Factura et al., 2010). In this system, human manure is collected (together or separately) in mobile, water-less sanitation devices. While collection in the storage tank of the toilet device, in a first process step, storage and sanitization of faecal matter and urine can be performed in low oxygen conditions in presence of anaerobic lacto-fermenting bacteria, supplied with external source of carbohydrates (e.g. molasses). In a following second process step of aerobic vermicomposting, the sanitized substrate may be mixed with aged Terra Preta soil, pulverised BioC, woody waste materials, stone meal and water to then be processed by earthworms and soil microorganisms into highly fertile Terra Preta compost (Factura et al., 2010). Supporting, Taghizadeh-Toosi et al. (2012b) reported, that NH_3 from compost or manure can be adsorbed and retained on

BioC surfaces, and was found to become bioavailable, when introduced into the soil matrix. Hence, it also seems viable, to charge BioC directly with nutrients from animal or human manure as post-treatment before soil amendment. Nutrient recovery from so far non-utilized wastewater could be increased, with beneficial impacts on soil fertility and the environment by prevention of nutrient entry into water bodies from untreated wastewater, although possible contamination of the compost with wastewater pollutants has to be dealt with.

Apart from nutrient loading of BioC before soil amendment, BioC properties can also be altered by alternative pre- and post-treatment of feedstock and produced BioC respectively.

It was reported, that specific surface area and total pore volume (and hence porosity) of BioC increased drastically from 9.36 m^2*g^{-1} and 0.012 cm^3*g^{-1} to 930 m^2*g^{-1} and 0.558 cm^3*g^{-1} respectively, with corresponding higher nutrient sorption ability, when the BioC feedstock (pine sawdust) was soaked in solution of phosphoric acid (H_3PO_4) prior to pyrolysis at 500°C (Zhao et al., 2017).

Similar results were reported by Chu et al. (2018), who found doubled specific surface area by post treatment of pine sawdust BioC with H_3PO_4, when compared to untreated BioC (around 800 m^2*g^{-1} to 400 m^2*g^{-1} at 650°C). However, when the feedstock material was treated with the acid prior to pyrolysis, the produced BioC show again increased specific surface area of around 1.600 m^2*g^{-1}. As a side effect, acid treatment also increased total P content in BioC significantly, with further enhances the fertilizing value of acid treated BioC (Chu et al., 2018).

Likewise, the sorption capacity of BioC can also be increased by steam treatment after BioC production. Alvarez et al. (2014) produced a BioC from rice husks with enhanced surface area by steam activation at 800 °C for 15 minutes. At this temperature, steam is directed through a fixed bed reactor filled with BioC, which gasifyes C atoms at the BioC surface, increasing the porous structure.

Interestingly, BioC can also be utilized as a feed supplement for livestock, firstly, improving animal health, feed efficiency and productivity, while, secondly, BioC is enriched in nutrients and bacteria from the digestion processes (Schmidt et al., 2019). Moreover, methane production in rumen of cattle can be significantly reduced by BioC feed supplement, which could decrease GHG emissions of livestock production (Leng et al., 2012).

3.3.5 Economics of BioC as soil amendment

BioC can either be bought from industrial suppliers or be self-produced from organic (waste) biomass on-site by pyrolysis in earth kilns or self-constructed pyrolysis system (Diekow, 2017).

Dosage of BioC application for plant growth enhancement strongly varies in literature, as positive effects of soil amendment depends strongly on local soil and environmental conditions as well as BioC properties.

While high dosage exceeding 100 $t*ha^{-1}$ seems unfeasible in regards of economics, application of 1 to 20 $t*ha^{-1}$ BioC could be expected to increase yields, as was reported by several authors, while being economically viable and processable, especially when BioC could be self-produced on-site, from agricultural waste materials (see Table 4). However, already small dosage of under 5 $t*ha^{-1}$ can have fundamental effect on plant growth (Glaser et al., 2002).

As many rural areas of developing countries are constrained in access to suitable feedstock for BioC production and farmers are often resource limited (Lehmann and Joseph, 2009), in the following, on-site feedstock production for BioC production integrated in a holistic agricultural management is discussed.

Production potential of BioC from different wood production systems is estimated in Table 3, but is highly dependent on multi-layered parameters. For wood biomass production potential these are soil and climate conditions, plant management and fertilization, planting density, tree species used, cultivation duration, yearly wood biomass harvesting quota and previous land usage (Chandrashekara, 2007; David and Raussen, 2003; Grattan and Grieve, 1992; Jama and Getahun, 1991; Jama et al., 2008; Kimaro et al., 2007; Lehmann et al., 2003; Nyadzi et al., 2003). Further, for the pyrolysis system and actual BioC production as well as (annual) BioC yield potential, factors of heating rate, co-pyrolysis material, pyrolysis temperature etc. are decisive (Chan and Xu, 2009; Downie et al., 2009; Gundale and DeLuca, 2006; Keiluweit et al., 2010; Preston and Schmidt, 2006).

Anyhow, under certain soil and environmental conditions, woodlot systems of *Acacia* sp., *Calliandra* sp. or *Sesbania* sp. trees could produce BioC feedstock for BioC production in a relative short time of two years rotation cycles, resulting in final applicable BioC quantities from 5.8 to 6.4 $t*ha^{-1}$ (David and Raussen, 2003), when pyrolysed with adequate technology. Interestingly, while no intercrop was cultivated during wood production, follow-up crops (wheat and maize) performed significantly better in comparison to natural fallow or continuous cropping due to increased soil N content (David and Raussen, 2003; Nyadzi et al., 2003).

While agroforest systems may show comparable (annual) BioC yield potential in comparison to wood lot systems (see Table 3), tree stems first have to develop sufficiently to allow frequent pruning for efficient production of wood biomass and green leaf manure or livestock fodder before start of cutting (Chandrashekara, 2007; Jama et al., 2008). Therefore, it may be feasible to start BioC feedstock production in a woodlot system with high tree densities, which is thinned out after around 2 years, yielding potentially 2.9 to 3.2 $t*ha^{-1}$ and 4.35 to 4.8 $t*ha^{-1}$ BioC, when 50 % or 75 % of trees are removed (considering values reported by David and Raussen (2003)). By thinning, feedstock for BioC is produced, while the woodlot system could be transformed into an alley cropping system with well-developed tree stems in rows and sufficient space for intercropping in the interspace, already enriched in soil N by the thinned out, leguminous trees (with 2 or 4 m row distance accordingly to the ratio of tree removal, when 10.000 $trees*ha^{-1}$ in 1 x 1 m spacing is assumed as in David and Raussen (2003)). Following, in an established agroforestry system with intercropping, annual BioC yield potential may range between 500 to 800 $kg*ha^{-1}$, assuming one-third to half of newly produced woody biomass from trees is pruned annually, as recommended depending on tree species by (Chandrashekara, 2007).

With prices starting around 300 $USD*t^{-1}$ BioC from commercial suppliers (Joseph et al., 2013) or costs around 40 $USD*t^{-1}$ in self-production with modern batch kilns from on-site waste materials (Joseph et al., 2009), calculation for BioC soil amendment economics is presented in Table 4.

Based on the findings of Major et al. (2010) the calculated amortisation time of 2.5 and 3.49 years for application with 8 $t*ha^{-1}$ and 20 $t*ha^{-1}$ self-produced BioC is drastically shorter than that for purchased BioC (see Table 4). Including certificates for CO_2 emission into the revenue stream, the investment costs for BioC production could directly become negative (assuming a price of 27.5 $USD*t^{-1}$ of CO_2 emission[1] prevented by sequestration and about 3.3 t $CO_2{*}^{-1}$ C with C content in BioC of 76 % (Gaunt and Cowie, 2009; Lehmann et al., 2003)).

Hence, usage of self-produced BioC at rates of up to 20 $t*ha^{-1}$ may become economically feasible for production of certain crops under certain soil and environmental conditions, after a short time. Further, as BioC also influences fertilizer efficiency and water status as well as resistance to pest attack,

[1] Price of European Emission Allowance in $USD*t^{-1}$, accessed on the 28.10.2019; EUR/USD = 1.1085, URL: https://www.eex.com/en/market-data/environmental-markets/spot-market/european-emission-allowances

Table 3: Estimated BioC production potential from different wood production systems (based on results from Lehmann et al. (2003), Jama et al. (2008), Nyadzi et al. (2003), Kimaro et al. (2007), David and Raussen (2003) and Jama and Getahun (1991)). * = C content in woody biomass was estimated at 45 % and used to calculate BioC yield potential with 53.5 % conversion factor of wood biomass C to BioC C, from Lehmann et al. (2003); ** = Assuming linear biomass production over time.

Wood production system	Region	Production conditions	Dominant plant spp.	Age (years)	Woody biomass (Mg/ha)	BioC yield potential * (Mg C/ha)	Annual BioC yield potential ** (Mg C /ha*a)	Reference
Primary forest	Manaus, Brazil	-	-	-	251.2	60.5	-	Fearnside et al. (1993) in Lehmann et al. (2003)
	Para, Brazil	-	-	-	222.3	53.5	-	
	Para, Brazil	-	-	-	225.1	54.2	-	Johnson et al. (2001) in Lehmann et al. (2003)
Secondary forest	Para, Brazil	following moderate-intensity pasture	-	3.5	12.9	3.1	0.89	Buschbacher et al. (1988) in Lehmann et al. (2003)
		following moderate-intensity pasture	-	8	30.4	7.3	0.91	
		following low-intensity pasture	-	8	81.8	19.7	2.46	
improved fallow / rotational woodlot system	Western Kenya	1.400 to 1.800 mm precipitation, tree density *C. paulina* =349.000; *T.candida* =180.000 trees*ha^{-1}	*Crotalaria paulina*	1	10.8	2.58	2.58	Jama et al. (2008)
			Tephrosia candida	1	8.4	2	2	
	Shinyaga, Tanzania	700 mm precipitation yearly, 3 month old seeldings at 833 trees*ha^{-1} in 3 x 4 m spacing, intercropped with maize in the first 3 years	*Acacia polyacantha*	7	70.9	16.9	2.4	Nyadzi et al. (2003)
			Leuceceana leucocephala	7	88.9	21.2	3.02	
	Tabora, Tanzania	928 mm precipitation yearly, 8 week old seedlings at 625 trees*ha^{-1} in 4 x 4 m spacing, intercropped with maize in the first 3 years, receiving NPK	*A. crassicarpa*	5	35	8.35	1.67	
			A. julifera	5	20.5	4.89	0.978	
			A. leptocarpa	5	40.9	9.75	1.95	
	Morogoro, Tanzania	800 mm precipitation yearly, 5 month old seedlings at 1111 trees*ha^{-1} in 3 x 3 m spacing	*A. crassicarpa*	5	51	12.16	2.43	Kimaro et al. (2007)
			Gliricidia sepium	5	29.15	6.95	1.39	
	Kabale, Uganda	1000 mm precipitation yearly, terraced slopes (8%), potted seedlings at 10.000 trees*ha^{-1}	*A. acuminata*	2	24.3	5.8	2.9	David and Raussen (2003)
			Calliandra calothyrsus	2	25.8	6.15	3.08	
			Sesbania sesban	2	26.9	6.42	3.21	
Agro-forestry	Mtwapa, Kenya	1260 mm precipitation yearly, 6 month old seedlings planted in plowed soil at 2 m distance between rows and 0.1 m within rows (50.000 trees*ha^{-1}), intercropped with either cassava, napier and bana grass at 10.000 plants*ha^{-1}	*L. leucocephala*	2.7	31.8	7.58	2.81	Jama and Getahun (1991)
			L. leucocephala + Cassava	2.7	24.8	5.91	2.19	
			L. leucocephala + Napier grass	2.7	24.1	5.75	2.13	
			L. leucocephala + Bana grass	2.7	20.4	4.87	1.8	

Table 4: Estimated amortisation of BioC application due to yield increase of maize, based on findings of Major et al. (2010). * = Maize price assumed to be 165 USD*t^{-1}, according to Index Mundi at 25.10.2019, URL: https://www.indexmundi.com/commodities/?commodity=corn; **a = Cost of commercial BioC = 300 USD*t^{-1}, as in Joseph et al. (2013), **b = Cost of self-produced BioC 40 USD*t^{-1}, on-site, as in (Joseph et al., 2009).

BioC amendment (t/ha)	**Plant spp.**	**Maize grain yield over 4 years and average per year (t/ha)**	**Yearly income (USD)***	**Cost for BioC amendment per ha **a,b**	**Amortisation time of BioC application (a)**
0	Maize (*Zea mays* L.)	17.6 (4.4)	726	0	-
8 (purchased)		20.7 (5.175)	854 (+128)	2400	18.75
8 (self-produced)		20.7 (5.175)	854 (+128)	320	2.5
20 (purchased)		23.16 (5.79)	955 (+229)	6000	26.2
20 (self-produced		23.16 (5.79)	955 (+229)	800	3.49

economic viability could actually be more profound than shown here due to savings in fertilizer, irrigation and pesticides or simply by better plant performance under such stresses, when compared to unamended soils.

3.3.6 Conclusion: BioC as additional soil amendment

As an aromatic, carbonaceous material, produced by pyrolysing biomass in low oxygen environment, BioC can serve as a beneficial soil amendment for enhancing soil fertility and soil structure. Applied to soils already in quantities of 1 to 20 t*ha^{-1}, depending on BioC feedstock, production, activation process and soil properties, BioC can improve infiltration and retention of water. It lowers volatilization and leaching of nutrients, enhances availability of soil nutrients by neutralising acidic soil pH and thereby immobilizes toxic elements concurrently. All, while plant available nutrient pools are enlarged and nutrient acquisition in general is simplified by BioC.

Further, as toxic soil constituents, detrimental for plant or microbial growth, can be adsorbed and fixed on the porous structure of BioC, it can contribute tremendously to soil detoxification and phytoremediation approaches. Concurrently, by enhanced nutrient availability and creation of favourable growing habitats with diverse sets of environmental conditions on the micro-scale, BioC can induce growth of soil microorganisms and shift their community composition, among others, towards PGPB and AM fungi. In combination with adjusted agricultural practice of intercropping with legumes, BioC can induce tripartite relationship between plants, PGPB and AM fungi, improving soil conditions, crop production and stress resistance.

Consistently with improved soil structuring by low bulk density of BioC, also HS are introduced by the soil amendment, which in cooperation with active, soil exploring fungal hyphae, enhance the rapid formation of water-stable organomineral macro aggregates. Those persistent aggregates may protectively enclose SOM and leachable nutrients, resulting in elevated soil fertility and long-term C sequestration for centuries or even millennia – from pyrogenic and non-pyrogenic sources, forming stable SOM, like the famous Terra Preta de Indio.

For suitable agricultural practice, fresh BioC can be activated by co-composting with agricultural waste or manure or utilized as livestock feed supplement, which again increases its ability for later stage soil improvement.

Ultimately, also by BioC induced resistance to abiotic and biotic stress, crop yields can be enhanced substantially, especially in regions of the tropics with highly acidic, hard-to-work on, fragile soils with high rates of erosion, SOM mineralization and declining fertility. Therefore, BioC from woody waste material, agricultural waste or even livestock bone waste may be a fundamental keystone for any holistic approach targeting productive and economical feasible restoration of degraded soils, as developed in this study.

3.4 Soil remineralization for functioning of soil biology

Fundamental processes of plant functioning are dependent on sufficient supply of essential nutrients. For adequate growth and yields as well as resistance and response to abiotic and biotic stresses, plants require essential nutrients, including macro nutrients of N, P, K, Ca, Mg and S in larger amounts and micro nutrients of Cu, Fe, Mn, Mo, Ni, Zn, B and Cl in very small or only trace amounts. Elements of selenium (Se), cobalt (Co), Si and Na are considered non-essential, but potentially beneficial, at least for certain plants (Dimkpa and Bindraban, 2016; Mengel et al., 2001; White and Brown, 2010).

While application of macro nutrients of N, P and K as conventional fertilizers is broadly established globally as common agricultural practice, status of Ca, Mg and S as well as status of micro nutrient in agricultural soils is often neglected and not taken care of (Vanlauwe et al., 2015). However, these elements are of equal essentiality for plant functioning.

Mn is involved in the enzyme activation for photosynthesis and plant respiration as well as functioning of the protein synthesis. Mg as part of chlorophyll is fundamental for functioning photosynthesis, while Mo is active in N_2 fixation and N metabolism of plants in general, similar to Co, which is also a constituent of vitamin B12. Further, non-essential Si can improve stalk stability of plants, which can improve yields (Amelung et al., 2018; Mengel et al., 2001).

Accordingly, when plants experience deficiency of essential micro nutrients (one or multiple) due to unavailability from detrimental soil conditions, non-adapted crop residue management, nutrient leaching or simple low endowment from parent rock material, crop yields and general plant growth and function can drastically decline (Vanlauwe et al., 2015). Equally, turning tables around, when supplied in sufficient amounts to plants, micro nutrients can enhance growth and resistance to stress factors of abiotic or biotic nature (Ashraf et al., 2014; Bagci et al., 2007; Giannousi et al., 2013; Kumar et al., 2009; Obrador et al., 2013; Raliya and Tarafdar, 2013; Servin et al., 2015; Tariq et al., 2014).

In omission trials in Burundi, a decline by 26 % in maize yield each was observed, when plants were fertilized with all macro and micro nutrients deficient in the growth substrate except either B or Cu. Here, it was indicated, that all essential nutrients need to be supplied to plants in order for basic functioning (Vanlauwe et al., 2015).

In wheat plants (*Triticum aestivum* L. cv. HD 2285), fertilization with 1.5 $mg*kg^{-1}$ Cu significantly increased all plant growth parameter in comparison to the unfertilized control, including grain yield by +62 %, while at higher Cu application rates (2.0 and 2.5 $mg*kg^{-1}$), decreasing content of other micronutrients (Fe, Mn and Zn) in leaves was observed (Kumar et al., 2009). In another study also using wheat grown in Zn and B deficient soil, Singh et al. (2014) reported plant growth enhancement by Zn and B in regards of total grain yield per plant (+31.8 % by Zn at 10 $mg*kg^{-1}$ and +39.1 % by B at 0.75 $mg*kg^{-1}$). In spinach plants (*Spinacia oleracea* L.), grown in Cu deficient substrate, increased plant dry mass by up to +102 % in comparison to untreated control was observed, when fertilized with chelated Cu at 3 $mg*kg^{-1}$ (Obrador et al., 2013). Again, in three different maize hybrids, the mean increase in grain yield by Zn treatment (foliar applied as 1 % ZnSO4 at 9 leaf vegetative stage) was +38 % over mean of untreated controls (Tariq et al., 2014).

Also, in conditions of abiotic or biotic stress, application of certain micro nutrients can support plant performance. Different rice cultivars under salt stress showed higher grain yields, when foliar treated with chelated Zn spray at 0.05 % concentration. While concentration of macro and micro nutrients (N, P, K, Ca, Mg, Mn, Fe, Cu, Zn) decreased in salt stress plants, Na was strongly accumulated. Zn treatment ameliorated these adverse effects for most of the cultivars. Accordingly, under salt stress, photosynthetic rate was improved by Zn treatment due to improved nutrient uptake of fundamental micro nutrients. Coherently, in Zn treated plants, also content of osmolytes (amino acids, soluble sugars, soluble proteins) were increased, with corresponding higher rates of water use efficiency, indicating improved water uptake and hence, osmoprotection from elevated salt levels in soils (Ashraf et al., 2014).

Interestingly, Zn treatment also showed the same effects in non-stressed plants. When compared to untreated, non-stressed controls, plants treated with Zn foliar spray accumulated more osmolytes, reduced Na uptake, but increased uptake of N, P, K and micro nutrients and had higher rates of photosynthesis, which all resulted in higher grain yield (Ashraf et al., 2014).

Similar, in findings of Bagci et al. (2007), it was reported that in severely drought stressed soil with high Zn deficiency, grain yields of different wheat genotypes were significantly improved by Zn soil application (23 $kg*ha^{-1}$), in both rainfed and irrigated open fields. It was indicated, that sufficient Zn supply can especially improve water use efficiency of stressed plants and further supports the alleviation of oxidative stress, as Zn is part of the antioxidant enzyme SOD (Bagci et al., 2007).

Several micro nutrients (among others, Zn, Cu, Mn and Fe) were found to be essential for the enzyme activity of the antioxidant systems of plants (Bagci et al., 2007; Mengel et al., 2001; Rahimizadeh et al., 2007). Hence, when sufficiently available for plant uptake or by direct application (e.g. by foliage spraying) of micro nutrients, the antioxidant system of plants under pathogenic attack or water stress could be strengthened, benefitting resistance and response to biotic stresses.

Foliar applied oxides of Cu and Mn both were found able to inhibit diseases progression of pathogenic fungi *Fusarium oxysporum f.* sp lycopersici on tomato plants (Servin et al., 2015). Similar, Giannousi et al. (2013) reported decreased infection of tomato plants with pathogenic fungi *Phytophthora infestants*, when Cu based formulations were foliar applied. However, in both studies by Servin et al. (2015) and Giannousi et al. (2013), the best results in regards of pathogen inhibition was reached not by application of bulk micro nutrients, but by nanoparticles (NP) formulation of the respective micro nutrients, as their uptake by stomata of plants or small plant openings in general could be more rapid and efficient due to smaller diameter (Raliya and Tarafdar, 2013; Servin et al., 2015).

Interestingly, application NP instead of bulk micro nutrients may also further enhance observed effects of plant growth and crop production. Raliya and Tarafdar (2013) reported positive effects of nano and bulk ZnO treatment (foliar spray with 10 $mg*l^{-1}$ nano- or bulk ZnO at 25 ml per plant, applied 2 weeks after germination) on cluster beans (*Cyamopsis tetragonoloba* L.). While bulk ZnO increased growth parameters only slightly, nanosized ZnO drastically improved all measured parameters. Shoot length, root length and dry biomass was increased by +7.1 %, +15.9 % and +10.8 % by bulk ZnO and +31.5 %, +66.3 % and +141.9 % by ZnO NP respectively, when compared to the untreated control. Chlorophyll content was also increased (+143 % and +276 % by bulk and NP ZnO). The authors found increased microbial soil life measured as counts of fungi, bacteria and actinomycetes, as well as increased activity of acid and alkaline phosphatase by both ZnO treatments. Accordingly, as P solubilization was enhanced indirectly due to ZnO treatment, total P uptake in plants was enhanced by +10.8 % for NP ZnO. Hence, as suggested by the authors, the production and utilization of new fertilizer from micro nutrient NP may seem favourable for application in agriculture in order to enhance plant growth and crop production (Raliya and Tarafdar, 2013).

This may come in handy, as under certain soil and environmental conditions, some agricultural soils already can show non-responsiveness to NPK fertilization due to lack in essential micro nutrients (Vanlauwe et al., 2015). Furthermore, different studies show, that crop responses to individual micro nutrient fertilization may be dependent on treated plant species, application rate and mode, micro nutrient and soil conditions (Dimkpa and Bindraban, 2016), but can have dramatic effects, when deficient and limiting crop growth or oversupplied in toxic concentrations. As the efficiency of macro nutrient fertilization may be drastically enhanced by application of deficient essential micro nutrients (Kumar et al., 2009; Obrador et al., 2013; Singh et al., 2014; Tariq et al., 2014), appropriate soil testing and adequate soil fertilization with micro nutrients as well as an adapted agricultural management system to utilize micro nutrients by enhanced soil biology should be considered in any agricultural operation.

Concluding, soil remineralization or foliage application with deficient micro nutrients can be seen as a great potential to stimulate plant growth, enhance resistance to abiotic and biotic stresses, while nutrition value of crops is increased and macro fertilization may become more efficient, thus may be reduced in quantity (Servin et al., 2015).

3.5 Biostimulants as plant additives

The term biostimulant refers to both biochemical substances and specific microorganisms with stimulatory effects on plant growth, not by their nutrition value but content of bioactive compounds or specific activity in soils. When applied to plants, seeds or growth substrate in specific formulations and usually only minor quantities, biostimulants can potentially activate and promote nutrition of plants, biotic and abiotic stress resistance and/or crop quality by modification of plant physiology and soil processes, resulting in increasing plant growth and performance (du Jardin, 2015; Halpern et al., 2015).

Up to until recently, a formal definition of biostimulants was lacking, thus no specific legislative framework for biostimulants was present. So far, commercial biostimulants were considered as "plant protection products" under EC regulation No. 1107/2009, similar to plant growth enhancers and herbicide safeners (du Jardin, 2015).

However, by the EC regulation No. 2019/1009 the rising interest and development in biostimulants was recognized on legislation level. A formal definition was included in new regulation, regarding biostimulants as products for stimulation of plant nutrition by mechanisms other than its nutrient content for improvement of nutrient use efficacy, abiotic stress tolerance, quality traits and/or nutrient availability in soils. In the future, (commercial) biostimulants will be regarded due to their value of nutrition improvement under regulation of fertiliser products, distinguished as microbial plant biostimulants and non-microbial plant biostimulants (Fertilising Products Regulation (FPR) (EU) 2019/1009, 2019). Although applied BoneC and BioC may possess stimulatory effects on plant nutrition and hence plant growth by other means than pure nutrient content, these materials should be considered as soil stimulatory amendments rather than biostimulants benefitting plants in the sense of the former given definition, reasoning that interaction with plants is indirect, via enhancing and protecting soil microorganisms serving as a "middleman" for plant growth stimulation and improving soil physical and chemical conditions, including nutrient amounts.

In recent reviews of Calvo et al. (2014) and du Jardin (2015), various biostimulants were classified due to their content, origin of active substances or activity in soils. Humic and fulvic acids, protein hydrolysates of plant or animal wastes, extracts from seaweed and plants, biopolymers including chitosan, inorganic compounds as well as beneficial fungi and bacteria were distinguished in different main groups of biostimulants (Calvo et al., 2014; du Jardin, 2015). Anyhow, as some biostimulants contain a complex mixture of several active substances, it may complicate the discovery of each mode of action and effect on treated plants (Yakhin et al., 2017).

PGPF and PGPB were already discussed as key elements of soil fertility, plant nutrition and defense resistance with biostimulatory effects (see chapter 3.2). In the following, interesting plant and soil additives from biochemical active substances as classified before are presented, but beyond that, also individual organic or inorganic compounds are discussed.

3.5.1 Humic substances: Humic and fulvic acids

HS display potential to benefit plant growth upon plant or soil application (Rose et al., 2014). HS are natural occurring compounds of humic acids, fulvic acids and humins in soils from the decomposition of vegetal, animal or microbial organic residues in the process of humification (Piccolo, 2001). Chemical structures of HS are not clearly defined, but in general are composed of heterogenous, small organic molecules of lignin and cellulose residues, which are linked highly complex by hydrophobic interactions and hydrogen bonds (du Jardin, 2015; Halpern et al., 2015; Rose et al., 2014). Mostly, HS are extracted artificially from humified organic matter like peat, leonardite or from compost (Rose et al., 2014), but also “humin-like” substances can be produced synthetically in physico-chemical processes reported by Eyheraguibel et al. (2008).

HS applied to plants as a foliar spray or to soil as a drench solution were found to benefit plant growth mainly by improved access to soil nutrients, both by affecting soil properties and plant physiology (Berbara and García, 2014; Halpern et al., 2015; Rose et al., 2014). Interaction effects of foliar spraying with 40 mg/l humic acid and N fertilization of peanuts (*Arachis hypogaea* L.) were reported accordingly (Moraditochaee, 2012).

Firstly, HS can improve the soil structure by enhanced formation of clay-humus complexes, benefitting aggregate stability and lessening aggregate destruction from wetting-drying cycles (Piccolo et al., 1997). In turn, this may also improve aeration of soil, root penetration of plants, water infiltration and causal to that, induces prevention of soil erosion (Bot and Benites, 2005).

HS can increase availability of nutrients like P, Fe and Zn by complexing with metal ions in the soil (Chen et al., 2004; Esteves da Silva et al., 1998). Also, HS can fix and immobilise heavy metals like Pb, Al, Ni and Cd (Berbara and García, 2014; Gerke, 1994), which further improves the availability of P in acidic, P fixing soils, while possible toxicity of heavy metal contamination can be reduced.

Delgado et al. (2002) found enhanced P buffer capacity of alkaline soils after treatment with humic and fulvic acid, which displayed higher levels of plant available P in the soil solution upon the application of inorganic P fertilizer. They concluded a HS induced inhibition of rapid formation of insoluble, crystalline carbonate apatite by HS activity, as primarily the formation of better-soluble amorphous CaP was observed in HS amended soil, leading to improved nutrient availability for crop production by enhanced organic matter content.

While both soil structure and nutrient availability in soils can be increased by HS application (and may improve plant performance alone), the efficiency of nutrient recovery from those soils can also be improved by HS induced alteration of plant physiology, affecting mainly root development.

According to findings of Berbara and García (2014), supported by findings of Schopfer et al. (2002), after soil application, humic acids are rapidly assimilated in plant root cells, inducing ROS (reactive oxygen species) production, which can regulate root growth processes and therefore act as a growth promoter. Further, Berbara and García (2014) stated, that cell proliferation and elongation was enhanced by ROS activity, while root differentiation of treated plants was delayed. Accordingly, enzymatic plasma membrane H^+-ATPase in root cells was induced by humic acid activity, contributing to root elongation and rapid uptake of nutrients by enhanced proton exudation into the rhizosphere (Canellas et al., 2002; Jindo et al., 2012a). Furthermore, Šamaj et al. (2004) reported enhanced Ca^{2+} influx by ROS activity and linked this to induction of lateral root growth and the formation of root hair.

As ROS production can be induced by humic acid application, concentration may remain in beneficial levels as resulting lipid POD, causing damage to cell membranes, was not observed by García et al. (2012), when humic acid was applied to rice plants. Here, the activation of the antioxidant system was measured as activity of enzymatic POD (García et al., 2012b, 2012a) and CAT (Cordeiro et al., 2011), with a corresponding decrease of H_2O_2 in plant tissue and beneficial reduced cell membrane permeability (García et al., 2012a). Yet, with high rates of humic acid application, ROS related tissue damage and negative effects on plant and root growth was detected, indicating a concentration dependence of growth promotion or plant damage by humic acid application (Berbara and García, 2014).

These effects of enhanced root growth were observed for both rice (García et al., 2012a, 2012b) and maize plants (Canellas et al., 2002; Cordeiro et al., 2011) treated with moderate levels of humic acids.

Enhanced growth of treated plants may be facilitated by enhanced nutrient uptake and hormonal activity of ROS. Also, abiotic stress can be regulated by humic acid, as effects of salinity and drought on rice both were alleviated under humic acid treatment (Aydin et al., 2012; García et al., 2012a).

Fulvic acids may exhibit similar effects in soils and plants, when used as a biostimulatory plant additive. Fulvic acid was found to compete strongly with hydroxide for the complexation with Fe^{3+}, which enables the formation of soluble metal complexes, rendering a higher plant availability of micro nutrients in soils treated with fulvic acid (Esteves da Silva et al., 1998). Accordingly, improved Fe uptake in rice seedlings was observed (Pandeya et al., 1998), while in general, higher supply of nutrients including N, P, K, Ca, Mg, Cu, Fe and Zn was found to be one effect of fulvic acid application in growing media, which resulted in higher growth of cucumber (Rauthan and Schnitzer, 1981).

With improved nutrition, plants treated with fulvic acid showed improved performance in stressed and non-stressed conditions of water deficiency, displayed by a higher photosynthesis rate in general and a higher chlorophyll content and gas exchange in specific, while the antioxidant system measured by elevated levels of SOD, POD and CAT was upregulated. The beneficial effects due to fulvic acid application resulted in improved water use efficiency and ultimately plant growth and yields of maize (*Zea mays* L.), under water stress as well as in well-watered conditions (Anjum et al., 2011).

All things considered, application of humic substances including humic and fulvic acids has great potential to facilitate root development of plants and enhance nutrient uptake in various pathways. Plant growth promotion can be established, but may vary according to parent material and extraction method of HS, as well as soil and environmental conditions and applied HS concentration and rate (Rose et al., 2014). Interestingly, humic acids from composts were found superior in plant growth promotion rather than from peat or non-composted organic sources (Jindo et al., 2012a; Rose et al., 2014). Further, humin-like substances, produced artificially in a physico-chemical process from wood waste to shorten production time of HS, were reported to remarkably enhance plant growth (Eyheraguibel et al., 2008). As the process shortens the production time and limits the heterogeneity of HS, according to the authors, this may be a sufficient approach to produce reliable biostimulants based on HS for large-scale application in a broad range of agricultural practices.

Finally, AM fungi may also benefit from HS treatment, as enhanced levels of root colonization were found after host plants were treated with HS. Additionally, other fungi and bacteria were depressed by HS activity, indicating a certain level of antibiotic properties (Gryndler et al., 2005).

Hence, HS in general, and humic acids and fulvic acids in specific, can be seen as biostimulants for promotion of plant growth and resistance to abiotic stress, although future research is needed to clarify heterogenous results of HS usage and to evaluate optimal application rate, concentration, timing and responsive plants under different soil and environmental conditions.

3.5.2 Amino acids from protein hydrolysates

Several authors reported plant growth stimulation effects of protein hydrolysates (PH), when applied to plants as foliar spray, soil drench or seed treatment (Ertani et al., 2013; Parrado et al., 2008; Schiavon et al., 2008). PH were observed to affect plant physiology by stimulation of N and C metabolism, both which is considered to enhance N use efficiency and overall photosynthesis and plant growth (du Jardin, 2015; Schiavon et al., 2008). Moreover, PH can act as chelating and complexing agents and induce microbial activity in soils (du Jardin, 2015; García-Martínez et al., 2010; Sharma and Dietz, 2006), improving access to soil nutrients (Halpern et al., 2015).

PH are produced from agro-industrial by-products of plant or animal residues in the process of chemical and enzymatic protein hydrolysis. The substances are heterogenous mixtures of proteins, majorly peptides and free amino acids (Calvo et al., 2014; du Jardin, 2015; Halpern et al., 2015), but can also contain fats, carbohydrates and macro and micro nutrients, as well as phytohormones, as detected by Ertani et al. (2013) and Parrado et al. (2008).

Applied to plants either as foliar spray, soil drench or solution for seed pre-treatment, the biochemical compounds contained are absorbed into the plant and display a variety of activities, which may lead to overall stimulation of plant growth and stress resistance.

García-Martínez et al. (2010) observed the stimulation of soil microbial activity and concurrent enhancement of phosphatase activity in PH treated soils, possibly benefitting P solubility. Further, amino acids contained in PH may chelate and complex metal ions, including Zn, Ni, Cu and Cd, contributing to nutrient availability and plant growth (du Jardin, 2015; Ghasemi et al., 2012) and possibly also to resistance to heavy metal contamination (Sharma and Dietz, 2006).

Schiavon et al. (2008) found enhanced N assimilation in alfalfa-PH treated maize plants, as reduction of NO_3^- was accelerated by upregulation of NO_3^- assimilation enzymes in leaves. Coupled with observation of enhanced sucrose content of plants, stimulated by upregulation of the C metabolism, and a responding balanced C:N ratio, the authors suggested indication for improved N use efficacy by PH treatment of maize plants. Accordingly, PH treatment could reduce required amounts of N fertilizer needed for stable or even increased crop production.

Observed effects of PH application may be related to their biostimulant properties, displayed in auxin- and gibberellin-like activity (Schiavon et al., 2008). A carob-PH was found to contain significant amounts of phytohormones (among others, auxin and gibberellin) and displayed stimulation of growth parameter of tomato plants such as plant height and number of fruits and flowers per plant (Parrado et al., 2008).

Consistently, auxin and triacontanol related growth stimulation of maize (*Zea mays* L.) was reported by Ertani et al. (2013). When treated with an alfalfa-PH (addition of 1 mg*l^{-1} PH to the growing substrate, 12 days after transplantation), fresh weight of maize roots and shoots was significantly increased, as well as other growth parameter (chlorophyll content, protein content and N metabolism). Interestingly, PH treatment improved plant performance in both saline and non-saline conditions, indicating elevated effects of plant growth and resistance to abiotic stress.

Further in Ertani et al. (2013), the stress response of PH treated plants was indicated, firstly, by improved biomass production (fresh weight of roots and shoots) in saline conditions, when compared to the untreated control. Secondly, under saline conditions, uptake of K^+ was elevated, while Na^+ accumulation was downregulated, resulting in a lowered K^+/Na^+ ratio. This effect was especially significant for respective $K^+$and Na^+ content in leaves, which influenced stomatal system positively.

Finally, proline content in roots was also significantly enhanced, as well as activity of phenylalanine ammonia-lyase (PAL), indicating upregulation of the antioxidant system. All in all, performance of maize under salinity was remarkably improved by treatment with PH (Ertani et al., 2013), which in general may could serve as a biostimulant for promotion of plant growth and stress response, although results under field conditions are still scare and so far, inconsistent. Further research should determine suitable source materials and management of application (concentration, rate, modus, timing), for a broad range of plant species in varying soil and environmental conditions.

Remarkably, PH may synergistically interact with AM fungi and exhibit positive effects on growth rates of crops in saline or alkaline conditions, as reported recently by Rouphael et al. (2017). In planting trials, lettuce (*Lactuca sativa* L.) grown in saline or alkaline conditions, was treated with a combination of commercial PH derived from legumes seeds (applied 6 days after transplantation of seedlings, as a foliar spray at a rate of 2.5ml*l^{-1}, four times in total, in weekly intervals) and commercial microbial inoculant containing AM fungi spores of *Rhizophagus intraradices* and biocontrol fungus *Trichoderma atroviride* (applied before seedling transplanting). Most significant results in terms of total fresh weight, shoot and root dry weight, along with enhanced growth parameters, like chlorophyll content, activity of antioxidant system and higher nutrient content, were obtained from the combination of the biostimulant formulas of PH and fungal inoculant, while adverse effects of abiotic stress were ameliorated (Rouphael et al., 2017).

Concluding, PH with its biostimulant properties may possess great ability to re-utilize agro-industrial by- and waste-products to enhance plant growth and resistance to abiotic stress of various plant species, while actual symbionts of AM fungi and beneficial soil bacteria might be promoted.

3.5.3 Chitosan from shellfish waste

Chitosan is derived by deacetylation of chitin, a polysaccharide found in exoskeleton of shellfish and cell walls of fungi (Hidangmayum et al., 2019; Iriti et al., 2009; Iriti and Faoro, 2009).

In several studies it was revealed, that exogenously applied chitosan may act as a biostimulant for plant growth and disease and stress resistance in crop production. Serving as an activator of the plant defense system, as a mediator of water use efficiency, as well as a complexing agent for heavy metals in soils, chitosan was found to improve physiological properties and photosynthetic processes of plants, leading to enhanced plant growth and nutrient content, in both presence and absence of biotic or abiotic stress (Hidangmayum et al., 2019).

Several authors found chitosan to activate plant defense shortly after foliar application, despite absence of actual pathogenic infestation or abiotic stress, indicating the eliciting ability of chitosan to prime a systemic acquired immunity (Iriti and Faoro, 2009). Therefore, chitosan may be considered as an elicitor for activation of the plant immune system, triggering systemic immunity (Iriti and Faoro, 2008).

Applied to plants, chitosan molecules may induce plant defense related signalling for accumulation of jasmonic acid, ABA and ROS, which in turn trigger various stress responses in plants (Iriti et al., 2009; Kuchitsu et al., 1995; Nojiri et al., 1996).

Bistgani et al. (2017) found elevated resistance to drought stress of chitosan sprayed thyme plants (water stressed *Thymus daenensis* Celak was sprayed three times a year with either 200 or 400 µl*l^{-1} chitosan dissolved in acetic acid). Content of MDA in plant tissue was reduced, which resulted in lower levels of lipid POD and corresponding, in improved membrane permeability, higher dry mass and higher yield of essential oils per plant under chitosan treatment, when compared to the water stressed, untreated control. Similar, seedlings of maize (*Zea mays* L.), soaked as seeds in chitosan solution pre-sowing, exhibited a reduced concentration of MDA and reduced membrane permeability under low temperature stress, when compared to untreated plants (Guan et al., 2009). Further evidence of chitosan related reduction of cell damage by abiotic stress was reported by Zong et al. (2017), who found reduction of MDA content under Cd stress in edible rape (*Brassica rapa* L.), when treated with chitosan.

The same authors also found chitosan induced upregulation of the antioxidant system of plants under varying abiotic stress, represented by elevated activity of either POD, CAT, SOD and/or proline (Bistgani et al., 2017; Guan et al., 2009; Zong et al., 2017), of which the latter may be induced by chitosan related activation of ABA synthesis (Iriti et al., 2009; Iriti and Faoro, 2008). These results of MDA reduction and activation of antioxidant activity by chitosan treatment are in accordance with Yang et al. (2009), who reported similar results for treated apple seedlings under drought stress. Further, leakage of electrolytes from plant tissue was decreased, indicating improved resistance to oxidative damage caused by stress encounter (Yang et al., 2009).

Consistent with these results, enhanced lignification of wounded wheat leaves by application of chitosan derivates was reported by Barber et al. (1989). These findings may be supported by Iriti and Faoro (2008) and Köhle et al. (1985). Köhle et al. (1985) showed, that chitosan treatment of soybean (cell culture of *Glycine max* cv. Harosoy 63) strongly induced the production of callose, needed for closure of membrane wounds and prevention of electrolyte leakage. Similar, Iriti and Faoro (2008) found enhanced callose production and a significant elevation of ABA levels in infested *Phaseolus vulgaris* L. plants, when treated with chitosan. Following, lesion of leaf area by the pathogenic tobacco necrosis virus was reduced drastically.

The eliciting properties of chitosan applied as a foliar spray for plant protection further may affect the water status of treated plants, especially viable under abiotic stress of salinity or drought. As proline is an important compound for osmotic adjustment, Bistgani et al. (2017) suggested, that part of the improved resistance to drought stress exhibited by treated plants was related to enhanced proline accumulation, largest under severe stress and highest rates of chitosan.

Under saline or drought stress, the stomatal system often is misfunctioning due to excessive accumulation of Na^+ and/or corresponding lack of K^+, leading to uncontrolled transpiration and water losses (Slabu et al., 2009). Chitosan may affect the stomatal system in two major pathways. Firstly, under water stress, enhanced levels of macro nutrients including K^+were found in chitosan treated plants of beans (*Phaseolus vulgaris*) by Abu-Muriefah (2013) and of cowpea (*Vigna ungiculata* L.) by Farouk and Amany (2012). Secondly, Iriti et al. (2009) found chitosan to exhibit anti-transpirant effects on beans (*Phaseolus vulgaris* L.) by reduction of stomatal opening, induced by enhanced ABA accumulation after application. Following, decreased stomatal conductance and overall reduced transpiration rates were found. This improvement of water use efficiency and a corresponding higher biomass-water ratio was also observed by Bittelli et al. (2001) for pepper plants (*Capsicum* spp.), sprayed on a weekly basis with chitosan. Lower stomatal conductance and reduced rates of transpiration led to reduced water usage by 43 % over the full growing period, when compared to the untreated control. Interestingly, no adverse effects on biomass production were observed.

Moreover, chitosan may also alter root growth physiology. In a study of Górnik et al. (2008), grapevine (*Vitis vinifera* L.) cuttings were treated in a chitosan solution for 24 h before transplanting. Treated cuttings displayed improved root growth parameters and established plants also showed higher levels of chlorophyll content in leaves.

Improved rooting ability may also contribute to enhanced uptake of nutrients as observed by Abu-Muriefah (2013) and Farouk and Amany (2012), both in the presence and absence of drought stress. Further, enhanced levels of chlorophyll in cowpea (*Vigna ungiculata* L.) were found by Farouk and Amany (2012) after chitosan application (three times with 250 $mg*l^{-1}$ chitosan solution; 40, 50 and 60 days after seeding (DAS)), again for both unstressed and water stressed plants. This may be indication for higher rates of photosynthesis capacity by chitosan application for certain plants, resulting in enhanced plant growth. Also, increased biomass production by chitosan treatment of maize (*Zea mays* L.), rapeseed (*Brassica napus*), perennial rye grass (*Lolium perenne*) and edible rape seed (*Brassica rapa* L.) under various abiotic stress conditions were reported (Guan et al., 2009; Kamari et al., 2012; Zong et al., 2017).

Zong et al. (2017) also reported metal complexing abilities of chitosan. They found reduced concentration of Cd in shoots and roots of treated edible rape, grown in contaminated soils. Thus, the reduction in Cd was depending on the applied rate of the chitosan derivate, with concentration of 50 and 100 mg*l^{-1} chitooligosaccharide with the best results.

All things considered, chitosan as a biodegradable, non-toxic product from shellfish waste may elicit a multitude of plant defense effects, prior to possible encounter of abiotic or biotic stress. In stressed and unstressed conditions, several studies strongly indicate positive effects of chitosan applied to plants, which ultimately may lead to enhanced plant growth and resistance to abiotic and biotic stresses. Therefore, chitosan may be considered as a biostimulant.

However, large scale application may not be recommended, as chitosan shows a high variability of results due to its dependencies on various factors of method, rate, concentration and timing of application, heterogenous source material and its processing, resulting in specific chitosan properties with possibly varying effects on plants (Hidangmayum et al., 2019). Therefore, in future research, a schematic analysis of chitosan biostimulants on various plant species in different soil and environmental conditions is needed to evaluate practical recommendations for utilization in agricultural production.

3.5.4 Botanical extracts of Seaweed, *Azolla* and *Moringa* leaves

3.5.4.1 Seaweed extract

Seaweed extracts are increasingly used in agriculture to improve growth and performance of crops, although application patterns and functioning are still object of research (Calvo et al., 2014; Craigie, 2011; Khan et al., 2009). Seaweed extracts are produced mostly from brown seaweeds like *Ascophyllum nodosum* or *Ulva lactuca* (Craigie, 2011; Kavipriya et al., 2011; Wally et al., 2013) by boiling or soaking of dried biomass in distilled water, autoclaving or simple by blending fresh biomass with water, followed by centrifugation and/or filtration (Godlewska et al., 2016; Jupri et al., 2014; Kavipriya et al., 2011; Rathore et al., 2009; Shabazi et al., 2015). Moniem and Abd-Allah (2008) also produced an extract from washed and centrifuged slurry of microalgae *Chlorella vulgaris*.

Extracts are usually diluted with water (1:500 to 1:1000 (Calvo et al., 2014; Craigie, 2011)) and applied as foliar spray to crops or as soil solution to the growing substrate of crops. However, several authors reported a multitude of varying dilution rates and application rates and procedures for different crops: Singular application of seaweed extract on seedlings of alfalfa (*Medicago sativa*) (Khan et al., 2013) or *Arabidopsis thaliana* (Wally et al., 2013), initial application 30 days after germination followed by second application at day 60 after germination of soybean (*Glycine max*) (Rathore et al., 2009), 3-week-intervals of application until harvest of grapevines (Moniem and Abd-Allah, 2008), twice in the vegetative state followed by application at flowering and fruit filling phase of rice (Jupri et al., 2014) or as a solution for seed germination of garden cress *Lepidium sativum* (Godlewska et al., 2016) or *Vigna radiata* (Kavipriya et al., 2011). Growth response in general was reported to be positive, either in height of plants, root growth, shoot growth, fresh mass, dry mass, nutrient content, and/or seed germination (Alam et al., 2013; Godlewska et al., 2016; Kavipriya et al., 2011; Khan et al., 2013; Moniem and Abd-Allah, 2008; Rathore et al., 2009; Shabazi et al., 2015).

Also, microbial communities were enhanced in their numbers (Alam et al., 2013), while *Rhizobium* induced root nodulation of alfalfa improved (Khan et al., 2013) and AM fungi root colonisation was stimulated (Kuwada et al., 2006), when seaweed extract was applied.

Plant growth promotion of seaweed extract, especially by induced root development (Alam et al., 2013; Khan et al., 2013) and subsequently enhanced nutrient uptake (Rathore et al., 2009), is linked to specific content of trace elements, amino acids, vitamins and phytohormones (cytokinins, auxins, gibberellins, ABA and polyamines) (Crouch and van Staden, 1993; Khan et al., 2009; Wally et al., 2013).

As application rates are low in general, it was suggested, that activity of phytohormones, especially of cytokinins and auxins, may be mainly responsible for plant responses to application of seaweeds, rather than nutrient content (Crouch and van Staden, 1993; Khan et al., 2011).

Yet, according to findings of Wally et al. (2013), phytohormone levels in adequately applied seaweed extract itself are insufficient in quantities to cause observed growth promotion of plants. Nevertheless, exogenous application of phytohormones contained in seaweed extract may trigger the upregulation of endogenous production of cytokinins and ABA metabolites in treated plants, majorly responsible for plant growth promotion (cytokinin) as well as abiotic stress response (ABA), as was reported by the same authors. Hence, seaweed extract can be seen as a true biostimulant, favouring upregulation of certain plant processes, cascading ultimately into enhanced nutrient uptake and plant growth. Anyhow, utilization of fresh seaweed extracts for biostimulatory treatment of plants may be restricted to coastal areas.

3.5.4.2 *Azolla* extract

Extracts from the water fern *Azolla* spp. also serve as a potential alternative biostimulant for seaweed extract, as it is easily cultivatable in ponds (even BoneC or bone ash could be utilised as a P source). Extracts from *Azolla* may display similar properties and content of bioactive substances of phytohormones, amino acids and trace elements as seaweed extracts. Stirk and van Staden (2003) found high cytokinin-like activity in *Azolla* extracts. Further, cyanobacteria (*Anabaena azollae*) and *Arthrobacter* bacteria, commonly associated with *Azolla*, were found by the same authors to produce the auxin IAA, possibly contributing to plant growth promotion effects of extracts from *Azolla*.

Accordingly, recent studies indicate plant growth promotion of *Azolla* extracts, similar to that of seaweed extracts (Bindhu, 2013; Hanafy and El-Emary, 2018; Taha and El-Shahat, 2017). Again, as with seaweed extract, different handling of preparation and application of the extract are reported, indicating still needed research for optimal techniques and confirmation of first results.

Extract from *Azolla pinnata* boiled in distilled water and filtered was applied in various concentration to transplanted tomatoes seedlings as a foliar spray. Application was repeated three times every 15 days until harvest (Hanafy and El-Emary, 2018). Similar, Bindhu (2013) also boiled and filtered *Azolla*, but used the produced extract for improvement of seed germination of *Pisum sativum*. Taha and El-Shahat (2017) used filtered extract from crushed and blended *Azolla pinnata* for soil application of established apricot trees (*Prunus armeniaca* L. cv. Canino), four times within the seasons (dormant bud stage, full bloom stage, after fruit set and one month prior to harvest).

In each case, improvement of plant growth and biochemical parameters or respective seed germination by application of *Azolla* extract was observed, when compared to the control. These studies give good indication of *Azolla* extract possibly serving as a biostimulant, easily available also in rural areas of landlocked countries, producible with local resources only. Anyhow, further research is needed to determine optimal conditions of production and application of *Azolla* extracts for responding plant species for treatment and to confirm recent results.

3.5.4.3 *Moringa* Leaf extract

Moringa is a fast growing soft-wood tree of the family of *Moringaceae*, order *Brassicales*, native to India, but also cultivated globally in many regions in the tropics and subtropics of Asia, Africa and Central America (Anwar et al., 2007; Busani et al., 2016; Makkar and Becker, 1996). Especially known for its rapid growth and high nutrition and medicine value of leaves and pods, *Moringa oleifera* is increasingly cultivated and used as a food, fodder, oil or herbal crop with multiple purposes, also in otherwise challenging conditions of low soil fertility and water stress (Anwar et al., 2007; Makkar and Becker, 1996; Soliva et al., 2005).

Similar to seaweed or *Azolla*, extracts from leaves of *Moringa oleifera* were also found to possess high quantities of nutrients, vitamins, amino acids and phytohormones (Abdalla, 2013; Abusuwar and Abohassan, 2017; Busani et al., 2016). Accordingly, *Moringa* leaf extract (MLE) applied to plants as a foliar spray, was found to exhibit stimulatory plant growth effects on various crops (Abdalla, 2013; Abdallah et al., 2017; Abusuwar and Abohassan, 2017; Biswas et al., 2016).

Biswas et al. (2016) reported significant improvement of growth parameters of Titan hybrid maize after application of MLE (fresh leaves grounded with little water, pressed and filtered) in two weeks interval, starting 2 weeks after germination until flowering. Grain yield increased significantly by +46 % (9.2 $t*ha^{-1}$ by respective treatment with MLE, compared to 6.3 $t*ha^{-1}$ by control).

In another study, filtered MLE from blended, fresh leaves, applied in the same pattern as Biswas et al. (2016) but to different cereals (*Sorghum bicolor* L. Moench, *Penisetum typhoideum* Rich and *Sorghum Sudanese*), was found to enhance biomass production remarkably (Abusuwar and Abohassan, 2017).

Further, Abdalla (2013) tested a MLE, produced from fresh leaves and twigs and distilled water, on rocket plants (*Eruca vesicaria subsp. sativa* c.v. Balady). The extract was applied twice as a foliar spray, at day 7 and 14 after planting (harvest at day 50 after planting). Again, growth parameters were enhanced substantially, while also content of photosynthetic pigments, total sugar and proteins and nutrients were increased. Interestingly, while in treated rocket plants, content of growth promoting hormones (cytokinins, auxins, gibberellins) were enhanced, growth inhibitor ABA was decreased by MLE treatment. Finalizing, the authors recommend the usage of MLE as a cheap, easy-to-produce organic biostimulant for various crops.

Finally, findings of Abdallah et al. (2017) indicated possible cross-effects of AM fungi symbiosis and MLE treatment for host plants. MLE (oven dried leaves of *Moringa oleifera* were macerated in distilled water, shaken and filtered), was applied 4 and again 6 weeks after germination on foliage of mycorrhizal and non-mycorrhizal fennel (*Foeniculum vulgare*). While mycorrhizal colonisation and MLE treatment alone increased all growth and flowering parameters of fennel, when compared to the respective uncolonized or untreated control, the best results were obtained, when mycorrhizal plants were treated with MLE (application of 60 $g*l^{-1}$ MLE resulted in herb dry mass increase of +109.1 % in mycorrhizal plants and +82.3 % increase in MLE treated non-mycorrhizal plants when compared to the untreated control). These results indicate possible synergistic, reinforcing effects of different biostimulants when jointly combined, which may lead to remarkable enhancement of plant performance, when integrated in agricultural systems.

All things considered, there is strong indication, that the biostimulant MLE may foster plant growth and performance. *Moringa* is an easy obtainable resource in many parts of the tropics and sub-tropics, especially in rural areas with otherwise little access to common agricultural production goods. Unfortunately, *Moringa* so far is often not or underutilized. Further, MLE production is cheap and affords only basic skills and equipment, but could possess great potential for stimulation of plant growth and performance. Hence, MLE can be considered as a biostimulant for intensification of restoration agriculture and promotion of AM fungi in developing countries.

3.5.5 Individual organic substances of glycerol and salicylic acid

3.5.5.1 Glycerol

Systemic acquired resistance (SAR) is a plantal mechanism to prevent secondary infection of plant tissue after primary infection with pathogens. Upon infection, mobile warning signals are generated and transferred through the plant to distal, so far uninfected plant tissue to activate plant defense mechanisms. In this mechanism, glycerol may play a major role (Chanda et al., 2011, 2008; Yang et al., 2013).

Glycerol (Propan-1,2,3-triol; $C_3H_8O_3$) is a non-toxic, sugar alcohol, commonly produced as a by-product in biodiesel production (Yang et al., 2012). Current uses in cosmetics or food additives are limited, thus spiked production of glycerol due to increase in global biodiesel production bears potential for a cheap, underutilized waste resource in agriculture, as several effects of glycerol on plant growth were reported (Ali et al., 2008; Tisserat and Stuff, 2011).

In several studies, a glycerol related induction of the activation of basal resistance and SAR of pathogen infected plants was observed, resulting in enhanced protection against fungal spread and damage. Chanda et al. (2011) in accordance with findings of Hu et al. (2014) showed, that glycerole-3-phosphate (G3P) is produced by the enzyme glycerol kinase from glycerol, serving in collaboration with salicylic acid (SA) as a mediate for lowering oleic acid concentration and increasing synthesis of ROS in infected *Arabidopsis* plants inoculated with a virulent strain of *Pseudomonas syringae*. Both processes are commonly associated with increased defense resistance of plants (Y. Zhang et al., 2015). Also, with increased G3P levels in plant tissue itself, susceptibility for pathogen infection is significantly lowered (Chanda et al., 2011; Yang et al., 2013; Y. Zhang et al., 2015).

These mechanisms generally occur endogenously in many plants upon infection. Anyhow, in recent studies, induction and enhancement of disease resistance was also achieved by exogenous application of glycerol as a foliar spray, prior to infection with pathogens, which reduced plant damage and corresponding, increased crop yield in comparison to untreated, infected plants (Chanda et al., 2011; Hu et al., 2014; Li et al., 2016; Y. Zhang et al., 2015).

Plants of cacao (*Theobroma cacao* L.) can be infected by the pathogen *Phytophthora capsici*, among others, causing significant yield loss on a global scale (Y. Zhang et al., 2015). However, in an experiment by the same authors, exogenous applied glycerol displayed effects of enhanced plant resistance. When glycerol was applied in dosages of 100 mM (9.2 $ml*l^{-1}$) to infected leaves (once every 24 hours for 3 consecutive days), enhanced resistance towards the infection was observed, due to enhanced ROS production. Yet, the effects were constrained to the treated leaves, not to other, untreated leaves. Therefore, SAR throughout the whole plant was not induced by glycerol treatment. Also, by higher dosage of glycerol (300 mM (27.6 $ml*l^{-1}$)) cell death of the leaf tip from sprayed leaves occurred, which was also observed by Li et al. (2016) for wheat.

Li et al. (2016) examined the effects of glycerol spray as a chemical agent for protection of wheat (*Triticum aestivum* L.) against infection with the fungus *Blumeria graminis* f. sp. *(Bgt)*, causing powdery mildew. Application of 3 % glycerol 1 or 2 days prior to infection led to enhanced resistance of wheat to the pathogen. Anyhow, activation of SAR throughout all the infected wheat plant was not observed, as effects were displayed only locally, in treated plant tissue prior to infection, not in distal, untreated plant material, which is similar with findings of Y. Zhang et al. (2015) on cacao. Hence, as no negative vegetative effects were reported, the full application of glycerol spray to plants, prior to infection could be performed to enhance plant resistance to pathogens.

These results are in accordance with Chanda et al. (2011), who next to *Arabidopsis* also treated plants of soybean successfully with glycerol to increase plant defense. Thus, this may be evidence for a broad effect of exogenous glycerol application for diverse plants as a biodegradable, non-toxic, cheap biostimulant for plant health.

However, Hu et al. (2014) demonstrated the alteration of root development of *Arabidopsis thaliana* seedlings cultivated in glycerol containing medium. They found inhibition of primary root growth by reduction of P in root compartments and accompanied enhanced growth of lateral roots, as well as elevated levels of G3P and H_2O_2 in glycerol treated seedlings. If this alteration of root development would impair plant performance or resistance to abiotic stress, was not reported by the authors.

Anyhow, there is indication, that glycerol applied as foliar spray in low dosages can also serve as a biostimulant not only for promotion of plant health, but also for promotion of plant growth and resistance to abiotic stress. Firstly, Eastmond (2004) showed, that seed germination in glycerol containing medium enhanced levels of glycerol in plant tissue of *Arabidopsis* mutants seedlings. These *Arabidopsis* mutants were unable to process glycerol into G3P. However, accumulation of high levels of glycerol in plant tissue decreased the susceptibility towards various abiotic stress, among others, salinity, drought and freezing.

Furthermore, Ali et al. (2008) reported enhanced fresh and dry mass of salt stressed *Ricinus communis* L. plants, grown from seeds soaked for 48h in glycerol solution (concentration of 10, 25 and 50 mM (0.92, 2.3 and 4.6 m*l^{-1})) for germination, in comparison to the control (seeds soaked in distilled water). In saline soil conditions, glycerol levels in shoots and roots increased significantly with and without exogenous application. Further, glycerol treated plants not stressed by salinity also grew better, with a substantial increase in growth parameters in fresh and dry weight and oil content (up to +64.4 % increase with 50 mM glycerol treatment). Therefore, both plant growth and resistance to abiotic stress was enhanced by glycerol application.

Finally, applied as a foliar spray, glycerol remarkably exhibited plant growth promoting effects on carrot (*Daucus carota* L.), maize (*Zea mays* L.) and spearmint (*Mentha spicata* L.) in a study of Tisserat and Stuff (2011). Application pattern of glycerol solution differed among plant species. Carrots were sprayed twice in the growing period (2.5 ml per plant and application), 2 weeks after plant establishment and again 4 weeks after the first treatment (2 weeks before harvest). Maize was treated 2 weeks after establishment, and again 2 weeks after first treatment (25 ml/plant and application). Harvest was performed 2 weeks after second treatment. 8-week-old spearmint plants were transplanted and were sprayed once with 25 ml glycerol solution per plant, 2 weeks after transplantation.

Concentration of 5 ml*l^{-1} (54 mM) glycerol showed best results for sprayed carrots and spearmint, as among other growth parameters, dry weight increased substantially (+158.4 % for carrots and +68.7 % for spearmint). Maize dry weight was maximal by foliage application of 0.5 ml*l^{-1} (5.4 mM) glycerol solution (increase of +154.6 % compared to untreated control). Although the mechanisms for plant growth promotion were not reported by the authors, glycerol was suggested as a plant growth promoting biostimulant for agricultural uses.

Concluding, currently considered as a cheap, disposable resource, great potential may lay in the utilisation of glycerol in agricultural practice, as low dosages already may trigger reported effects of plant growth and health promotion. Hence, also resource-poor farmers in remote areas could become users and improve crop yields with only very low investment costs. Still, there is much unclarity of glycerol application serving as a biostimulant, as results vary. Concentration, timing, application rates, responding plant species, convenient soil and environment conditions, all need to be further examined for optimal usage of glycerol in agriculture, so it can be utilised as a potential biostimulant to boost yield, productivity and profitability as well as resistance to pathogen attack and abiotic stress.

3.5.5.2 Salicylic acid

As discussed before, next to G3P synthesised from glycerol, SA is also an important compound, needed to induce plant defense mechanisms upon biotic stress (Chanda et al., 2011; Shakirova et al., 2010).

SA (ortho-hydroxy benzoic acid; $C_7H_6O_3$) is a naturally occurring phenolic compound, present in different basal levels in various plants (Sharma, 2014). Synthesis of SA within plants can be induced by pathogenic attack or influence of abiotic stress, like salinity or water deficit (Bandurska, 2013). Rapid movement of the mobile molecule from infected plant tissue to non-affected, distal tissue occurs, activating responsive defence mechanisms and stress countermeasures in collaboration with G3P (Ohashi et al., 2004).

The endogenous process of SA activity though is activated upon primal infection or presence of stress. To allow plants to pre-adapt to a forthcoming stress situation, exogenous SA treatment of plants prior to encountering stress may have significant, positive impact on plant response and defence capability against biotic and abiotic stresses, as was shown in recent studies (Gunes et al., 2007; Hayat et al., 2008; Shakirova et al., 2003). Further, in coherence with those findings, SA induced plant growth promotion was also found (Gunes et al., 2007; Shakirova et al., 2003; Singh and Usha, 2003), indicating possible utilization of SA as a biostimulant for plant health and growth in agricultural practice.

Among other compounds, SA was found co-responsible to induce the activation of SAR, which again can be seen as a cascade of defense related processes and mechanisms initiated in plants.

One of the most important compounds related to plant health is the SA intermediate ABA. In several studies, increase of ABA content in plant tissue due to SA accumulation was reported (endogenously by abiotic stress or exogenously by application), causing the activation of different anti-stress effects (Shakirova et al., 2013, 2010).

The authors of Shakirova et al. (2003) observed a rapid, SA induced increase in ABA and IAA content of wheat seedlings, which preceded corresponding upregulation of proline production, both in saline and non-saline conditions. Proline serves as an osmolyte, associated with improve water and defense status of plants (Hayat et al., 2008). Anyhow, subjected to salinity, wheat seedlings (*Triticum aestivum* L. cv. Saratovskaya 29) with SA treatment (3 hours soaking in 0.05 mM (6.9 mg*l^{-1}) SA solution) showed superior root growth. Here, ABA content was maintained at elevated levels, as well as IAA and cytokinins, while untreated controls displayed a sharp, transient rise of ABA and a stable and progressive decline for IAA and cytokinins respectively, resulting in growth inhibition in saline conditions. Furthermore, the authors found, that SA treatment not only improved the plant performance under salinity, but also the recovery rate after withdrawal of the abiotic stress factor. SA treated seedlings rapidly overcompensated salinity induced growth decline, while untreated plants struggled to enhance growth rates to former levels in unstressed conditions. Hence, it was concluded, that SA activated ABA mediated improvement of hormone and water status, which contributed remarkably to enhanced growth of SA treated plants, in both saline and non-saline conditions. This may be a fundamental function of protection and preadaptation against forthcoming stress by SA application (Shakirova et al., 2013, 2003).

Additionally, SA can enhance activity of the antioxidant system, preventing damage caused by stress induced generation of ROS, as increased production of enzymes POD and PAL, as well as SOD and proline, were observed in SA treated plants, both prior and upon stress (Hayat et al., 2008; Shakirova et al., 2013), similar as in chitosan treated plants (Bistgani et al., 2017; Guan et al., 2009; Zong et al., 2017).

Further, Shakirova et al. (2013) found increased levels of root lignification in wheat seedlings following treatment of seeds by soaking for 24 h prior sowing in SA solution. The acceleration of lignin deposition in cell walls and a respective improvement of barrier function of affected roots was associated to ABA induced enhanced production of H_2O_2, POD and PAL, which are considered key factors in lignin biosynthesis. Accordingly, examined uptake and translocation of toxic soil constituents like Cd was lowered in SA treated plants, when compared to untreated controls. Thereby, Cd related hormonal imbalance of cytokinins, auxins and ABA resulting in inhibition of root growth and overproduction of ROS was prevented in SA treated plants, which grew significantly better, comparable to untreated controls without Cd pollution. Hence, these results may indicate, that by SA treatment, effects of Cd toxicity in contaminated soils could be fully equalized for wheat plants (Shakirova et al., 2013).

Moreover, under conditions of abiotic stress, upregulated production of ROS can deteriorate membrane lipids of root cell walls by lipid POD. This enhancement of membrane permeability can lead to leakage of water and exudates from affected root compartments (Hayat et al., 2008), contributing to dehydration and inhibition of growth of plants. However, results of Gunes et al. (2007) indicate, that exogenous SA treatment of salt stressed maize can alleviate salinity induced damage of membranes, which is in accordance with findings of Mishra and Choudhuri (1999) on rice cultivars.

These findings are also in accordance with reports of Hayat et al. (2008), who treated tomato seedlings (*Lycopersicon esculentum* L. cv. K-25) with SA foliage application, after the plants experience water stress for 10 days. Analysis of data showed positive effects of SA on membrane stability and hence, reduced leakage of electrolytes from root tissue. In coherence with increase of proline and higher stomatal conductance of leaves, an increased transpiration rate and overall relative water content of SA treated plants was found, as well as higher water use efficiency. This was true for plants with and without water stress prior to SA treatment, indicating, that SA in general can remarkably improve the water status of plants, regardless of presence of stress. Reduced uptake of Na^+ and Cl^- from saline soils found by Gunes et al. (2007) may contributes to improved water status of plants, as Na^+ in high concentration otherwise can exhibit detrimental effects on the stomatal system of plants.

Along with observed water status of plants, exogenous SA application may also promote uptake of nutrients according to findings of Gunes et al. (2007). Also, increased values of chlorophyll were detected, when SA was applied to plants (Fariduddin et al., 2003; Hayat et al., 2008; Singh and Usha, 2003), as well as other photosynthetic parameters (Ribulose-1,5-bisphosphat-carboxylase/-oxygenase (Rubisco) activity, stomatal conductance, photosynthetic rate; Singh and Usha, (2003)). At the same time, due to SA induced activation of antioxidant system, senescence of plant leaves was delayed in Huang Kum pears (*Pyrus pyrifolia* Nakai), which may extend the total duration of photosynthesis of treated plants (Imran, 2007). This all may be indication of elevated photosynthetic capacity of a broad range of plants induced by exogenous SA treatment (Fariduddin et al., 2003; Khan et al., 2003; Singh and Usha, 2003). Ultimately, this may in turn could improve yields of various plants, as was observed for maize (Khan et al., 2003), soybean (Gutiérrez-Coronado et al., 1998), onion (Dixit et al., 2018), wheat (Shakirova et al., 2003; Singh and Usha, 2003) or tomato (Javanmardi and Akbari, 2016), among others.

All things taken into account, SA treatment can adverse the negative effects of biotic and abiotic stress towards affected plants. Increased growth rates with higher dry yield and other growth parameters were found in several studies, proving the potential of SA to serve as a biostimulant, both in stressed and unstressed conditions. SA treatment prior to actual occurrence of stress (salinization, drought, toxification or pathogenic attack) can activate several mechanisms as pre-adaptation of stress response. Hence, vegetal stress symptoms upon primal stress encountering can be mediated and stress induced damage considerably lowered. Further, growth parameters can be increased, possibly resulting in elevated crop production. To exhibit beneficial effects, many studies showed, that already applications as solutions for seed treatment, soil drench or foliage spray in nano or micro quantities are sufficient. Therefore, as costs for SA are low, the utilization of SA as a general biostimulant may be feasible, also in extensive, low-resource agricultural operation focused in this study.

Anyhow, studies on interaction of SA treatment of mycorrhizal plants reported elevated levels of mycorrhiza activity, when host plants were treated with SA pre-sowing. SA priming was found to enhance the levels of sugar transported to roots of mycorrhizal plants (Garg and Bharti, 2018).

A study of Garg and Bharti (2018) investigated the interactions of SA-priming and AM fungi symbiosis. Interestingly, the authors found enhanced levels of AM fungi activity, when seeds of the later host plant were treated with SA solution pre-sowing. SA treated plants transported larger amounts of photosynthates to their rhizosphere than untreated plants, both in saline and non-saline conditions. Hence, AM symbiosis with SA treated host plants was enhanced, leading to increased growth parameters (increased seed weight, root and shoot dry weight, higher chlorophyll and P content, higher sugar content in leaves) and defence parameters (enhanced antioxidant system, lower membrane permeability, lower NaCl content in leaves, higher $K^+/Na^+$and Ca^{2+}/Na^+ ratios), when compared to untreated plants, or single treated plants with AM fungi or SA alone. Ultimately, equalization of stress conditions from salinity was accomplished most successfully by co-operation of SA and AM fungi effects. As these effects were displayed in saline and non-saline conditions, evidence may be found, that SA in cooperation with AM fungi promote plant growth, but also contributes to plant defence against abiotic stress.

These findings are in accordance with findings of Zhang et al. (2018), who found established AM fungi networks to transport SA as a defence related signal molecule between infected and uninfected host plants of orange trees, where defence mechanisms (e.g. antioxidant system measured as PAL activity and lignin concentrations) increased accordingly, prior to pathogenic infestation.

Effects of SA on AM fungi are highly desired to rapidly establish full colonisation with AM fungi, and in turn, enhance plant growth, health and resistance to biotic and abiotic stress, pre-adaptive, prior to occurrence to minimize plant damage. All things considered, SA could be considered as a beneficial biostimulant for promotion of plant growth and stress resistance and promotor of AM fungi establishment.

SA utilization could boost intensification of crop production of a broad range of agricultural systems. Yet, SA application is still underexamined in terms of mode, rates and timing of application, suiting plant species and interaction with other possible biostimulants, which all should be determined for optimal growing conditions in different ecosystems with varying soil and environmental conditions in future work.

3.5.6 Individual inorganic substances of hydrogen sulfide and titanium dioxide NP

3.5.6.1 Hydrogen sulfide

Hydrogen sulfide (H_2S) is a toxic gas, famous for its foul odour. However, in recent studies, evidence for a potential role in mediation of plant growth and defense response surfaced. Depending on concentration, exogenous application of a H_2S donor could in fact benefit plant processes of abiotic stress resistance and growth promotion (Chen et al., 2011; Dooley et al., 2013; Fotopoulos et al., 2014; Jin et al., 2013; Li et al., 2012; Thompson and Kats, 1978; Zhang et al., 2009a, 2009b).

In contaminated soils, uptake and accumulation of non-essential heavy metal Cd can have detrimental effects on plant growth by excessive production ROS, causing oxidative stress and consequently, inducing programmed cell death by lipid peroxides of cell membranes (Gallego et al., 2012).

Anyhow, H_2S application (as $NaHS$) was found to alleviate Cd toxicity of stressed alfalfa plants (*Medicago sativa* L. cv. Victoria). This was regarded, firstly, to decreased Cd uptake by roots of H_2S treated seedlings, and, secondly, by activation of the defense mediating antioxidant system by higher activity of antioxidative enzymes (SOD, POD, ascorbate peroxidase (APX)), which all counteract lipid peroxidation (Li et al., 2012). Further, in a subsequent experiment of Zhang et al. (2009a), it was shown, that plant responses of defense activation after H_2S treatment are related to H_2S or HS^- derived from NaHS, and not to other S or Na containing compounds. This results were later confirmed by Chen et al. (2011) and Li et al. (2012).

Similar, in experiments with poplar trees (*Populus euphratica* Oliver), Sun et al. (2013) found ameliorating effects of H_2S on Cd toxicity as well, as the NaHS pre-treatment induced upregulation of the antioxidant system (enhanced activity of antioxidant enzymes glutathione reductase (GR), APX and CAT), which decreased levels of lipid POD measured as MDA content. By corresponding lowered levels of ROS, in particular H_2O_2, also Cd accumulation in plant cells was reduced, as H_2O_2 can activate certain pathways responsible for Cd flux. Further, the same authors also reported enhanced Cd compartmentation in cells of H_2S treated plants. They observed enhanced flux and sequestration of Cd from cytoplasm into vacuoles within plant cells, hence compartmentalized, which further is considered as an effective strategy to cope with toxic Cd accumulation in plant cells (Sun et al., 2013).

Other factors of abiotic stress also can be counteracted by H_2S application. The authors of Jin et al. (2011) and Jin et al. (2013) reported improved drought resistance of H_2S fumigated *Arabidopsis thaliana*, when experiencing 14 days of severe drought. The rate of survival was significantly higher in H_2S fumigated plants, when compared to the control. Also, stomatal closure was induced by H_2S, as the stomatal aperture size was reduced remarkably. Hence, it was speculated, that H_2S may act as a signalling molecule for the guard cells of stomata. In extension of the first study, Jin et al. (2013) further investigated the interlinkage of H_2S and ABA. According to their findings, stomatal closure by ABA is dependent on sufficient concentration of H_2S. These findings are in accordance with Garcia-Mata and Lamattina (2010), who found that exogenously applied H_2S as NaHS fosters ABA induced stomatal closure in various plants, while in plants with no or little endogenous H_2S synthesis, ABA induced stomatal closure was partly blocked. Concluding, H_2S treated plants showed higher relative water content, indicating enhanced water status and improved resistance to drought.

Under osmotic stress, also the decline of chlorophyll content in leaves of sweet potato (*Ipomoea batatas* L. cv. Xushu 18) was observed, which in turn was recovered by NaHS treatment. Here, also concentration of H_2O_2 and MDA was reduced, indicating elevated activity of the antioxidant system (Zhang et al., 2009b). However, also under non-stressed conditions, exogenous application of H_2S may affect plant growth. Chen et al. (2011) observed increasing chlorophyll content in treated *Spinacia oleracea*, with 100 µM NaHS displaying the strongest effect. Following, also photosynthetic parameters improved (photosynthetic net rate, stomatal conductance, light saturation point, carboxylation efficiency, activity of Rubisco), indicating elevated photosynthesis capacity. Finally, remarkable promotion of plant growth was observed. Hence, it was speculated by the authors, that H_2S can play a significant role in promotion of photosynthesis and therefore, ultimately also in plant growth (Chen et al., 2011).

It was further demonstrated by Zhang et al. (2009a), that H_2S treatment can alter plant physiology in terms of root morphology. Culturing of seedlings of sweetpotatoe (*Ipomoea batatas*), willow (*Salix matsudana* var. tortuosa Vilm.) and soybean (*Glycine max* L.) in 0.2 mmol*l^{-1} NaHS solution increased formation and growth of adventitious roots, which was related to elevated levels of IAA and nitric oxide (NO).

The observed effects of H_2S in stressed or non-stressed conditions may all promote plant growth and performance in their entirety. Corresponding, Dooley et al. (2013) found substantial enhancement of growth (roots, stems, leaves and fruits) of wheat (*Triticum aestivum* L. cv. USU-apogee), maize (*Zea mays* L.), pea plants (*Pisum sativum*) and bean plants (*Phaseolus vulgaris*), when treated with H_2S (seed soaking in H_2S solution of various concentration for growth period of 7 days with daily exchange of solution). The time and rate of germination also improved with H_2S treatment of seeds, with 100 µM H_2S determined as the optimal concentration.

Conclusive, H_2S applied as a solution from $NaHS$ may serve as an eliciting defense signal for priming against abiotic stress conditions. The activation of the antioxidant system was observed for H_2S treated plants of several species by enhanced production of antioxidant enzymes and corresponding, lowered content of MDA and POD. Oxidative bursts by rapid production of ROS can be prevented, which also could support plants to inhibit uptake of toxic heavy metals like Cd, among others. Further, prevention of translocation by enhanced compartmentalization in plant vacuoles was observed for Cd, displaying diverse mechanisms of coping with heavy metal contamination. Also, improved water status of stressed plants by regulation of the stomatal system through H_2S supported ABA activity became evident. All these mechanisms of improved resistance to abiotic stress may further translate into enhanced plant growth. Consistently, higher recovery of stress induced reduction of chlorophyll content was measured in H_2S treated plants, which, together with enhanced photosynthetic capacity and improved root growth, can significantly promote biomass production of various plant species, under different soil and environmental conditions.

Although most mechanisms underlaying the actions of H_2S in plant tissue are not fully understood yet, there is clear indication of beneficial exogenous application of H_2S to foster promotion of plant growth and performance. Therefore, H_2S may be considered as a biostimulant.

Anyhow, as experience of H_2S treatment under field conditions are still lacking, further research should examine suiting handling of the biostimulant in regards of mode of application, rate, concentration, duration and timing, as well as possible synergistic co-operation or interference with other promising biostimulants.

3.5.6.2 Titanium dioxide nanoparticles

NP were found able to elicit a series of effects beneficial for plant performance, mainly based on their particle size and surface charge (Juárez-Maldonado et al., 2019; Khodakovskaya et al., 2009).

Among others, metallic oxide NP can have profound effects when exposed to plants, especially on germination rate, seedling growth, disease suppression and photosynthesis ability (Elmer and White, 2016; Raliya et al., 2015; Raliya and Tarafdar, 2013). Yet again, especially titanium dioxide (TiO_2) NP became focus of interest for utilization in plant production as a biostimulant, as a diverse set of interesting effects may be elicited upon application as foliar spray on growing plants or solution for seed germination pre-treatment. Plant performance may be profoundly supported by different mechanisms, all leading to improved levels of plant production (Abdel Latef et al., 2017; Frutos et al., 1996; Ghooshchi, 2017; Owolade and Ogunleti, 2008; Yang et al., 2007, 2006).

Titanium (Ti) is a non-essential, but potentially beneficial element for plant growth (Lyu et al., 2017). It is contained in soil minerals of anatase and rutile as TiO_2, in many soils globally, leading to natural levels of up to 30 $µg*l^{-1}$ in soil solution, yet in plant unavailable form (Kabata-Pendias and Mukherjee, 2007).

However, when exposed to seeds in nanometer size of its particles, TiO_2 was found to impose positive impacts on overall seed germination and seedling growth. In trials by Zheng et al. (2005), the authors found improved germination parameters of aged spinach (*Spinacia oleracea*) seeds, when soaked in solution of both nano-TiO_2 or ordinary, non-nano TiO_2 for 48 hours as pre-treatment prior to planting. While germination rate, dry weight of seedlings and vigor index were poor for the control treatment, and only slightly enhanced by the non-nano TiO_2 treatment, best results with significant improvement for all parameters were obtained by the nano-TiO_2 treatment at a concentration of 0.25 %. Similar to other NP of C nanotubes (Khodakovskaya et al., 2009) and graphene (M. Zhang et al., 2015) affecting seed germination positively, the underlaying mechanisms was ascribed by the authors to the capability of nano TiO_2 to penetrate the seed coating due to its small particle size and facilitate increased water and oxygen uptake, improving seed germination (Zheng et al., 2005).

This fundamental work of Zheng et al. (2005) was part of a series of studies by a group of authors investigating the effects of TiO_2 NP on *Spinacia oleracea.* In the course of the studies, the authors of the group reported elevated photosynthesis rates as one of the main mechanisms for observed enhancement of plant productivity by TiO_2 NP application and concluded several underlaying mechanisms of TiO_2 NP beneficial to the photosynthesis system (Gao et al., 2006; Hong et al., 2005; Yang et al., 2007, 2006; Ze et al., 2011; Zheng et al., 2007, 2005).

Zheng et al. (2005) found enhanced photosynthetic rate of treated spinach seedling, in coherence with significantly increased chlorophyll content and Rubisco activity, which all lead to enhanced fresh and dry weight (+63 % and +76 % in comparison to untreated control) with optimal TiO_2 NP concentration of 0.25 %.

Other authors confirmed, that TiO_2 NP application can have a tremendous, beneficial impact on chlorophyll synthesis and following, photosynthesis and plant growth, in treated plants of spinach (Gao et al., 2006; Yang et al., 2007, 2006; Zheng et al., 2007), broad beans (Abdel Latef et al., 2017), cucumber (Cui et al., 2013), triticale (Gooshchi2017), mung bean (Raliya et al., 2015) and vetiver (Shabbir et al., 2019).

Further, Rubisco, as a key enzyme in photosynthesis, could be stimulated by seed pre-treatment and later foliar application of TiO_2 NP (at two-leaves-stage of seedlings, once, with solution of 0.25 % TiO_2 NP with 5 nm particle size) in its activity due to enhanced content of Rubisco activase and Rubisco carboxylase activity (Gao et al., 2006; Zheng et al., 2005). Furthermore, TiO_2 NP may improve the photosynthesis apparatus of spinach plants by enhancing content of light harvesting complex II in thylakoid membranes of spinach, allowing higher levels of light absorption and more rapid transfer and conversion of light energy into chemical energy (Zheng et al., 2007). Similar results were obtained by Ze et al. (2011) for TiO_2 NP treated *Arabidopsis thaliana*.

Another mechanism for enhanced photosynthesis by TiO_2 NP is protection from oxidative stress. By excessive light and non-optimal environmental conditions, photosynthetic capacity of plants can be decreased by chloroplast aging induced by enhanced levels of ROS. Yet, TiO_2 NP application on spinach showed protective mechanism by activation of antioxidant system (increased activity of antioxidative enzymes SOD, CAT and POD) in the chloroplast, inhibiting elevated levels of ROS and in the following, cell membrane damaging lipid POD measured as MDA. Hence, cell membrane permeability of chloroplast thus was lessened in spinach plants subjected to TiO_2 NP treatment in comparison to control (Hong et al., 2005).

However, increased content of photosynthetic compounds, enhanced photosynthetic rate and thereby improved plant growth by photocatalytic TiO_2 NP was closely related to alteration of N metabolism in treated plants, as Frutos et al. (1996) and Yang et al. (2006, 2007) suggested.

In the study of Yang et al. (2006), fresh and dry weight of spinach was considerably increased, when treated with nano anatase TiO_2 solution (TiO_2 NP size around 5 nm) of 0.25 % as seed pre-treatment and later foliar spray. In TiO_2 NP treated plants, significant higher amounts of NO_3^- were accumulated, as well as higher amounts of enzymatic nitrate reductase. Nevertheless, NH_4^+ content was slightly reduced. However, the trend of NH_4^+ correlated with enhanced activity of enzymes for organic N synthesis (glutamate dehydrogenase, glutamate synthase and glutamic-pyruvic transaminase) and following, enhanced content of protein and chlorophyll in treated spinach plants, both which are organic N compounds synthesized from NH_4^+. These results may indicate enhanced utilization of absorbed, inorganic N for synthesis of organic N compounds in plant cells due to TiO_2 NP activity, benefitting overall plant growth.

Similar results of enhanced nitrate absorption and nitrate reductase activity were indicated by findings of Frutos et al. (1996). They showed, that TiO_2 ascorbate foliar spray (concentration of 0.042 µM TiO_2 at application rate of 1 ml per plant, 40 and 70 DAS) could improve the growth of red pepper plants (*Capsicum annuum* L., cv. Bunejo) – for both deficient and non-deficient N nutrition conditions. Remarkably, although supplied with nutrient solutions containing only 50 % of the N rate supplied in the control (100 % N), TiO_2 treated plants showed similar biomass production and total-N and nitrate concentration like control plants with 100 % N nutrition and no TiO_2 treatment – in distinct contrast to untreated 50 % N plants with little biomass and N content. Accordingly, TiO_2 was able to compensate the N deficiency by higher N efficiency of absorption and utilization. When N was not deficient (100 % N), TiO_2 application improved biomass production of plants by +56 %. The authors concluded, that TiO_2 can significantly enhance plant yield or may reduce N fertilizer amounts needed by roughly 50 % without yield depression. Hence, a more profitable production of crops seems possible with TiO_2 application, while N contamination of soils and water bodies may be reduced.

Further investigation of Yang et al. (2007) about the N metabolism changes in plants induced by TiO_2 NP application revealed, that TiO_2 NP may also enable photocatalytic fixation of N_2 in treated plants. In their experiments, content of total-N, chlorophyll and protein of spinach plants were all drastically reduced, when grown in nutrient solution without N nutrition. Oxygen evolution as an indicator for photosynthesis was reduced as well, all leading to diminished plant growth with low fresh and dry weight of spinach plants. However, in absence of exogenous applied N nutrition, TiO_2 NP (5 nm particle size) applied as a foliar spray to spinach plants was able to partly alleviate effects of severe N deficiency. Total-N content, and following, content of chlorophyll and protein, oxygen evolving rate and finally, fresh and dry weight was significantly improved (+91 % and +99 % when compared to untreated control). This growth response was possible due to increased N status of treated plants, potentially followed from the ability of TiO_2 NP to reduce N_2 from the atmosphere to NH_3 by its photocatalytic activity in spinach leaves, when exposed to sunlight as the authors suggested. In general, this process of physiochemical N_2 fixation on TiO_2 containing surfaces is also indicated by work of Schrauzer et al. (1983), Schrauzer and Guth (1977) and Yuan et al. (2013). Anyhow, in general, more research is needed to elaborate proposed effects of photocatalysis of N_2 fixation by TiO_2 NP for a broad variety of crops and plants, application mode of TiO_2 NP with optimal concentration, rate, timing and management of treated plants as well as compatibility with other biostimulants, especially AM fungi and PGPB.

Next to alleviating effects on plant nutrition, TiO_2 may also support treated plants in their response to abiotic and biotic stress by a set of mechanisms, including the possible elicitation of a priming effect regarding the antioxidant system of treated plants, as findings of Abdel Latef et al. (2017) suggest.

The authors conducted experiments with salinity stressed broad beans (*Vicia faba* cv. Misr-1) and TiO_2 NP (foliar spray of 5 ml solution with 0.01, 0.02 and 0.03 % per plant, 3 and 10 days after exposing plants to salt stress by $NaCl$ solution added as irrigation water on day 7 after germination, harvest at day 28 after treatment). Next to improved plant growth and biomass production in unstressed soil conditions (+44 % root dry weight with 0.01 % TiO_2 NP), their results also indicated a priming effect for the regulation of the antioxidation system and osmoprotection in broad bean plants, all induced by TiO_2 NP treatment (Abdel Latef et al., 2017).

In conditions without exposure to salts stress, in TiO_2 NP treated plants, considerably higher content of antioxidative enzymes SOD, CAT, APX and POD was observed, with corresponding lower generation of H_2O_2 and, coherent to that, slightly lower levels of lipid POD measured as MDA. As a second measure of abiotic stress response, the content of osmolytes like soluble sugars, amino acids and proline was increased in treated plants, which, in case of stress exposure, would regulate the osmotic potential in plants, improving water uptake. Concluding, as activity of abiotic stress related enzymes is stimulated and osmolytes are accumulated without actual abiotic stress imposed to the plants, TiO_2 NP may serve as a priming agent in plants (Abdel Latef et al., 2017).

Further, when actually exposed to salinity, TiO_2 treated plants showed a strong antioxidative response by increased content of SOD, CAT, APX and POD. Levels of ROS were above unstressed, sprayed plants, but well below that of stressed controls. MDA was significantly reduced in comparison to untreated plants. Osmolytes concentration was also increased significantly for 0.01 % TiO_2 NP application in comparison to the untreated control, indicating osmoprotection of plants induced by the biostimulant. Taken together, as plant growth in regards of shoot and root dry weight was increased (+77 % and +75 % in comparison to stressed control), TiO_2 NP could serve as an ameliorative agent against oxidative stress (Abdel Latef et al., 2017).

Also, TiO_2 NP were found able to alleviate stress of Cd soil contamination for triticale plants, when applied twice as a foliar spray at 0.02 % concentration during the growing season. Grain yield was increased, as well as chlorophyll content and activity of SOD and CAT with responding lowered content of MDA. All despite higher accumulation of Cd in leaves and seeds of triticale, indicating ameliorating effects on Cd toxicity for plants by TiO_2 NP treatment and possible utilization of TiO_2 NP as an agent to support Cd phytoremediation (Ghooshchi, 2017)

In regards of biotic stress response, Cui et al. (2013) and Owolade and Ogunleti (2008) found protective effects of TiO_2 NP against plant pathogens. Cui et al. (2013) reported photocatalytic disinfection by TiO_2 NP through ROS production, when treated leaves of cucumber (*Cucumis sativus* L.) were illuminated. Accordingly, penetration of leaves by bacterial pathogens was significantly reduced, leading to less lesions and overall enhancement of biomass production of cucumber. Similar to that, Owolade and Ogunleti (2008) found less pathogenic incidence and severity of Cercospora leaf spot and Brown blotch with corresponding higher plant biomass production for cowpea (*Vigna unguiculata* Walp), when treated with TiO_2 NP foliar spray.

With its antibiotic properties against bacteria, viruses and fungi, TiO_2 NP were investigated in several studies for their effect on soil microorganisms, focusing on beneficial soil microorganisms of PGPB and AM fungi.

In soil spiked with TiO_2 NP, possible effects on biological N_2 fixation by rhizobia and root colonization by AM fungi in symbiosis with red clover (*Trifolium pratense* var. Merul) were examined (Moll et al., 2016). Neither rhizobia nor AM fungi showed signs of detrimental effects of the soil applied NP. Root nodulation and N_2 fixation levels of treated plants were unaffected, as well as root colonization of AM fungi showed no difference to the control. P content in plants were also not affected, overall leading to unaffected biomass production of red clover. These results were supported by findings of Burke et al. (2015), who also did not find negative effects of applied TiO_2 NP on rhizobia and AM fungi activity with soybean (*Glycine max*) and again by Moll et al. (2017), who reported no adverse effects by TiO_2 NP soil application on wheat growth and performance of associated AM fungi. Contrasting, Priyanka et al. (2016) reported inhibitory effects of soil applied TiO_2 NP on mycorrhizal symbiosis in rice plants (*Oryza sativa* L.), as AM fungi mycelium formation was reduced by TiO_2 NP treatment. Further, reports of Feng et al. (2013) suggest, that certain other NPs, e.g. of FeO, may improve AM fungi root colonisation, depending on NP concentration, but may also diminish AM fungi symbiosis performance by possibly binding fungal exudated glomalin, which could reduce its beneficial effects on soil formation and hence, water and nutrient acquisition for the vegetal partner.

With TiO_2 NP as a new, emerging biostimulant gaining track in agricultural application, higher concentrations are to be expected in soils. Anyhow, no detrimental effects regarding nodulation, N_2 fixation rate or mycorrhizal colonisation rate respectively were shown for a series of plant species and their respective symbiotic soil partners of rhizobia or AM fungi. However, as studies dealing with the topic are still scarce, further research is needed to determine specific behaviour of said soil microorganisms regarding TiO_2 NP application to a broad variety of crops and soil and environmental conditions, including the mode of application of the biostimulant, as most studies conducted testing of TiO_2 NP as a soil drench, not foliar spray.

Different effects may be exhibited by TiO_2 NP, when applied to plants. Accelerated seed germination by TiO_2 NP pre-treatment can increase vigor and seedling growth. Improved N metabolism with enhanced accumulation and possibly photocatalytic N_2 fixation could greatly induce plants' access to N and synthesis of organic N compounds, active in photosynthesis processes. Corresponding, the photosynthesis system may be stimulated, leading to enhanced biomass production of TiO_2 NP treated plants. Moreover, it was shown, that TiO_2 NP can mediate stress response of plants by priming and activation of the antioxidant system as well as regulation of the osmotic system.

First studies hint, that TiO_2 NP may not affect AM fungi and other beneficial soil microorganisms negatively, but intensified research is needed to determine optimal application mode of TiO_2 NP as well as compatibility with other biostimulants.

Although not considered detrimental in human consumption (orally) by the European Safety Authority (EFSA) (Aguilar et al., 2016), a recent study may show potentially constrains of TiO_2 NP usage in food items (Bettini et al., 2017). Further, inhalation of TiO_2 NP was classified as suspected carcinogenic by the European Chemicals Agency (ECHA), which may prohibit TiO_2 NP spray application in agriculture. Therefore, more research is needed to determine hazardousness of TiO_2 NP solution used as a foliage spray for crops in the described approach.

It seems likely, that TiO_2 NP can lessen N fertilizer demand for crop production (Frutos et al., 1996). This could be a fundamental benefit and key stone in the holistic system proposed in this work, as soil fertility and available soil N in the beginning of the restoration activity is likely to be very limited in degraded, to-be-restored soils with low organic matter content and only starting vegetation. Performance of pioneering plants could be drastically improved by TiO_2 NP biostimulant application, when their N demand could be reduced by increasing their N efficiency (absorption and utilization of NO_3^-), like was shown for spinach (Yang et al., 2006) and red pepper (Frutos et al., 1996), all while photosynthesis is improved and abiotic and biotic stress resilience may be enhanced. Therefore, TiO_2 NP, as a non-expensive material needed in only minor amounts, could serve as a true biostimulant – with the potential to act as a photocatalyst of N_2 fixation on the side (Yang et al., 2007).

3.5.7 Conclusion: Biostimulants

In this chapter, a broad range of exogenously applicable non-microbial biostimulants for promotion of plant growth and stress resistance from different origin, exhibiting diverse activities in plants and soil were presented (see Table 5), which could potentially increase biomass production of crops tremendously at very little or neglectable costs, boosting productivity and profitability of agricultural operation. Along with biostimulatory PGPF and PGPB discussed in chapter 3.2, biostimulants in general may possess great ability to intensify agricultural production by enhanced nutrient efficiency, while minimizing dependency on costly and limited production goods of synthetic fertilizer and pesticides needed in bulk quantity.

Further, to some extent, some biostimulants could be produced locally, on-site, partly from waste products or natural products so far underutilized. As needed quantities are generally low, investment costs may become negligible and thus, utilization of biostimulants may boost the economic and ecological viability of crop production in many regions so far struggling due to limited access to production goods by lack of sufficient finances and suitable transportation infrastructure. Concluding, biostimulants may be a key factor in intensification of agricultural production in the 21st century, especially in those regions struggling with a changing environment and declining soil fertility and corresponding low yields.

However, with increased biomass production of plants, increased uptake and depletion of soil nutrients is inevitable. Therefore, to manage nutrient balances in soils, adapted plant selection, utilization of alternative fertilizer and specific crop management systems are fundamental for sustainable agricultural operation.

In general, knowledge and experience with handling of the various biostimulants is still lacking, especially under field conditions. Future research should determine optimal conditions for application of biostimulants in crop production, regarding mode, rate, concentration, duration and timing of application, for various plant species under differing soil and environmental conditions.

Further, so far only some studies examined the interaction of different non-microbial biostimulants in combination with AM fungi (humic substances (Gryndler et al., 2005), protein hydrolysates (Rouphael et al., 2017), *Moringa* leaf extract (Abdallah et al., 2017), salicylic acid (Garg and Bharti, 2018; Zhang et al., 2018), titanium dioxide nanoparticles (Moll et al., 2016)) and found positive or neutral responses of synergistic co-operation. Hence, in future research, application of multiple non-microbial and microbial biostimulants in dual and multiple combination among themselves should be examined for potential synergistic or detrimental effects, so that the full potential of biostimulants can be utilized for tackling one of the greatest challenges of humankind in the 21st century: Nourishing a growing number of people in a sustainable way.

Table 5: Effects of selected biostimulants on biomass production on various crops, special impact and estimated material costs per ha and season. If no application rate or quantity per plant or ha was reported in the reference, 25 ml solution per plant and 2.5 plants*m^{-2} (25.000 plants*ha^{-1}) were assumed for single foliar spray application (625 l*ha^{-1}) and 0.5 ml solution per plant for seed preparation treatment. Prices in USD per kg or l of material were obtained from suppliers of alibaba.com at single kg prices: Humic acid (1), fulvic acid (2.5), protein hydrolysate (5), chitosan (25), seaweed powder (3.5), *Moringa* leaf powder (10), Salicylic acid (10), Glycerol (5), NaHS (10) and TiO2 NP (60 and 30 for 5 and 20 nm particle size respectively). FM = fresh mass; DM = dry mass.

Bio-stimulant	Plant spp.	Plant stage at application	Application mode, concentration and rate	Biomass production	Special impact	Material costs per ha and season	Reference
Humic-like substances	Maize (*Zea mays* L.)	germinated seeds in hydroponic solution	self-prepared humic acid growing solution with 50 mg/l C for hydroponic-cultured seeds	+66 % plant FM +85 % plant D	water efficiancy increased (g DM/l water consumed)	no material cost calculated, as material was self-produced	Eyhera-guible et al. (2008)
Humic acid	Beans (*P. vulgaris* L.)	soil amendment	Soil amended with humic acid (K-Humate with 75 % HA and FA, 20 % water soluble K_2O) at 0.1 % w/w	+12.9 % root DM +12.3 % shoot DM	reduction of soil EC and elektrolyte leakage, increase in proline, chlorophyll and nitrate content of plants under salt stress	2175 USD/ha, with 1 ha of 15 cm soil depth and 1.45 g/cm³ = 2175 t soil * 0.1 % HS (w/w) = 2175 kg HS (one-time application) at 1 USD/kg HS	Aydin et al. (2012)
Humic acid	Peanut (*Arachis hypogaea* L.)	seedlings, 30 days after sowing	foliar spray of 40 mg/l HA, at 30 days after sowing and blooming stage	+83.6 % seed yield +18.5 % straw yield	interaction effect with N fertilization, +86.4 % and +354.4 % in HA+N treated plants in comparison to N treated plants and to untreated controls, respectively	0.05 USD/ha, with 2.5 plants/m², each receiving 2 x 25 ml spray with 40 mg HA/l at 1 USD/kg HA	Mora-ditochaee (2012)
Fulvic acid	Maize (*Zea mays* L.)	three-week-old seedlings after transplantation	single 1.5 mg/l (v/v) fulvic acid foliar application with 25 ml per plant, 6 days after imposing water stress	+10.9 % shoot FM +17.7 % shoot DM +8.8 % grain yield in water-saturated soils (75% SFC); +11.9 % shoot FM +29.6 % in shoot DM +18.8 % grain yield in water-deficient soils (35% SFC)	decrease in chlorophyll content alleviated in water-stressed plants treated with FA	0.0024 USD/ha, with 2.5 plants/m², each receiving 25 ml spray with 1.5 mg FA/l at 2.5 USD/kg FA	Anjum et al. (2011)

Table 5 (continued): Effects of selected biostimulants on biomass production on various crops, special impact and material costs per ha and season. For details see table 8, p.83.

Bio-stimulant	Plant spp.	Plant stage at application	Application mode, concentration and rate	Biomass production	Special impact	Material costs per ha and season	Reference
Protein hydrolysate	Maize (*Zea mays* L.)	two-weeks-old seedlings	growing solution with 1mg/l protein hydrolysate from alfalfa	+19 % shoot FM +77 % root FM	effects of salinity partly alleviated	not calculated as application quantity per plant or m² not reported	Ertani et al. (2013)
Protein hydrolysate	Lettuce (*Lactuca sativa* L.)	20-days-old seedlings after transplan-tation	foliar spray with 2.5 ml/l protein hydrolysate at weekly intervals over four weeks, starting 6 days after transplantation	+13 % root DM		31.25 USD/ha, with 2.5 plants/m², each receiving 4 x 25 ml spray with 2.5 ml PH/l at 5 USD/kg PH	Rouphael et al. (2013)
Chitosan	Cowpea (*Vigna ungiculata* L.)	seedlings, 40 days after sowing	foliar spray with 250 mg/l chitosan and 0.5 % surfactant Tween-20, sprayed until dripping of plants, at day 40, 50 and 60 after sowing	+25 % shoot FM +20 % shoot DM	effects of water stress partly alleviated	58.59 USD/ha, with 2.5 plants/m², each receiving 3 x 25 ml spray with 250 mg chitosan/l at 25 USD/kg chitosan and 5g Tween-20/l at 5 USD/kg Tween-20	Farouk et al. (2008)
Chitosan	Maize (*Zea mays* L.)	seeds	germination solution with 0.5 % (w/v) chitosan, with 0.24 ml solution per seed	+34.2 % shoot DM +31.0 % root DM	activation of antioxidant system	0.75 USD/ha, with 2.5 plants/m², each receiving 0.24 ml seed pretreatemen solution with 5g chitosan/l at 25 USD/kg chitosan	Guan et al. (2009)
Chitosan	Thyme (*Thymus daenensis* Celak)	transplanted seedlings	foliar spray with 400 µl chitosan/l, dissolved in acetic acid, sprayed three times, at 50% flowering, full flowering and full bloom	+17.9 % DM +23.4 % oil yield	effects of water stress partly alleviated, +5.7 % DM +31.9 % oil yield in water-stressed soil compared to untreated, stressed control	not calculated, as preparation of foliar spray was not reported	Bistgani et al. (2017)

Table 5 (continued): Effects of selected biostimulants on biomass production on various crops, special impact and material costs per ha and season. For details see table 8, p.83.

Bio-stimulant	Plant spp.	Plant stage at application	Application mode, concentration and rate	Biomass production	Special impact	Material costs per ha and season	Reference
Seaweed extract	Alfalfa (*Medicago sativa* L.)	seedlings	growing solution with 1 g/l seaweed extract powder of *Ascophyllum nodosum*	+118 % root DM +112 % shoot DM	nodulation improved	8.75 USD/ha, with 2.5 plants/m², each receiving 100 ml seaweed solution with 1 g/l *A. nodosum* extract powder at 3.5 USD/kg	Khan et al. (2013)
Seaweed extract	Wheat (*Triticum* spp.)	seeds	germination solution with 0.1 % (w/v) seaweed extract from powdered *Nizimuddinia zunardini*, with 0.5 ml solution per seed	+178 % root FM +33 % root DM	seed germination improved	0.0125 USD/ha, with 2.5 plants/m², each receiving 0.5 ml seed pretreatemen solution with 1 g seaweed powder/l at 1 USD/kg	Shabazi et al. (2013)
			germination solution with 0.1 % (w/v) seaweed extract of powdered *Gracilaria corticata*, with 0.5 ml solution per seed	+142 % root FM +42 % root DM	seed germination improved		
			germination solution with 0.2 % (w/v) seaweed extract of powdered *Ulva fasciata*, with 0.5 ml solution per seed	+223 % root FM +7 % root DM	chlorophyll content enhanced		
Seaweed extract	Soybean (*Glycine max*)	seedlings, 30 days after sowing	foliar spray with 15 % (v/v) seaweed extract from grinded and filtered *Kappaphycus alvarezii* (Doty) as mother solution, at day 30 and 60 after sowing, with 650 l/ha for each application	+58 % grain yield		5 USD/ha, when solution is produced from dry seaweed powder of *Kappaphycus alvarezii* solved in water, assuming 5% DM of seaweed and 15 % seaweed extract (v/v) concentration, at double application of 650 l/ha, with 1 USD/kg	Rahtore et al. (2009)

Table 5 (continued): Effects of selected biostimulants on biomass production on various crops, special impact and material costs per ha and season. For details see table 8, p.83.

Bio-stimulant	**Plant spp.**	**Plant stage at application**	**Application mode, concentration and rate**	**Biomass production**	**Special impact**	**Material costs per ha and season**	**Reference**
Azolla extract	Pea (*Pisum sativum*)	seeds	germination solution with 0.05 % (w/v) extract from boiled and filtered *Azolla*	+118 % plant FM +30 % plant DM	seed germination improved	no material cost, as material was self-cultivated	Bindhu et al. (2013)
Azolla extract	Tomato (*Solanum lycopersicum*)	transplanted seedlings	foliar spray with 0.05 % (w/v) extract from boiled and filtered *Azolla,* at day 15, 30 and 45 after transplantation	+140 % plant FM +34 % plant DM	seed germination improved	no material cost, as material was self-cultivated	Hanafy et al. (2018)
Moringa leaf extract	Rocket (*Eruca vesicaria subsp. sativa*)	7-days-old seedlings	foliar spray with 2% (w/v) *Moringa* extract, 7 and 14 days after germination	+68 % plant FM +51 % plant DM		no material cost, as material was self-cultivated	Abdalla et al. (2013)
Moringa leaf extract	Fennel (*Foeniculum vulgare* Mill.)	four-weeks-old seedlings	foliar spray with 0.6 % (w/v) powdered *Moringa* extract, at week 4 and 6 after germination	+59 % plant FM +82 % plant DM		no material cost, when self-cultivated; 75 USD/ha, with 2.5 plants/m², each receiving 2 x 25 ml spray with 6 g Moringa leaf powder/l at 10 USD/kg Moringa leaf powder	Abdallah et al. (2017)
Salicylic acid	Maize (*Zea mays L.*)	soil amendment	soil amended with 10^{-4} M salicylic acid (0.0138 g SA/kg or plant)	+28 % plant DM	effects of salinity partly alleviated	3.45 USD/ha, with 2.5 plants/m² each receiving 0.0138 g SA/plant or 600 USD/ha with 0.0138 g SA/kg soil at 10 USD/kg SA (assuming 1 ha, 30 cm soil depth with 1.45 g/cm³)	Gunes et al. (2007)
Salicylic acid	Tomato (*Lyco-persicon esculentum*)	transplanted seedlings	foliar spray with 10^{-6} M salicylic acid (0.000138 g SA/l), starting two weeks after transplanting, for five times	+38 % plant yield	higher brix index and vitamin C content	< 0.001 USD/ha, with 2.5 plants/m² each receiving 25 ml of spray with 10^{-6} M SA/l at 10 USD/kg SA	Javajeri et al. (2016)

Table 5 (continued): Effects of selected biostimulants on biomass production on various crops, special impact and material costs per ha and season. For details see table 8, p.83.

Bio-stimulant	Plant spp.	Plant stage at application	Application mode, concentration and rate	Biomass production	Special impact	Material costs per ha and season	Reference
Glycerol	Castor (*Ricinus communis*)	seeds	germination solution with 50 mM glycerol	+55 % plant FM +64 % plant DM	effects of salinity partly alleviated	0.29 USD/ha, with 2.5 plants/m², each receiving 0.5 ml seed pretreatemen solution with 4.6 glycerol/l at 5 USD/l glycerol	Ali et al. (2008)
Glycerol	Maize (*Zea mays* L.)	two-weeks-old seedlings	foliar spray with 25 ml/plant of 0.5 ml/l glycerol, at week 2 and 4 after germination	+84 % plant FM +155 % plant DM		3.13 USD/ha, with 2.5 plants/m², each receiving 2 x 25 ml spray with 0.5 g glycerol/l at 5 USD/l glycerol	Tisserat et al. (2013)
Glycerol	Carrot (*Daucus carota* L.)	two-weeks-old seedlings	foliar spray with 2.5 ml/plant of 5 ml/l glycerol, at week 2 and 6 after germination	+106 % plant FM +158 % plant DM		3.13 USD/ha, with 2.5 plants/m², each receiving 2 x 2.5 ml spray with 5 g glycerol/l at 5 USD/l glycerol	Tisserat et al. (2013)
Glycerol	Spearmint (*Mentha spicata* L.)	eight-weeks-old seedlings, after transplantation	foliar spray with 2.5 ml/plant of 5 ml/l glycerol, at week 8 after germination	+47 % plant FM +69 % plant DM		1.56 USD/ha, with 2.5 plants/m², each receiving 1 x 2.5 ml spray with 5 g glycerol/l at 5 USD/l glycerol	Tisserat et al. (2013)
NaHS	Spinach (*Spinacia oleracea*)	seedlings (hydroponic)	hydroponic solution with 100 µM NaHS for 30 days, renewal of solution every 3 days	+160 % biomass production		not calculated, as preparation and quanity of solution was not reported	Chen et al. (2011)
TiO_2 NP	Spinach (*Spinacia oleracea*)	seeds and seedlings (two- or three-leaf stage)	seed pretreatment solution and foliar spray of 0.25 % (w/w) TiO2 NP (5 nm particle size), at two- or three-leaf stage of plants	+99 % plant DM	photo-catalytic, abiological N2 fixation	18.75 USD/ha, with 2.5 plants/m² each receiving 5 ml spray with 0.25 g TiO_2 NP/l at 60 USD/kg TiO_2 NP (5 nm particle size)	Yang et al. (2007)
TiO_2 NP	Broad bean (*Vicia faba*)	10-days-old seedlings	foliar spray of 0.01 % (w/v) TiO2 NP (20 nm particle size) solution with 0.1 % (w/v) Tween-20 as a surfactant at day 10 and 20 after germination, 5 ml per plant	+62 % root DM	salinity partly alleviated	1.93 USD/ha, with 2.5 plants/m² each receiving 5 ml spray with 0.01 g TiO_2 NP/l and 1g Tween-20/l at 30 USD/kg TiO_2 NP (20 nm particle size) and 5 USD/kg Tween-20	Abdel Latef et al. (2017)

3.6 Conclusion: Literature review

P is one of the most crucial nutrients in regards of efficient crop production in agriculture. Considering the declining quantity and quality of mineable reserves and resources of fossil P, its shortage likely will threaten global food supply in future decades with increasing intensity. Hence, alternative P fertilization strategies and approaches for utilization of fixed soil P reserves for maintaining or even enhancing global crop production need urgent consideration (Cordell et al., 2009).

Further, taking a rapidly growing world population into account, with high growth rates especially in SSA, along with changing environmental conditions and high rates of continuous soil degradation by non-adapted, conventional agricultural practice all over the world, the need for a fundamental shift in agriculture with substantial recycling of nutrients and productive restoration and conservation of fertile soils deems essential for future development of humankind (Cordell et al., 2011).

In this chapter, it was shown, that BoneC from pyrolysis of recycled animal bones possess interesting properties and features as an organic soil amendment with P fertilizer value, especially for low-input farming practice with lacking financial resources or infrastructure. However, BoneC only may deem suitable as a possible alternative for inorganic P fertilizer, by all means, if its fertilizer recovery rate can reach levels comparable to inorganic P fertilizer, over the short-term. It needs to meet functional, ecological and economical demands of intensive crop production to produce high yields after a minimal transition time, within the first season of application. Only then it may seem feasible for resource-poor farmers with high pressure of securing the daily livelihood to adapt BoneC fertilizer as a new technology in their farming practice.

Without adaption of the agricultural system, plain BoneC clearly cannot compete with any water-soluble P fertilizer in this regard. Turning tables around, however, it may can in synergistic collaboration with other alternative soil additives like BioC and stimulatory plant sprays, all fundamentally fostered and mediated by beneficial soil microorganisms of AM fungi, Sebacinales and PGPB as part of the soil food web, which also trigger numerous other beneficial effects on soil status and plant performance on the side.

BioC can improve soil fertility tremendously by adding highly porous pyrogenic carbon structures to the amended soils, with great inner surface area, able to ad- and de-sorb nutrients, water and organic C compounds. By their liming effect and diverse moisture and oxidation conditions, inner and outer BioC surfaces can serve as an easy-to-access nutrient adsorption pool and concurrently, as niche soil habitats for improved proliferation of various beneficial, soil forming and nutrient solubilizing microorganisms, among others, fungal hyphae and PGPB.

Singly, but more efficient in co-operation, both PGPB and AM fungi can increase solubilization of inorganic and organic P sources in soils by excretion of enzymes, organic acids and protons. Especially in drought stressed, P fixing soils with limited diffusion capacity, P acquisition for plants may primarily depend on activity of soil microorganisms – less on diffusion and water solubility of fertilizer.

In this regards, AM fungi mycelia, interconnected into spacious CMN colonizing plants of the same and different species, the same or different growth status, can serve as a true biological soil infrastructure, sharing nutrients, water and information between the linked host plants, with great benefit of the overall plant community in biomass production, crop yields, biodiversity and stress resistance – of which the latter can also be improved by Sebacinales activity.

These remarkable abilities and effects of synergistic soil microorganisms, all in combination, can in fact increase P acquisition from BoneC fertilizer in challenging soil conditions to levels comparable to inorganic P fertilizer. Hence, with suiting management and under certain conditions, BoneC could constitute a viable P fertilizer alternative, produced locally, from underutilized, recycled waste materials. Its contribution to future agriculture production in rural areas of developing countries may be essential in the decades to come.

Moreover, in cooperation, BioC and soil life may foster the formation of highly-stable organo-mineral macro aggregates, enriching C based stable SOM levels and water status of soils long-term – all, which can affect desired soil restoration and plant performance significantly. For proper functioning of soil life and plant growth, essential micro nutrients (in bulk or nanoparticle formulation) should be considered for soil remineralization or foliage application, as often lacking in intensively used agricultural soils.

Anyhow, for avoidance of declining yields over the short-term as well as ongoing intensification of agricultural production and true "cutting edge" economic feasibility of the new approach, crop yields may be further boosted to high levels with the usage of microbial and non-microbial biostimulants, which, in turn, once again were found to improve the activity of beneficial soil microorganisms.

Ultimately, to foster functioning of all multi-layered interactions of all single measures contributing to nutrient supply, soil formation and plant growth promotion, all system components need to be embedded in a holistic approach. In this approach, special focus is put on the adjustment of the agricultural management practice towards organic, regenerative agriculture - nurturing soil biology, not harming it, building up long-lasting, fertile soil, not degrading it.

4 Development of holistic approach for productive soil restoration

In a study with acidic, P fixing soils, evidence was presented by Zwetsloot et al. (2016), that P from BoneC can be recovered efficiently by AM fungi, in comparable rates to water-soluble, inorganic P fertilizer, significantly higher than without AM fungi. In field situation, similar effects may occur, when abundancy and activity of suiting P solubilizing soil microorganisms is high. Hence, to increase P availability from BoneC and P loaded soils in general and to assure sufficient plant yields in levels competitive to conventional practice, agricultural management and plant selection of the holistic approach should be tailored to meet requirements of AM fungi, P solubilizing bacteria and the soil food web in general. Apparently, this intention is the fundamental key feature of recently developed practice of regenerative agriculture.

4.1 Regenerative agriculture

Regenerative agriculture mimics natural processes of successional establishment of soil biology, which is followed by succession establishment of plants, accordingly. Early succession of bacteria dominated, unfertile, highly oxidized soils of a degraded state, is followed by a balanced or fungi dominated, fertile soils eventually, with high production capacity due to no detrimental practice of physical and chemical soil disturbance, resulting in a balanced and favourable redox potential. As the major principle in regenerative agriculture, soil biology comprised by the rich soil food web is nurtured and protected from any harm caused by conventional agriculture practice, as it is mainly responsible for nutrient and C cycling, constituting the natural pathway for plant nutrition and hence is fundamental for plant growth (Ingham et al., 1985; Neher, 1999; Setälä and Huhta, 1991).

The establishment of a species rich perennial cover cropping system with legumes and non-legumes, along with crop rotation regime and adapted crop residue management as well as usage of soil amendment with aerobic compost or compost tea, all supports the activation of rich soil life, which then is maintained and further nurtured by conservative no- or reduced-tillage and direct seeding and avoidance of inorganic fertilizer, pesticides and herbicides usage.

The major importance of high biodiversity and high plant species richness for efficient and sustainable ecosystem functioning, especially in regards to formation of stable SOM, was shown by a number of authors. Eisenhauer et al. (2017) reported increased total root biomass and quantity of root exudation of labile C in agricultural plots with high plant diversity, increasing the biomass (and therefore ratio) of fungi and bacteria in the soil. As more plant derived C was allocated to the rhizosphere by root exudation, the total biomass of the plant increased, indicating an overcompensation of the C costs for stimulus of soil life with beneficial properties on soil aggregation and formation of stable SOM.

With increasing fungal biomass in soils under regenerative agriculture, soil aggregation can be improved due to the physical stabilisation of soil particles into aggregates from enmeshing fungal hypha and fine roots in a first, physical step. In a second, bio-chemical step, polymers and necromass from microbial activity are either exudated or deposited onto or into the stabilized aggregates, acting as glue-like substances for binding of the particles (Jastrow et al., 1998; Rasse et al., 2005). In fact, these substances of microbial metabolism are a major fraction of stable SOC in soils (Liang et al., 2017).

Further, these formed macro aggregates tend to offer beneficial conditions of a moist, almost anoxic and reduced micro site for the anabolic process of polymerization, in which stable SOM is synthesized from root-derived labile C, organic N (fixed by symbiotic or associative diazotrophs) and other elements by microbial activity (Liang et al., 2017).

Interestingly, AM fungi were found to act as the mediator of root-C exudation in soils, as a major fraction of labile C from roots is not exudated directly in the rhizosphere, but channelled to the AM fungi, transported through the mycelium and eventually exudated partly into the hyphosphere (Kaiser et al., 2015), potentially directly into the created soil aggregates, out of reach of decomposing microbes due to small pore size (Rasse et al., 2005). Hence, the priming effect of increased decomposition of SOM by availability of labile C for enhanced microbial activity was lessened in soils with high species richness. New, stable SOM was formed, while old SOM was protected from decomposition, elevating total levels of stable SOM (Lange et al., 2015). BoneC and BioC compounds, serving as microbial hotspots as described in chapter 3.3.3.2., may exhibit similar properties as formed soil aggregates or will be included in the overall aggregate after all. Hence, the formation of stable SOM in soils could benefit from amendment with these materials also in this regard.

In regenerative agriculture, detrimental soil compaction from impermeable plough pans is prevented, along with following prevention of waterlogging and anaerobic soil conditions, often occurring in heavily tilled soils, poor in stable SOM (Finch et al., 2014). Water status and thus protection from soil erosion is improved by enhanced infiltration and water storage capacity in well aggregated, organic rich soils, covered by dense vegetation and mulch material (Bot and Benites, 2005; Liniger et al., 2011).

Also, evaporation rates by soil covering can be decreased, possibly benefitting mobility of soil life and nutrients due to elevated moisture levels in soils (Liniger et al., 2011; Lourenço de Freitas et al., 2014). Nutrient availability is improved, as soil life is activated (Ingham et al., 1985; Setälä and Huhta, 1991), while little to almost no nutrient leaching and volatilization, especially in fungal dominated soils under regenerative agriculture (De Vries et al., 2013, 2011).

Conventional N fertilizer application was found to reduce content of soil organic N (SON) due to a change of the soil C:N ratio, which can shift the equilibrium between immobilization (enrichment) and mineralization (decline) of SOM, especially in monocultures (Mulvaney et al., 2009). However, the authors argue, that a large fraction of plant N may not be derived from fertilizer application but from SON, which would further impede production potential of conventionally managed fields over long term. This argumentation may find indication in the reported declining N fertilizer efficiency in conventional managed fields (Tilman et al., 2002).

Other way around, increasing levels of both stable and labile SOM, can serve as energy and nutrient buffer and source for soil life and plant growth (Ingham and Rollins, 2006; Six et al., 2006). Accordingly, in soils with regenerative management principles, under certain conditions, sufficient yields at high profitability could be enabled, when compared to conventional farming (Bullock et al., 2007; Pimentel et al., 2005; Skinner and Dell, 2016). Weigelt et al. (2009) even found higher yields in high-diversity low-input fields in comparison to low-diversity high-input fields. High species richness outcompeted N fertilization yield-wise.

The intended regeneration of degraded soils and ecosystems, while bearing a sufficient harvest with low input material and high profitability, seems doable the most in an adapted, holistic approach with regenerative agricultural practice as its base. Nevertheless, to further strengthen the holistic approach developed in this study in regards of closing nutrient cycles and utilization of fixed soil P, formation of stabile SOM, transition times and productivity, the principles of regenerative agriculture as the core of the management system are extended by compatible measures of agroforestry, System of Crop Intensification and biological pest control.

4.2 Agroforestry

To activate primary and secondary beneficial effects of AM fungi and the soil food web in general, the nurturing and support of microbial proliferation in soils must be a top priority of the agricultural management system. Therefore, principles of agroforestry are integrated in the holistic concept.

Agroforestry is an emerging agricultural technique combining more than one farming activity in one field operation by the combination of specifically selected and arranged perennial plants and trees for production of wood, forage, nuts or fruit crops in repeated strips (often along slope contour lines or north-south direction) and annual crops or pasture in the interspace (Carvalho et al., 2010; Liniger et al., 2011; Soni et al., 2016) (see Figure 12). It is often integrated with livestock management for silvopastoral agroforestry, extending the forage production capacity of a given land by quantity and duration of production (Soni et al., 2016). Agroforestry allows multi-story farm design, increasing the agricultural surface per hectare, with synergistic effects on soil status, micro climate, biodiversity, biological pest control and overall plant productivity. In practice, the design and spacing of agroforestry systems needs to be adapted to local soil and environmental conditions, as well as intended farming operation.

Rates of soil erosion can be reduced drastically, when fields are under agroforestry management, potentially by factor 3 to 4 in comparison to conventional cropland, as was shown in a modelling study in the Philippines (Delgado and Canters, 2012).

When aligned along contour lines of the terrain according to principles of Rain Water Harvesting, agroforestry with perennial trees and plants can increase the retention and thus, infiltration of precipitation water, preventing excessive, destructive surface runoff (Liniger et al., 2011). Overall, the water status of soils may be improved, while top soils are protected from erosion.

Perennial vegetation of agroforestry systems also increases water status of soils by shading of the interspace. By shading, also excessive heating and UV radiation of the soil surface is prevented, which could potentially damage near-surface soil biology (Liniger et al., 2011). Also, it was found, that trees or hedgerows can substantially decrease wind velocities on their leeside, reducing wind erosion processes and high rates of evaporation (Tamang et al., 2010). Concluding, agroforestry may create local micro climates, beneficial for plant growth and proliferation of soil life (Liniger et al., 2011; Tamang et al., 2010).

With improved water status and permanent, nurturing host plants, soil biology in fields under agroforestry is enhanced, with again benefitting effects on overall soil status.

Soil life activity and the overall diffusion driven availability of soil nutrients may benefit significantly from elevated levels of soil moisture and increased exudation of C sources, additionally triggered by grazing or pruning of trees (Ingham and Rollins, 2006). Several authors found enhanced activity of AM fungi in agroforestry fields, when compared to monocultural fields of the same crop. Both, Cardoso et al. (2003) and Muleta et al. (2008) found general higher levels of mycorrhizal activity in soils of coffee agroforestry systems in Brazil and Ethiopia, and specifically, higher levels of spores also in deeper soil layers due to enhanced root formation by deep rooting trees. Hence, as speculated by the authors, due to enhanced promotion of spores into deeper soil layers, the overall active soil volume for fungal the water supply from deeper soil layers by effects of AM fungi mediated hydraulic redistribution may

Figure 12: Silvopastoral agroforestry system with holistic planned grazing of the interspace, La Luisa farm, Codazzi, Cesar, Colombia. CIPAV archive.

increase (Egerton-Warburton et al., 2007; Querejeta et al., 2003). Interestingly, in agroforestry systems, tree rows may function as a reservoir and seed bank for fungal and bacterial infection of adjacent soils with lacking substrate-producing ability (Seiter et al., 1999). Ingleby et al. (2007) showed the ability of AM fungi to extend their mycelium from their tree host towards adjacent annual crops in the near distance for the establishment of a shared CMN. Here, tree rows of agroforestry systems may serve as the starting point for the establishment of donor-receiver plant-fungi systems described by Walder et al. (2012), with trees as the main C donor and annual crops as the main nutrient receiver. Importantly, further it was shown, that pruning of the tree did not reduce fungal colonization, although C source strength was reduced (Ingleby et al., 2007). However, to shorten the lengthy process of full network establishment, annual crops also should be inoculated with suiting AM fungi propagules according to principles of Ingham and Rollins (2006) to fuse into an overall CMN by anastomosis rapidly as discussed in chapter 3.2, when the expanding networks of a tree row and the interspace overlap.

All things considered, along with the creation of habitats of pest predators, improved biodiversity and hence improved biological pest control, all in all, agroforestry systems may enhance the overall productivity of the agricultural system, as not one single crop, susceptible to failing due to drought or pest attack is cultivated, but many of different species, over an extended growing period (Carvalho et al., 2010). However, growing in close spacing, perennial and annual plants may compete for the same nutrient and water resources in the interspace (Jose et al., 2000). Therefore, agroforestry systems need to be managed properly to reduce competition for needed resources without decline in plant productivity of the single components. In future research, suitable techniques should be evaluated to ensure deep rooting of perennial plants and trees, while a shared mycorrhizal network can be established and maintained for the mediation and alleviation of competition of nutrient and water distribution between plants in the interspace.

4.3 System of rice and crop intensification

The agricultural approach “System of Rice Intensification” (SRI) was developed to improve overall management of paddy rive cultivation in Africa and Asia in terms of lowered input materials, higher water efficiency and achievement of higher yields and higher profitability for farmers (Uphoff and Thakur, 2019). Promising results since then were adopted by many farmers especially in South and South-East Asia. Understanding the principles and convinced by observed increases in yield and profitability due to lower external input materials, farmers adopted the system for other crops like teff, sugarcane, wheat, maize, etc. and now being referred to as “System of Crop Intensification” (SCI) (Abraham et al., 2014).

In SCI, the conventional management of plants is altered, so that plant root growth is enhanced and soil life is stimulated. Firstly, by increased space between and within row distances, overall plant density is reduced, which again reduces the competition for soil and water resources between neighbouring plants accordingly, but enhances the potential space for the rhizosphere of each plant. Secondly, by early transplantation of germinated seedlings or direct seeding into fields, root growth of juvenile rice plants was found to be less affected by transplanting and accordingly, was improved in comparison to conventional practices. Here, SCI may improve the overall root development of plants, closer to their full genetic potential, activating soil volumes otherwise not utilized by weak and disturbed root growth. Finally, overall soil life is stimulated by partly or full abandonment of conventional fertilizer, pesticides and herbicides, but enhanced application of organic fertilizer materials such as livestock or green manure and inoculation of beneficial soil biology such as plant growth promoting fungi and bacteria. Here, also aerobic soil conditions are to be maintained to nurture beneficial, aerobic soil microorganisms (Abraham et al., 2014; Adhikari et al., 2018; Balamatti and Uphoff, 2017; Uphoff and Thakur, 2019).

Benefits of SCI are mainly related to improved rooting structure of plants, enabling improved nutrient cycling capacity of soils and access to soil water resources, as discussed before for regenerative agriculture. Resistance to abiotic stress of drought and heavy storm was reported to be improved as well as biotic stress from pest and pathogenic attack (Adhikari et al., 2018; Balamatti and Uphoff, 2017).

Substantial yield improvements by SCI are reported for many crops of teff, maize, wheat, sorghum, rice, sugarcane, in different countries like Ethiopia, Nepal, Pakistan, India and China (Abraham et al., 2014; Adhikari et al., 2018; Balamatti and Uphoff, 2017; Uphoff and Thakur, 2019). However, a meta-analysis by Uphoff of Chinese studies on SRI in China found, that rice yields increased only by about +10 %, compared to conventional practices (Wu and Uphoff, 2015). Due to lower inputs, higher water use efficiency and similar labour efforts, profitability of agricultural operation may rise significantly by the extensive SCI method. Albeit, SCI may be used to improve yields, especially in regions of general low productivity, but actual improvement of yields may be depending on current status of production levels.

Concluding, principles of SCI, mainly lowered plant densities and early establishment of seedlings or direct seeding, are considered as another key element of the proposed system in this thesis and are considered compatible with other measures of the regenerative agricultural system as they are partly shared between themselves. However, future research should determine root growth behaviour of SCI seedlings planted in soils with active, already established CMN as discussed in chapter 3.2, sufficiently supplying nutrients and water to new host plants at very low C costs. Here, the need for extensive root growth may become minor, which may affect the root development negatively and could counteract intended effects of SCI.

4.4 Biological pest control

Agricultural production can be susceptible to weeds and pest and pathogen attack, especially when in unnatural monocropping layout with declining soil quality and fertility. Thus, crop production can be diminished, potentially reaching levels of complete loss of yields, with according detrimental to catastrophic effects of the livelihood of farmers (Bouwmeester et al., 2003; Kim et al., 2002).

Yet, conventional pest control mostly relies on heavy tillage operation and chemical pesticides, fungicides and herbicides, which again increases the need for external input materials, at high costs with possible detrimental side effects on beneficial soil life, especially mycorrhizae (Banerjee et al., 2019). Therefore, in the holistic approach aiming to empower soil life activity, conventional weed and pest control is best avoided, but various measures of biological pest control are considered and integrated into the pest management system for further enhancement of protective effects partly already elicited by certain elements of the holistic system as discussed in chapter 3.

As already shown, effects of AM fungi, organised in CMN, as well as Sebacinales and PGPB may contribute fundamentally to biological pest control of the holistic approach, beyond effects of improved plant nutrient (Jung et al., 2012) and soil health (van Bruggen and Finckh, 2016).

Both AM fungi and Sebacinales were found to prime the host plant immunity system against potential pest and pathogen attack (Jung et al., 2012; Waller et al., 2005). The required systemic resistance may even be acquired by neighbouring “receiver” plants from interconnected “donor” plants through distribution of chemical warning signals by channelling through the fungal network (Song et al., 2013). Accordingly, AM fungi were found to decrease number of suitable infection sites for pathogenic microorganism by rapid colonization and hence occupation of host roots (Smith (1988) cited in Harrier and Watson (2004)), further to improved closure of wounds (Wick and Moore, 1984) and finally to enhance rates of root lignification (Dehne et al. (1978) cited in Akthar and Siddiqui (2008)). Once the symbiosis is fully established, exudation of C sources and strigolactones to the soil matrix by the host plant is reduced drastically (Baker and Cook (1982) cited in Akthar and Siddiqui, 2008; Lendzemo et al., 2007; López-Ráez et al., 2012). Furthermore, when nutrient allocation, solubilization and transport is facilitated predominantly within the hyphosphere by fungal mediation, only small fractions of available nutrients may be present in the soil solution.

With lacking C and nutrient sources and reduced sites of potential root infection, pathogenic soil microorganisms may lack needed resources for their proliferation and diminish in quantity and activity, substantially lowering potential biotic stress severity. All, while beneficial aerobic soil microorganisms are nurtured by AM fungi and measures of regenerative agriculture and SCI, while anaerobic conditions and the proliferation of anaerobic pathogenic microorganisms are lessened (Ingham and Rollins, 2006).

Similar, non-mycorrhizal plants like many agricultural weeds would also lack solubilized nutrients in fungal dominated soils (Ingham and Rollins, 2006). This in turn could mean, that plants, actively harbouring beneficial soil symbionts, gain better access to soil P and other nutrients over plants without symbiotic partner. Hence, weedy species may encounter a drawback and become less competitive in comparison to desired mycorrhizal crops. Accordingly, AM fungi were observed to reduce growth of weeds in combination with mycorrhizal sunflower plants (Rinaudo et al., 2010) and can contribute tremendously to the decline of *Striga* weed severity in crop cultivation (Lendzemo et al., 2007; López-Ráez et al., 2012).

However, biological pest control by these secondary effects of integrated systems elements may be enhanced by specific management of agricultural production and emerging techniques to control pest and diseases by sustainable, low-cost approaches.

By intercropping of specifically selected and arranged companion plants, biological pest control of crops can be enhanced by push-pull systems, which were found viable for cereal farming in SSA. Plant feeding insects are repelled by semiochemicals produced from intercropped, non-host "push" plants, serving as companion plants to cereals, which may also attract predators of pests by additional chemical signalling and habitat creation. Simultaneously, suiting trap plants located at the boundary of the threatened crop field again produce attracting semiochemicals associated with the original host plant to "pull" repelled pests from the field. Here, pest population can be diminished by natural insecticides produced by the trap plant or regular utilization of plants as forage material, preventing laid pest eggs from hatching. A well-known push-pull practice is cultivating *Desmodium* spp. as the "push" plant with maize as the main crop along with Napier grass (*Pennisetum purpureum* spp.) at the field boundary as the "pull" plant. Here, leguminous *Desmodium* also enhanced N supply to plants and suppresses germination of *Striga* weed, contributing to weed control (see Figure 13; Pickett et al., 2014). In general, high biodiversity in crop fields was found to reduce pests and weed pressure (Lundgren and Fausti, 2015; Skinner and Dell, 2016).

However, to further enhance protection from substantial yield losses due to heavy weed, pest and diseases infestation of agricultural fields under organic management, someone may turn to biopesticides.

Biopesticides can be produced from material of natural living organisms like plants or microorganisms, in some cases with only little investment or equipment needed. Their mode of action among others can be toxicity or antifeeding, but are not considered to cause problematic toxic residues or development of resistance by pests. A well-received and widely recommenced botanical insecticide is produced from seed kernels or leaves of neem tree (*Azadirachta indica* spp.), with many components acting for insect control (Senthil-Nathan, 2015). Also, some biostimulants discussed in chapter 3 may elicit plant-protective properties, e.g. TiO_2 NP was found to reduce severity of pathogen attack on treated plants (Cui et al., 2013; Owolade and Ogunleti, 2008).

Certain biopesticides may also be utilized against tsetse fly incidence. Tsetse flies (*Glossina* spp.) may be one of the most notorious pests in affected areas of SSA, as it can transmit parasitic protozoa of *Trypanosoma*, causing the "sleeping sickness" in human and cattle. With moist forest areas as one of the main natural habitats of the flies (Cecchi et al., 2008), areas under agroforestry management as proposed in this study may face tsetse fly infestation. However, Mbewe et al. (2018) reported effective reduction of tsetse fly population density by fly killing traps composed of small sticky targets of blue and black netting material. It further was shown by Maniania et al. (2006; 2013) that tsetse fly population can also be reduced by contamination with the fungus *Metarhizium anisopliae* (Metsch.) Sorok. (Hypocreales: Clavicipitaceae) by prepared traps. Another measure for repellence of tsetse flies was presented by Saini et al. (2017), who developed a collar for livestock, which dispenses a chemical compound regularly, mimicking the odour profile of the tsetse non-host waterbuck (*Kobus ellipsiprymnus defassa*).

Suitable options for control of tsetse flies seem available at low cost, but further research must examine the potential of tsetse incidence in agroforestry system with livestock management in affected areas of SSA as well as efficiency of possible coping measures mentioned here. In general,

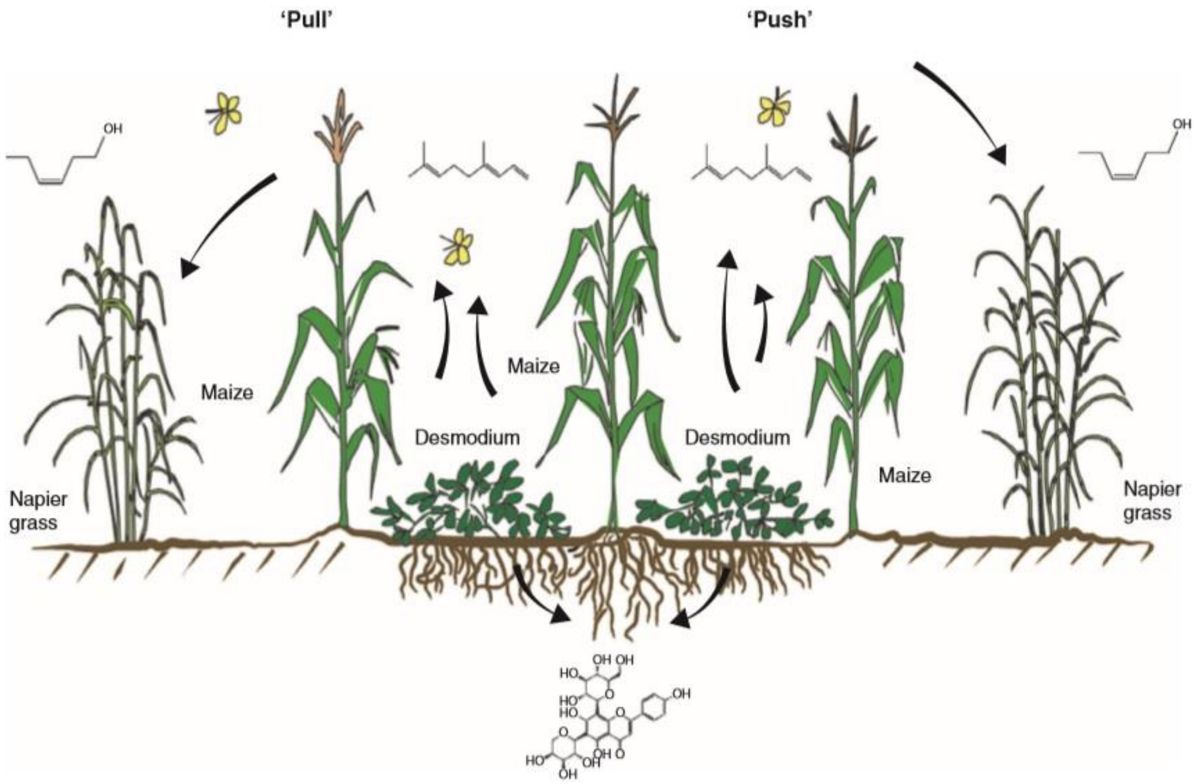

Figure 13: Push-Pull System for enhanced biological pest control by intercropping of *Desmodium* spp. as "push" plant with maize as the main crop and adjacent Napier grass as the "pull" plant, from Pickett et al. (2014), page 128.

integrated pest management should include a rich set of measures, preventive and reactive, to protect farmers from high yield losses due to pest, diseases and weed infestation. However, the most effective method to increase resistance of plants to biotic stress may be optimal supply with water and macro and micro nutrition by creation of healthy, fertile soil, rich in stable SOM and active beneficial soil life, so plants can develop to their fullest capacity, which includes a high capacity to repell pest and diseases of plants under organic management (Muneret et al., 2018), while outperforming weeds in fungal dominated soils (Ingham and Rollins, 2006) with support of AM fungi (Jung et al., 2012; López-Ráez et al., 2012; Othira et al., 2012; Rinaudo et al., 2010).

4.5 Effects of the holistic regenerative agricultural system

With a shift towards regenerative agriculture, physical and chemical soil disturbance is avoided by minimum or no-tillage and no usage of synthetic fertilizer and pesticides. Instead, amendment with compost and organic matter re-establishes a functional, C and nutrient cycling soil food web with bacteria, fungi, nematodes, protozoa and microarthropods as well as macro fauna like earthworms (Ingham and Rollins, 2006; Ingham et al., 1985; Moos et al., 2017). Specific application of BioC amended compost and compost tea, serving as microbial inoculum and food source, supports the establishment of this beneficial soil biology, in adjusted ratios of bacteria to fungi needed for the intended, specific successional stage of each part of the cropping system (Ingham and Rollins, 2006).

Adapted plant selection including diverse species of annual and perennial leguminous cover crops, intercrops, pasture grasses, cash crops and vines, bushes and trees, will foster the formation of a permanent mycorrhizal soil infrastructure in undisturbed soils (Heijden, 2004; Heijden and Horton, 2009), with high capability of P solubilization (Feng et al., 2002), scavenging and allocation of nutrients (Perez-Tienda et al., 2012) and water (Ruiz-Lozano et al., 2012), suppression of weeds (Lendzemo et al., 2007; Rinaudo et al., 2010) and pests (Harrier and Watson, 2004), contribution to soil formation by production of glomalin (Rillig and Mummey, 2006) and finally, plant growth promotion potential (Walder et al., 2012). Atmospheric N_2 is fixed by soil bacteria in symbiosis with plants or associative in created soil aggregates and retained in microbial or plant biomass until decay (Whitehead and Day, 1997). An adapted mulching system utilizing nutrient and C rich crop residues enables avoidance of bare soil, nutrient cycling and improvement of AM root colonization, with great potential to increase crop yield (Salami and Osonubi, 2003). Nutrient cycling is performed by soil life according to needs of their own metabolism and interlinked crops and perennial plants (Ingham et al., 1985; Setälä and Huhta, 1991).

Soil amendments of BoneC and BioC and created soil aggregates in general represent a suitable "hotspot" habitat at their inner and outer surface areas for desired soil microorganisms, with multiple niche conditions of moisture, oxygen, redox potential, nutrients and pore space (Joseph et al., 2010). By both, BoneC and BioC, P availability in amended soils can be enhanced (Atkinson et al., 2010; Leinweber et al., 2019), as well as microbial biomass (Steiner et al., 2008a). Moreover, contributing to rapid soil formation processes and protection of stable SOM in soils (Liang et al., 2010), BioC plays a major role in improved water status (Kammann et al., 2011) and long-term fertility of soils (Glaser and Birk, 2012), as well as C sequestration (Fang et al., 2015) and prevention of GHG emission (van Zwieten et al., 2010b).

The water status of soils is also to be improved by implementing physical measures of rain water harvesting soil works such as terracing, contour trenches and planting pits with the aim to retain and infiltrate any precipitation water on-site (Mekdaschi Studer and Liniger, 2013). In combination with these physical measures, no-tilled, but bioturbated, well aggregated soil rich in stable SOM and soil life can prevent surface runoff and accordingly soil erosion, while water storage and nutrient retention is improved (Bot and Benites, 2005). Deep rooting perennials or trees can access water and nutrients from deeper soil layers (Egerton-Warburton et al., 2007), which would remain unutilized in a conventional system. Arranged in a silvopastoral agroforestry system, those plants may constitute strong C donors for the establishment and maintenance of multiple, overlayered CMN systems, nurturing annual crops in the intermediate space from the day of germination (Heijden, 2004; Heijden and Horton, 2009; Walder et al., 2012), while reducing wind velocities (Tamang et al., 2010) and producing forage or mulch material (Salami and Osonubi, 2003).

Resilience of plants and crops to drought stress is further enhanced by improved access to water, which allows a permanent vegetative soil cover – another principle of regenerative agriculture. This again contributes to prevention of soil compaction and erosion. Microorganisms benefit in regards of soil temperature, avoidance of UV radiation (Liniger et al., 2011) and, most importantly, continuous exudation of labile C via root-fungi exudation for metabolism of soil life (Ingham and Rollins, 2006). Soil is more and more aggregated and enriched in stabile SOM, again increasing effects of water and nutrient retention, preventing excessive leaching or volatilization (De Vries et al., 2013; Mulvaney et al., 2009; van der Heijden, 2010).

Rotational grazing by livestock (or pruning) as a measure of regenerative agriculture continuously enriches the soil with organic matter and allows root growth continuation and elevated rates of root exudation of grazed or pruned plants to soil, when at least 50 % of above-ground biomass remains intact for efficient photosynthesis (Crider, 1955). The natural nutrient cycling performed by the soil food web is enhanced with beneficial effects on soil health and nutrient supply (Pramanik et al., 2017).

Further, by induced prevention of soil crusting and compaction as well as lowered bulk density by BioC addition and enhanced soil aggregation by microbial activity, soil aeration can be improved. Anaerobic conditions from water logging by poor soil structure are prevented, which benefits beneficial, aerobic over detrimental, anaerobic soil microorganisms (Ingham and Rollins, 2006).

With a beneficially balanced soil biology, including PGPF of mycorrhizae and root endophytes like Sebacinales, and the improved root growth capacities of species rich plant communities managed by SCI principles, susceptibility of crops towards pest and pathogen attack can be decreased, as well as the occurrence of weed plants (Harrier and Watson, 2004; Ingham and Rollins, 2006; Lundgren and Fausti, 2015). Availability of surplus nutrients in plant available form as from inorganic, water-soluble fertilizer is reduced by close interlinkage of plant exudation and microbial nutrient cycling and corresponding transient nutrient immobilization (Ingham et al., 1985). Hence, nutrient supply to pathogenic microbes may be limited, as well as number of infection sites, as plant roots are already highly colonized by competing beneficial fungi (Smith (1988) cited in Harrier and Watson (2004)). Moreover, some PGPB were found to exhibit protective effects against root damaging competitors (Bashan and De-Bashan, 2005; Pandey et al., 2006). Also, certain biostimulants (Iriti and Faoro, 2009; Y. Zhang et al., 2015) and compost tea (Al-Mughrabi et al., 2008; Ingham and Rollins, 2006) were found to improve resilience to biotic stress.

The discussed measures of agricultural management and soil amendment may increase plant productivity singly. However, when applied in combination, a boost in plant production may be possible, in a reasonable short time of transition. Nevertheless, as one aim remains to produce a viable harvest within the first season of shifting to the new agricultural system and to further foster the profitability and acceptability of the proposed system, agricultural production may be intensified by additional usage of biostimulants (Guan et al., 2009; Hanafy and El-Emary, 2018; Mona, 2013; Rose et al., 2014; Schiavon et al., 2008; Shakirova et al., 2003; Tisserat and Stuff, 2011).

4.6 Practical implementation of holistic approach

Phase 1: Starting from degraded soil

Degraded soils often show lack of permanent vegetation cover. As nutrient availability and soil structure is poor, crop production is low, while weedy species bloom (Ingham and Rollins, 2006). Diminishing quantity of soil food web biomass by tillage operation, coupled with increased N losses by leaching, volatilization and loss of N containing SOM, enhance the demand for application of inorganic N fertilizer (De Vries et al., 2013). Rates of water infiltration and storage capacity are low, resulting in easy erosion by surface runoff or wind. Further, high soil temperatures and strong UV radiation harm soil life, while evaporation rates increase. The overall water status of those mineral soils is poor, as SOM is lacking (Bot and Benites, 2005). By heavy tillage operation, strong compaction layers can form belowground, impermeable for exploration by roots or percolation of water (Finch et al., 2014; Ingham and Rollins, 2006). Moreover, just above the compaction layers, anaerobic zone by retained water can affect yields negatively by creating a disease enhancing environment (Husson, 2014; Lone et al., 2016). The soil volume beneath those layers is inactivated, with no contribution to nutrient or water supply. Any plant growth is restricted to the unfertile, depleted topsoil layers, which increases competition between plants (Casper and Jackson, 1997; Ehleringer et al., 1991) (see Figure 14).

Conventional managed soils may contain enhanced concentration of fertilizer pollutants and pesticide residues, such as salts, heavy metals or organic contaminants (Hilber et al., 2009; Ingham and Rollins, 2006; Nicholson et al., 1994; Rothbaum et al., 1979), which can all harm plant growth in excessive

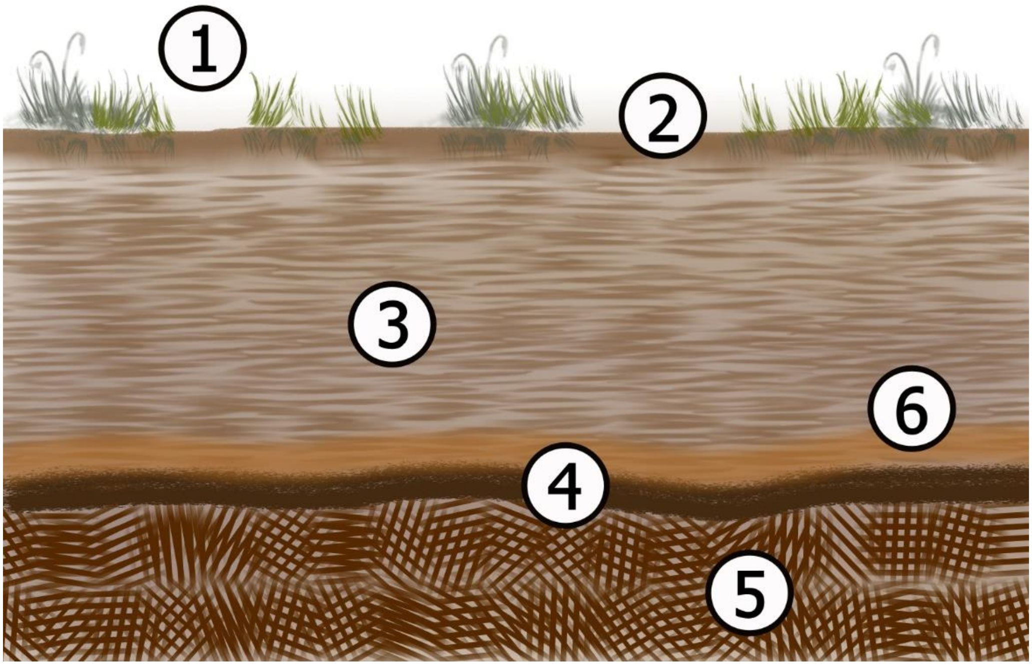

Figure 14: Conceptual diagram of degraded soil before establishment of the holistic system. Sparse vegetation (1), soil crusting (2), poor, compacted, horizontal soil structure (3) and impermeable ploughing pan (4), all restrict plant roots from growing into untapped deeper soil layers (5). Anaerobic soil layer is formed above ploughing pan due to lack of drainage (6). Wibbing (2020).

concentration. All in all, under this soil conditions, crop production is highly dependent on mechanical seed bed preparation, water-soluble nutrients, mechanical and chemical weed removal and chemical pest protection. Hence, profitability is low, while work load, energy demand and input materials are high as well as the environmental burden (Pimentel et al., 2005).

Phase 2: Preparation of soil

For the establishment of the holistic approach, one-time preparation tillage by subsoiling may be necessary, depending on the soil status (Finch et al., 2014; Ingham and Rollins, 2006). While mixing in BioC amended compost and BoneC, the compacted soil can be loosened in depth below the compaction layer, while beneficial soil biology including AM fungi propagules is inoculated by application of compost tea and fungal inoculates concurrently, in accordance to the later crop species present (perennials and trees = fungal dominated compost and compost tea; crops and grasses = bacterial and fungal balanced compost and compost tea; Ingham and Rollins (2006)). Soil works adapted to local climate and morphology are performed (construction of swales, terraces, contour lines, planting pits, etc.; see Mekdaschi Studer and Liniger (2013)), while the soil is mulched with organic material (Bot and Benites, 2005; Liniger et al., 2011) (see Figure 15).

Already in this process step, fundamental changes may occur in treated soils. The subsoil below the former compaction layer is activated and can infiltrate and store percolating water. The soil surface is loosened up. In aerobic conditions, the inoculated beneficial soil microorganism from compost and compost tea can strive, protected from splash erosion, high soil temperatures and UV radiation due to the mulch (Liniger et al., 2011). Earthworm population can start to restore, supporting the transportation of organic material from the surface into the soil matrix (Moos et al., 2017). Soil aggregates are formed, from incorporated organic material, mineral soil particles, BioC particles and fungal and bacterial compartments. Contributing, due to addition of Ca_2^+ and Mg_2^+ contained in BoneC, and hence lowering concentration of Na^+, dispersed clay particles may flocculate and are prevented from being washed of in deeper soil layers, where they could cause soil compaction and causal to that,

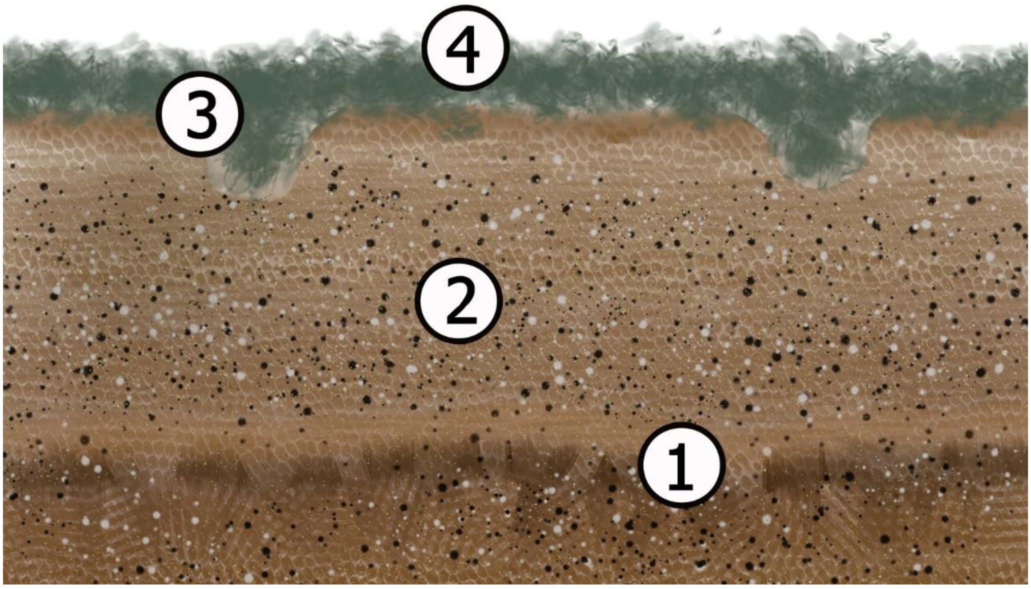

Figure 15: Conceptual graphic of soil preparation phase for the establishment of the holistic system. Compacted soil is deep tilled to crack the ploughing pan (1), while soil amendments (biochar, bone char and cow manure or compost) are mixed into the soil along with inoculum for soil biology including mycorrhizal propagules (2). Trenches or planting pits are constructed as measures of Rain Water Harvesting (3). The soil surface is covered with green mulch (4). Wibbing (2020).

possible formation of waterlogged anaerobic zones (Amelung et al., 2018). This would contribute to soil aggregation and retention of nutrients and water. Erosion processes are prevented by protection against splash erosion and no formation of surface runoff. Infiltration of precipitation is enhanced, as retention time on-field is increased by physical measures. Overall water status of soils improves.

Phase 3: Establishment of planting system

For planting, as a pre-treatment, seeds can be primed by inoculated with microbial and non-microbial biostimulants. By direct seeding into the mulch layer, seeds are protected from drying, cold temperatures and feeding animals. High germination rates with rapid formation of roots were observed, when mulched (Ashman et al., 2018). With the first exudation of C by plant roots, SOM levels of the soil rise and the soil food web starts C and nutrient cycling (Ingham et al., 1985). First mycorrhizal networks are established by chemical root signalling of germinated plants (Akiyama and Hayashi, 2006). Extraradical hyphae explore the soil, interconnecting plants of the ecosystem into spacious networks (Heijden and Horton, 2009). Perennial plants and deep rooting trees can utilize deep soil layers as the compaction zone was broken up. Hence, the competition for nutrients, water and space in shallow soil layers is reduced, benefitting all plants and crops (Casper and Jackson, 1997; Ehleringer et al., 1991). Free-living and symbiotic diazotrophic microorganisms start fixation of atmospheric N_2, enriching the soil in N (Dos Santos et al., 2012). Surfaces of BoneC and BioC are colonized by soil microorganism, including PGPB and AM fungi, which contribute to nutrient availability by solubilization of organic P and other nutrients from BoneC and mineral fractions of soil (Feng et al., 2002; Sharma et al., 2013), while glomalin producing fungal hyphae support the formation of stable macro aggregates (Denef et al., 2001; Rillig and Mummey, 2006). The formation of organo-mineral humus complexes enables the long-term sequestration of non-pyrogenic (from vegetal origin) and pyrogenic C (from BioC) (Glaser et al., 2002), while emission of N_2O can be significantly reduced in comparison to conventional management (see Figure 16; De Vries et al., 2013; Kammann et al., 2012).

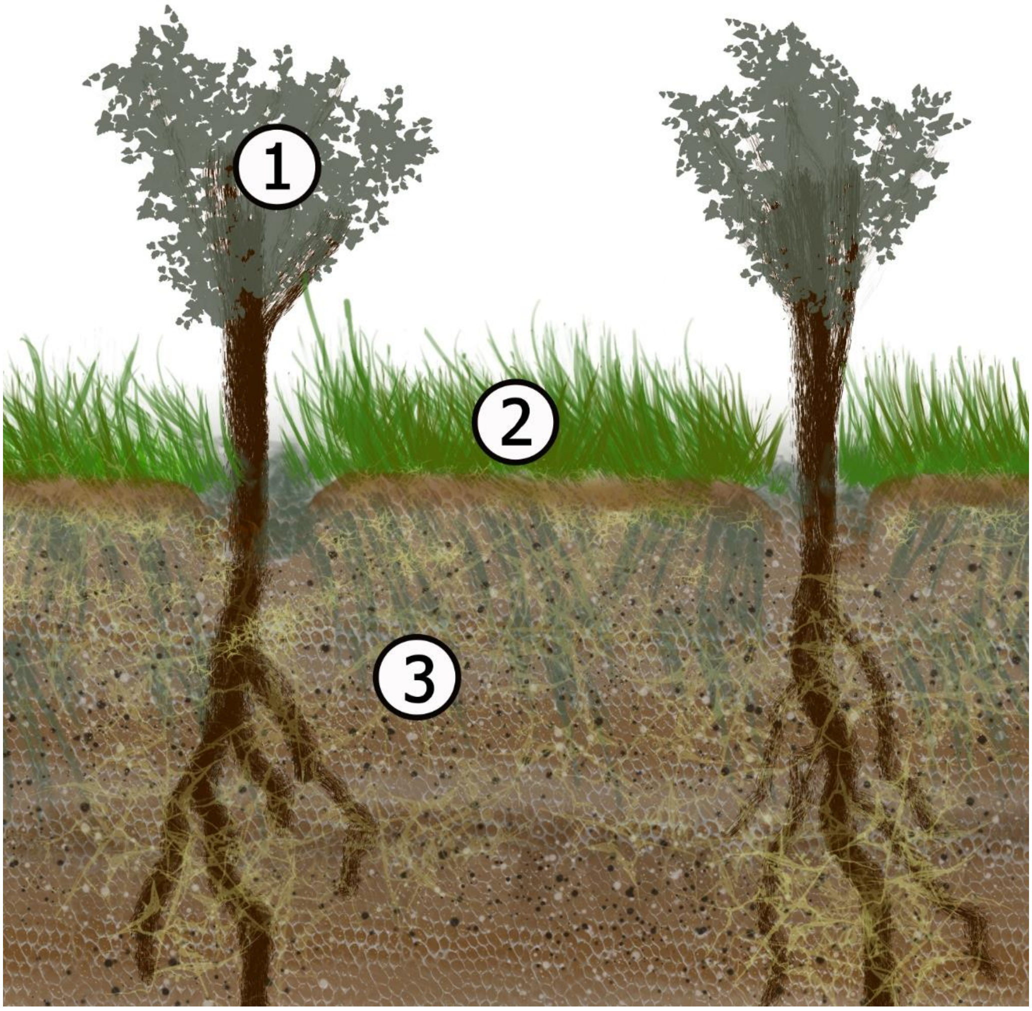

Figure 16: Conceptual graphic of plant implementation phase for the establishment of the holistic system. Perennial plants of the agroforestry system (1) and mycorrhizal pasture plants (2) establish a spacious common mycorrhizal network (3), which utilizes added nutrient sources of bone char and cow manure or compost. Wibbing (2020).

The soil surface is covered by vegetation, further reducing soil erosion processes (Liniger et al., 2011). Perennials and trees, arranged in agroforestry systems, can shade the understory plants in the interspace to create beneficial microclimates with reduced evaporation and wind velocities (Liniger et al., 2011; Tamang et al., 2010). By suiting selection of intercropped plants, a beneficial donor-receiver mycorrhizae plant system may be formed (Walder et al., 2012), while deep rooting plants with access to deep groundwater layers may serve as a biological irrigation system for shallow rooted plants (Egerton-Warburton et al., 2007). As it was found by De Vries et al. (2013), crop rotation can lower the biomass of the soil food web with less effective prevention of nutrient leaching. Hence, in this setting, perennial plants and trees may act as harbouring plants for a richer, more fungal dominated soil food web, which can interact with the poorer, bacterial dominated soil food web in the interspace cultivated with rotated annuals, as indicated by findings of Seiter et al. (1999). This may also lead to improved drought resilience of the whole ecosystem, as fungal dominated soil food webs were found to adapt to a changing climate after experiencing drought stress (De Vries et al., 2012).

Phase 4: Management of established system

Depending on soil conditions and the farmers goals, annual crops can be rotated by season. Residues of harvested crops should remain to a large extent on-field as mulch for the new crop.

As the CMN may be sustained mostly by perennials plants and trees, new emerging seedlings can directly attach to the unharmed fungal networks in no-tilled soils and gather needed nutrients, water and information from the start, at almost no costs (Heijden, 2004; Walder et al., 2012). Moreover, as BioC can retain chemical signal molecules, root colonization can be improved (Akiyama et al., 2005).

Regular pruning of the perennial plants by cutting or holistic planned grazing with livestock enables increased nutrient cycling by the soil food web, as C exudates from remaining live roots and re-absorbable nutrients from dieback roots are enhanced. To reduce root competition in the shallow soil volumes between perennials and annuals, horizontal roots of perennials or trees should be cut regular in the establishment phase of the plants by cutting with appropriate machinery. Accordingly, higher microbial biomass and activity and consistently, higher phosphatase activity and finally, higher concentration of plant available nutrients were found in soils of pruned tea plants, in comparison to unpruned plants (Pramanik et al., 2017).

Soil life activity, soil nutrient and C levels, soil aggregation and soil water holding capacity increase over time. All in all, a permanent high soil fertility can be created. Healthy soil is built up, rich in available macro and micro nutrients, while plant growth can be further enhanced by regular application of biostimulants and compost tea.

4.7 Conclusion: Holistic approach with regenerative agriculture

The proposed holistic approach is a multi-layered, agricultural management system for the productive restoration of degraded land and water resources, primarily in tropical regions in developing countries.

By synergistic combination of regenerative agriculture management and soil amendment with locally produced BoneC and BioC, as well as compost, healthy, fertile soils, rich in stable SOM, can be created. By nurturing and protection of P solubilizing microorganisms - among others, AM fungi and PGPB - nutrient availability from BoneC fertilizer and fixed soil P may be tremendously improved, to comparable levels of TSP, needed for intensive, annual crop production. Hence, under this management regime, BoneC may become a suitable alternative for inorganic P fertilizer in regards of P supply to plants, especially in rural areas and for resource limited small-scale subsistence farmers. Regarding needed quantities, Simons et al. (2014) showed at the example of Ethiopia, that substantial fertilizer amounts, ranging from 28 to 58 % of annual national P fertilizer supplies, could be covered by thermal treated bones (measures by P content of conventional fertilizer and bones).

By this interacting combination of soil amendments of BoneC, BioC and microbial inoculum, plant selection and design, regenerative agriculture management, topped by usage of biostimulants, beneficial synergies may be created to maximize improvements of soil structure, fertility, health, resilience to stress and crop yields concurrently – at high profitability and short transition times.

There is strong indication, that by such an integrated system, crop yields in high quality and competing quantity at higher profitability and lower environmental pollution may be produced (De Vries et al., 2013; Pimentel et al., 2005; Rembiałkowska, 2007; Weigelt et al., 2009), especially in soils of low fertility (Setälä and Huhta, 1991) – while restoring degraded soils and creating local value from underutilized waste materials. Livelihoods in rural areas of the developing world could be strengthened, with potential positive effects on food and water security, the micro and macro climate, rural poverty and migration processes.

The enhanced formation of stable SOM levels of BioC amended soils bears potential for substantial C sequestration. Considering a SOM content of 1 % and a density of 1.45 kg*dm^{-3} of soil as well as the C content of SOM to average 50 % (Pribyl, 2010) with a C:N ratio of 10:1, 30 cm of the top layer of one hectare of field would contain around 21.75 t C and 2.175 t N. When the content of stable SOM can be increased as by the proposed system in this study, for each % increase in stable SOM content, large amounts of additional C can be sequestered in agricultural soils, increasing simultaneously soil fertility and hence soil production capacity. Alongside C, also large amounts of water can be stored (135 to 225 m^3 water*ha^{-1} for each percent of stable SOM contained), further strengthening the drought resistance of the holistic system (Schneider, 2019).

Fossil P resources, declining in quantity and quality, along with other production goods of wasteful, energy intensive fertilizer, pesticides and heavy machinery, do not need to be the sole base of modern agriculture, which often represents a burden for soils, ecosystems and farmers. With an adapted, holistic system, organic P from recycled waste may be a suitable low-cost alternative.

Instead of continuing soil degradation by conventional practice, a new agriculture system based on the productive recycling of organic P from waste streams and utilisation of fixed soil P in general, as proposed in this study, should be considered for implementation in suiting regions, aiming at productive restoration of the most important resource of humankind: Fertile soils.

5 Experiments

Pot experiments were conducted to examine effects of soil amendments (BoneC, BioC, CM) and biostimulatory plant additives (*Moringa* leaf extract, *Azolla* extract, GLY spray, SA spray) on growth of a local variety of pigeon pea (*Cajanus cajan*).

5.1 Introduction and objective of the study

P fertilizer are a necessity in today's agricultural practice to ensure high crop yields. However, due to a facing decline in quantity and quality of conventional P base materials and no common P recycling system from waste streams fully established, future supply (and distribution) of P for agricultural production in amounts needed to establish and secure food security for a growing global population is a major task in the decades to come.

A large fraction of future population growth will take place in rural areas of developing countries in Asia and Africa, whose inhabitants are predominantly depending on subsistence by low-input small-scale farming. Yet, in these areas with the most population pressure and hence the highest pressure to increase crop production, access to conventional modern agricultural production goods is already restricted by lack of finances and suitable infrastructure for transportation. Furthermore, traditional organic fertilizer materials like livestock manure often also are lacking in quantities sufficient for adequate plant P supply due to "off-site" livestock management by open grazing or pastoralism, breaking close nutrient cycles for "on-site" crop production. Availability of affordable fertilizer materials to boost crop production to levels needed for sustaining a growing number of people often is inadequate in rural areas – declining global P resources for conventional fertilizer production only would worsen this situation.

However, agricultural production needs to be intensified in its productivity per area cultivated in general, on a global scale, while further destruction of natural ecosystems (e.g. by deforestation) must be prevented. Thus, needed intensification of crop production in rural areas by simply enhancing area under conventional cultivation finds it limitations in the reasons mentioned above.

In this scenario, BoneC as an alternative, recycled P resource as discussed in chapter 3.1 may become of vital importance. As a so far untapped, locally-producible resource from waste streams of both, rural and urban areas, in almost any place in the world, it could serve as an alternative fertilizer material for enhancement of crop production, with secondary benefits in terms of job creation and poverty alleviation.

Also, BioC charged with human urine or co-composted, as discussed in chapter 3.3, could support availability of P fertilizer materials in rural areas, shortening P cycles and benefitting soil fertility in general, again enhancing crop yields.

Supporting these alternative soil amendments in the intensification of crop production, biostimulants may be used as discussed in chapter 3.5, especially in cases, where conventional production goods are too expensive, simply not available or are restricted from usage as in organic farming practice. As some biostimulants can be self-produced on-site, with only very basic equipment, and are only needed in minor quantities reducing infrastructure and investment costs, they may be considered as highly beneficial for boosting crop production and economic viability of operation by a very low cost-to-benefit ratio, making it affordable also for resource-limited farmers in rural areas.

The object of the study was to evaluate BoneC as an alternative fertilizer material containing P, embedded into the setting of small-scale agriculture operation, suitable for farmers in rural areas of developing countries. Further, economically and ecologically sound BioC and biostimulants should be investigated as additional support for plant growth promotion.

The working hypothesis was, that BoneC can represent an effective alternative to CM as the common agricultural fertilizer material used by small-scale subsistence farmers in Lake Chamo region, Ethiopia, by increasing biomass production of legumes in well managed, biological active soil, within the first season of application. Further, it was speculated, that a mixture of charged BioC, BoneC and CM can increase plant growth by its effects on soil fertility and nutrient load as well as beneficial impact on P solubilizing soil microorganisms, compared to single application of the fertilizer materials. Finally, it was hypothesized, that in amended soil, biostimulants of *Moringa* leaf extract, *Azolla* extract, glycerol spray and salicylic acid spray can be utilized to stimulate plant growth for enhanced biomass production.

Therefore, it is tested in this study, if BioC and BoneC produced from local resources by low-cost methods can enhance the biomass production of the legume plant Pigeon pea (local variety of *Cajanus cajan*) in comparison to CM and thus, could be considered as suitable soil amendments with fertilizer value, serving as beneficial additives or alternatives for CM. Further going, it was tested, if a mixture of the three fertilizer materials can increase plant growth to higher levels than application of single fertilizers. In a second experiment, treatments of the different biostimulants are compared to the untreated control group in their effect of plant growth stimulation in fertilized soil.

5.2 General methods

5.2.1 Description and preparation of experimental growth substrate

Standard growth substrate was obtained from the B horizon of a local soil, next to the experimental site at the compound of the Catholic Church Arba Minch, Ethiopia (6°00'08.4"N, 37°32'20.8"E). It was mixed thoroughly in the ratio of 4.5 to 1 (w/w) with substrate obtained from the litter-freed A horizon of a field in in the same compound under cultivation of perennial Pigeon pea (*Cajanus cajan*), serving as inoculation media for beneficial soil biology. Before experimental setup, the substrate was sieved through a 5 mm sieve for removal of stones and substrate homogenization.

BioC was obtained from pyrolysis of dried *Acacia* wood in a self-constructed slow-pyrolysis kiln as described by Diekow (2017). In the process, wood stalks were stacked densely in the kiln and lit in the top using dried grass and bark material. Pyrolysis process reached up to 580 °C and lasted around 80 minutes. Once the pyrolysis stopped (and the combustion of BioC started), BioC was removed from the stove, put in a hole in the ground and covered with soil for 48 hours. Afterwards, BioC was grinded to a maximum particle size of 1.5 mm. For activation, BioC was soaked in fresh human urine at a ratio of 1 to 1.2 (w/w) for 1 week. The slurry was sun-dried for 1 week after soaking and stored as a dry powder until start of the experiment.

BoneC was produced from local cow bones in a self-constructed slow pyrolysis kiln as described by Diekow (2017). In the process, unrendered, fresh bones were pyrolysed in the kiln with small amount of dried *Acacia* wood as a starter material over a period of 2 hours, reaching temperatures of up to 650 °C. Once the pyrolysis stopped (and the combustion of BoneC started), BoneC was removed from the kiln, put in a hole in the ground and covered with soil for 48 hours. The dried BoneC then was manually grinded to a maximum particle size of 1.5 mm and stored as a dry powder until start of the experiment.

Dry CM was collected from a local farmer and stored for 6 months prior to application. Before application, the manure was sieved to a particle size of 1.5 mm to homogenize the material and to remove any other particles.

The mixed substrate was classified by the Wondo Genet College of Forestry and Natural Resources Soil Laboratory, Hawassa University, Ethiopia, as clay soil (sand 26 %, silt 20 %, clay 54 %), with an organic fraction of 0.88 %, a pH of 7.1 and an electric conductivity of 83.7 $\mu S*cm^{-1}$ of dry substrate.

The central laboratory of Hamburg University of Technology, Germany, analysed total element content of the different soil substrates and soil amendments by aqua regia digestion with hydrofluoric acid and following ICP-OES or ICP-MS analysis. Total sample N was analysed with Kjeldahl procedure. Plant available P was further analysed with $NaHCO_3$ extraction according to Olsen et al. (1954) as described in Estefan et al. (2013) and determined photometrically or ICP-MS. Extractable micronutrient content was determined by NH_4HCO_3-DTPA analysis according to Soltanpour and Schwab (1977) as described in Estefan et al. (2013) with ICP-OES. Total C content of samples was determined by NCHS analyser. Results of soil substrates and soil amendments are presented in Table 6 and Table 7, respectively.

5.2.2 Preparation of plant pot compartments

2 litre water bottles were used as pot compartments. Bottles were cut 18 cm above ground. With a bottle diameter of 9 cm, plant pots had a total volume of app. 1.145 l. Any label was removed from the bottle, which was thoroughly cleaned. 5 holes (diameter 3 mm) were drilled in the very bottom of the bottle (4 at the sides, 1 in the bottom), serving as drainage and air ventilation opening.

For each pot, 1.1 kg of the mixed growing substrate and the respective soil amendments for each treatment were thoroughly mixed and filled in the pot singly to avoid heterogenic substrate composition at a bulk density of app. 1.12 $g*cm^{-3}$.

5.2.3 Experiment design and pot establishment

In a randomized complete block design, each of four blocks served as one true replicate, containing all the possible treatments as experimental units. Each experimental unit consisted of 12 pots for observation at the centre of the experimental unit, surrounded by 12 equally treated pots, serving as boundary plants without observation purpose. Pots were placed in a fenced experimental ground in the compound of the Catholic Church Arba Minch, Ethiopia (see Figure 17). Small trenches between the experimental units were established for drainage of precipitation water. Environmental conditions were equal over all the blocks.

After pot establishment, substrate was watered daily with 70 ml water per pot for 2 weeks prior to seeding. Emerging weeds were removed manually.

Figure 17: Experimental ground in compound of Catholic Church Arba Minch, Ethiopia. Wibbing (2018).

5.2.4 Establishment and cultivation of plants

Local seeds of Pigeon pea (*Cajanus cajan*) were purchased from Simeon Sittotawu Mandida PLC of pasture and forage seed supplying organization in Wollaita Soddo, Ethiopia. Seeds were soaked between wetted towels for 24 hours in the dark prior to planting. Three seeding holes at equal distance to one another with a depth of 3.5 cm were drilled in each pot. In each hole, one pre-germinated seed was placed and slightly covered with soil substrate. 10 DAS, emerged seedlings were reduced to one per pot by removal of the smaller ones, when seedlings started to grow a third leaf. After seeding, pots were irrigated daily with 70 ml of stored tap water each. Weeds were removed manually on a daily basis. All experiments were performed in late 2018 to early 2019, with 2 weeks incubation time after pot establishment, 10 days of germination time after sowing and 6 weeks of growth before harvest, with double application of biostimulants 14 and 28 DAS in case of the second experiment.

Daily noon temperature and daily precipitation were recorded with a MONSUN weather station (TFA Dostmann, Germany) during experiments. Average noon temperature during the first experiment (testing of soil amendments) was 29.41 °C. Total precipitation was 92 mm. In the second experiment (testing of biostimulants), average noon temperature was 30.91 °C and total precipitation was 7 mm.

Table 6: Elemental composition of soil substrates used in the experiments. * = Kjeldahl procedure; **a = Aqua regia digestion with ICP-OES; **b = Aqua regia digestion with ICP-MS; *** = $NaHCO_3$ with ICP-MS; ****= Ammonium Bicarbonate-DTPA with ICP-OES.

Sample	Total soil N * Kjeldahl-N	Total-P **a PO_4^{3-}	Olsen-P *** PO_4^{3-}	K **a	Ca **a	Mg **a	micronutrients **** Fe	Mn	Zn	Cu	Pollutants Cd **b	U **b	Total C C	pH (H_2O)
	g/Kg						mg/Kg				mg/Kg		g/Kg	25°C
Growth substrate	0.71	1.26	0.018	3.09	6.55	7.23	31.1	83.1	1.10	7.49	<1	<1	13.0	7.1
Amended substrate with BioC, BoneC and CM	0.97	6.05	0.034	4.02	8.92	8.12	49.40	26.50	1.79	7.15	<1	<1	18.0	7.4

Table 7: Elemental composition of soil amendments used in the experiments. * = Kjeldahl procedure; **a = Aqua regia digestion with ICP-OES; **b = Aqua regia digestion with ICP-MS; *** = NaHCO3 with photometer; ****= Ammonium Bicarbonate-DTPA with ICP-OES.

Sample	Total soil N * Kjeldahl-N	Total-P **a PO_4^{3-}	Olsen-P *** PO_4^{3-}	micronutrients **** Fe	Mn	Zn	Cu	Pollutants Cd **b	U **b	Total C C	ash content 815 °C, 48h	pH (H_2O)
	g/Kg			mg/Kg				mg/Kg		g/Kg	%	25°C
urine-charged Bio char	6.4	10.46	1.71	11.6	42.4	68.4	0.23	<1	<1	670	-	9.6
Bone char	3.0	461	2.28	28.4	7.6	60.9	0.527	<1	<1	38	92.27	10.2
Cow manure	8.7	8.14	1.04	51.5	143	30.3	6.04	<1	<1	110	-	7.3

5.3 Combined application of soil amendments for plant growth promotion

5.3.1 Introduction

BoneC is a novel P fertilizer from organic waste streams of animal slaughter, with additional fertilizing properties of Ca and Mg, as well as beneficial impact on general soil fertility by improving soil structure, water holding capacity, microbial habitat creation and accumulation of toxic heavy metals by absorption (Leinweber et al., 2019; Morshedizad and Leinweber, 2017). However, BoneC is not generally recommended as fertilizer in conventional production as its P solubility in general is low and cannot compete with conventional P fertilizer like TSP in regard of P supply to crops (Zimmer et al., 2019).

Nevertheless, when access or usage of conventional fertilizer products is restricted, as in the case in rural areas of southern Ethiopia or organic farming practice in general, utilization of BoneC as a locally-producible alternative fertilizer from unused waste streams could become viable. Therefore, it is tested, if BoneC can compete with CM as the common fertilizer practice in regard of fertilizer efficiency measured as increase in fresh biomass production and if additional usage of BioC in a soil additive mixture with CM can further boost biomass production, hence contributes to intensification of agricultural production in rural areas.

5.3.2 Materials and methods: preparation of substrate, planting units and statistics

During establishment of experimental pots, growing substrates were treated with all possible combination of biochar (BioC), bone char (BoneC) and cow manure (CM) (labels; 1 = control; 2 = BioC; 3 = BoneC; 4 = CM; 5 = BioC*BoneC; 6 = BioC*CM; 7 = BoneC*CM; 8 = BioC*BoneC*CM). Feasible application rates for each amendment material were determined in preliminary experiments (data not shown) and were not considered as bulk supplied materials per hectare, but as individual rates applied for each planting hole of intercropped Pigeon pea in a precise manner at a plant density of 2.47 per m^2, which was reported suitable by Meena et al. (2015). Concluding, BioC and CM were both applied at 7.8 g per pot (7.09 $g*kg^{-1}$ soil substrate) or 192 $kg*ha^{-1}$ each, while BoneC was applied at 4.7 per pot (4.27 $g*kg^{-1}$ soil substrate) or 116 $kg*ha^{-1}$.

Four replicates per treatment were used, with one replicate (experimental unit) in each block and each replicate containing 12 single observations. Total number of observation pots was 384, of which three failed to grow or sustain plants over the course of the experiment (one in each treatment of BioC, BioC*CM and BoneC*CM).

Explorative data analysis by histogram and normal plots showed approximate normality distribution of fresh mass residues. Assumption of normality of residues was supported by Shapiro-Wilk test ($p >$ 0.05) for all observation objects. Only the group of CM treatment violated the assumption of normality distribution slightly (Shapiro-Wilk test; $p = 0.02$). Variances were found to be homogenous between all groups (Levene test; $p > 0.05$). Visual box-plot analysis indicated outliners in group of BoneC (fresh mass = 0.63 g), in group of BioC*CM (fresh mass = 0.85 g) and in group of BioC*BoneC (fresh mass = 0.86 g and 6.42 g), but were found not significant for the result of the analysis. Due to only slight violation of assumptions as well as same group size and generally robust ANOVA procedure as well insignificant blocking factor, data were subjected to three-way between subject ANOVA without block interaction terms (Lüpsen, 2019).

5.3.3 Results

Data are mean ± standard error per plant, unless stated otherwise. All single treatments of BioC (2.45 ± 0.71 g; +26.3 %), BoneC (3.88 ± 1.29 g; +100 %) and CM (3.35 ± 1.06 g; +72.7 %) increased above ground fresh mass production of Pigeon pea plants in comparison to untreated controls (1.94 ± 0.65

g), but only the main effects of BoneC (*F* (1,1370) = 104.537; *p* < 0.001) and CM (*F* (1,370) = 44.064; *p* < 0.001) were statistically significant (see Table 8 and Table 9).

Although the highest fresh biomass yield per plant was achieved by application of the full soil amendment mixture of BioC*BoneC*CM (4.15 ± 1.18 g; +113.9 %), the increase over all other groups was not statistically significant (*F* (1,370) = 2.689; *p* = 0.102) (see Table 8 and Table 9). However, Tukey-test revealed, that the full mixture was statistically different from groups not containing BoneC, including CM treatment (see Figure 18).

Further analysis of interaction terms revealed, that only the two-way interaction term of BoneC and CM (BoneC*CM; *F* (1,370) = 17.456, *p* < 0.001) was statistically significant (see Table 9). Following pairwise comparison with Bonferroni adjusted *p* values revealed, that BoneC main effect on plant growth was statistically significant in presence of CM (+BoneC and +CM; *F* (1,370) = 18.23; *p* < 0.001; mean difference to +CM = 0.674 g/plant, 95 % CI, 0.364 to 0.984) as well in absence of CM (+BoneC and -CM; *F* (1,370) = 103.993; *p* < 0.001; mean difference to -CM = 1.605 g/plant, 95 % CI, 1.296 to 1.915). In contrast, the simple main effect of CM is only statistically significant, when BoneC is not applied simultaneously (+CM and -BC; *F* (1,370) = 58.338; *p* < 0.001; mean difference to -BC = 1.206, 95 % CI, 0.895 to 1.516) (see Table 10).

Table 8: Fresh biomass of Pigeon pea plants (g) by application of soil amendments bio char, bone char and cow manure. Shown are the mean values ± SD (and the label) for all treatments of ± BioC, ± BoneC and ± CM. Different letters following means indicate significantly different means due to treatment effect according to multiple comparison Tukey-test (p < 0.05).

Bio char treatment	Cow manure treatment	**Fresh biomass (g/plant)** Bone char treatmtent	
		-BoneC	+BoneC
-BioC	-CM	1.94 a ± *0.65* (control)	3.88 bc ± *1.29* (BoneC)
	+CM	3.35 b ± *1.06* (CM)	3.99 bc ± *1.13* (BoneC*CM)
+BioC	-CM	2.45 a ± *0.71* (BioC)	3.72 bc ± *1.27* (BioC*BoneC)
	+CM	3.45 b ± *1.25* (BioC*CM)	4.15 c ± *1.18* (BioC*BoneC*CM)

Table 9: Results of three-way between-subjects ANOVA without block interaction terms for treatment effect of different soil amendments (BioC, BoneC and CM) on fresh mass production of Pigeon pea plants. A significant effect of the treatments is indicated by highlighting.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Observed Power[b]
Corrected Model	211,392[a]	10	21.139	17.869	.000	1.000
Intercept	4318.205	1	4318.205	3650.232	.000	1.000
BioC	2.213	1	2.213	1.870	.172	.276
BoneC	123.667	1	123.667	104.537	**.000**	1.000
CM	52.127	1	52.127	44.064	**.000**	1.000
BioC * BoneC	2.243	1	2.243	1.896	.169	.279
BioC * CM	.048	1	.048	.040	.841	.055
BoneC * CM	20.651	1	20.651	17.456	**.000**	.986
BioC * BoneC * CM	3.181	1	3.181	2.689	.102	.373
block	6.836	3	2.279	1.926	.125	.497
Error	437.708	370	1.183			
Total	4970.486	381				
Corrected Total	649.100	380				

a. R Squared = .326 (Adjusted R Squared = .307)

b. Computed using alpha = 0.05

c. Coefficient of variability cv = 0.079

d. Standard error of the means s = 0.067

Table 10: F-Test and Pairwise comparison for BoneC*CM interaction effect on fresh mass production of Pigeon pea plants. A significant effect of the treatments is indicated by highlighting.

Simple effect of CM on ± BoneC		Sum of Squares	df	Mean Square	F	Sig.	Mean Difference	Std. Error	95% Confidence Interval for Difference[b]	
									Lower Bound	Upper Bound
- BoneC	Contrast	69.014	1	69.014	58.338	**.000**	1,206*	.158	.895	1.516
	Error	437.708	370	1.183						
+BoneC	Contrast	3.589	1	3.589	3.034	.082	.274	.157	-.035	.584
	Error	437.708	370	1.183						
Simple effect of BoneC on ± CM										
-CM	Contrast	123.023	1	123.023	103.993	**.000**	1,605*	.157	1.296	1.915
	Error	437.708	370	1.183						
+CM	Contrast	21.566	1	21.566	18.230	**.000**	,674*	.158	.364	.984
	Error	437.708	370	1.183						

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

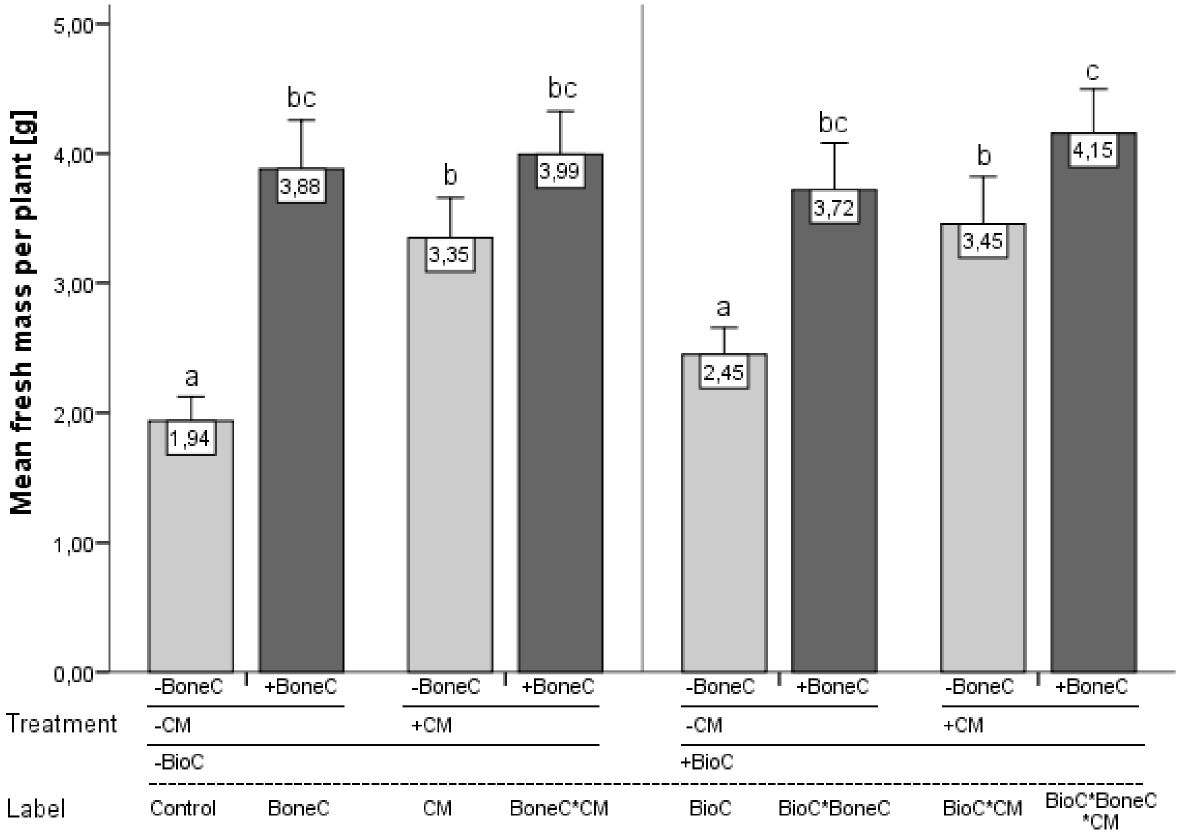

Figure 18: Mean fresh mass per Pigeon pea plants (g) treated by application of different soil amendments of bio char, bone char and cow manure. Plants were fertilized with bone char or no bone char (± BoneC), cow manure or no cow manure (± CM) and bio char or no biochar (± BC). Different letters above bars indicate significantly different means due to treatment effect according to multiple comparison Tukey-test ($p < 0.05$). Error bares: 95 % CI.

5.3.4 Discussion and conclusion

The results indicate, that under the experimental soil and environmental conditions, growth of leguminous Pigeon pea plants can be enhanced by the tested soil amendments of BioC, BoneC and CM. While the effects of single application of BoneC and CM showed drastic enhancement of +100 % and +72.7 % fresh biomass per plant in comparison to the untreated control, the effect of BioC only was minor (+26.3 %) and statistically not significant, although the application of charged BioC added slightly more plant available Olsen-P to the growing substrate as BoneC or CM treatment (+4.34, + 3.49 and +2.64 mg Olsen-P per pot for BioC, BoneC and CM application rates with 6.46 mg Olsen-P per pot contained in the original growth substrate). Content of total-N per pot was also similar for the treatments BioC, BoneC and CM (830.9, 795.1 and 848.9 mg per pot), and only slightly larger than in the control (781 mg total-N per pot).

As the experiment duration was only over an 8 weeks period, it cannot be ruled out, that the full effect of BioC due to soil structuring and advanced enhancement of soil biology adding to improved nutrient supply could become significant only over time (Kammann et al., 2015). Therefore, BioC may be considered as a companion soil amendment, along with amendments of higher effect on plant growth for intensified plant production within the season of application.

It also was shown here, that BoneC may be used as an alternative fertilizer for CM, ranging the same levels of biomass production in the experimental conditions. This may become feasible, when farmers do not have access to adequate quantities of CM for their operation, but can utilize slaughterhouse waste often available in rural areas at no costs as a valuable low-cost resource.

Pairwise comparison of significant BoneC*CM interaction effect revealed, that fertilizing practice of CM application can be significantly improved by additional application of BoneC as the dominating factor, as biomass production of Pigeon pea was increased by +19.1 % in BoneC*CM treatments in comparison to single CM treatment, but only by +2.8 % in comparison to single BoneC treatment. Here the general high Ca content of BoneC may enabled higher biomass production, which could indicate Ca deficiency of the soil substrate in the experiment. Accordingly, BoneC could also serve as a Ca fertilizer in rural areas, where access to lime or gypsum is limited. However, if quantity of CM is not sufficient in agricultural operation in rural areas, BoneC should rather be used in unfertilized soils rather than added in soils fertilized with CM to extend the total agricultural area under fertilization. If CM is plentiful available, both materials could be combined for displayed yield enhancement.

By utilization of BoneC as an additional fertilizer material, the usage of P remains of livestock can be substantially increased. While the large fraction of total P in the lifecycle of cattle remains in the manure, around 18.6 % of total P is contained in the bones (2-year-old cattle), depending on the age at slaughtering (calculation see Appendix A). Considering a significant fraction of the manure is lost due to off-site grazing or leaching of the liquid phase due to lacking manure management, the ratio of recoverable P from cattle remains may shift strongly in favour of bones. By usage of BoneC, the amount of available fertilizer material based on their P content could be increased by considerably more than +20 % in rural areas, considering BoneC and CM alone.

The highest fresh biomass yield of all treatments was derived by the full combination of BioC, BoneC and CM. However, while the results were not statistically significant in comparison to other treatments also containing BoneC, statistically significant differences of means were found for plants not treated with BoneC, including treatment only with CM, in which comparison the production of fresh biomass was enhanced by +23.8 %. As CM application is considered here as the common agricultural fertilizer method for improvement of yields in small-scale subsistence farming in rural areas, the additional (single time) amendment of soils with BioC and BoneC along with CM could become feasible when increasing the production per area is the top priority and sufficient amounts of amendment material are available.

Further, the secondary effects of BoneC and BioC in regards of improved soil structuring, stimulated soil biology, enhancement of SOM content and thus increased nutrient and C cycling, water storage capacity and P solubilization as well as biological N_2 fixation, may become fully active only over time, past the first season of application, as findings of Cabeza et al. (2011) indicate by increased second-year P uptake from recycled P fertilizer material. Hence, the full potential of yield increasing prospects of BoneC in combination with BioC and CM may not be fully displayed here.

Therefore, although already useful as a fertilizer in the first season of application, the true value of BoneC, as a BioC from animal bones, may be regarded as the mid- to long-term P and Ca supply for plants and the enhancement of stable SOM formation in treated soils, which was not examined here, but should be considered in future multi-season trials. Further, to verify the nutrient recovery efficiency of applied BoneC under field conditions of the holistic approach, application of BoneC should be tested in restored soils, with fully established root and mycorrhizal systems. In field conditions the effect of P supply to plants may be further strengthened as root growth is not restricted as in this pot experiment and a more diverse soil biology with AM fungi and beneficial soil bacteria may be formed.

Despite the significant growth effect of BoneC compared to the control, the quantity of total and plant available P contained in applied BoneC at rates used in this experiment in relation to the total soil P reserves is only minor (17.44 kg total-P*ha^{-1} and 0.085 kg Olsen-P*ha^{-1} in applied BoneC; 1.355 kg total-P*ha^{-1} and 19.37 kg Olsen-P*ha^{-1} at 30 cm soil depth and 1.1 kg*dm^{-3} as soil P reserves, see Table 6 and Table 7). Hence, to secure substantial P supply in agricultural production over the long-term, soil P reserves need to be utilized efficiently. Here, BoneC actually may serve as an alternative fertilizer material to kickstart plant growth in degraded soils with limited resources. Applied BoneC could stimulate growth of perennial plants, which then again could establish and nurture a rich soil food web including AM fungi and P solubilizing bacteria over time, through enhanced exudation of C sources. The capacity to utilize P and other soil nutrients from soil reserves would be gradually increased, also with increasing stable SOM content of amended soils, while P contained in crop residues or animal waste products is recycled on-site. Ideally, the bulk fraction of P supplied to plants would be gradually shifted from external fertilizer materials to internal soil resources, when the system is appropriately established and managed according to principles discussed in this work. However, future research should evaluate the effects of BoneC and BioC soil amendment and elevated stable SOM formation on availability of fixed soil P reserves over multiple seasons under field conditions.

Concluding, with BoneC application as the significant main effect on enhanced biomass production of Pigeon pea in this experiment, BoneC may serve as a feasible, alternative fertilizer material to CM in organic agriculture operation, especially in places where access to other P and Ca containing fertilizer materials is restricted or available quantities are low. This safe, non-polluted fertilizer material could substantially contribute to the intensification of agricultural production in regions lacking access to fertilizer materials. However, for high efficiency of P recovery from BoneC, an adapted agricultural system with specific, P solubilizing, mycotrophic plants should be considered.

5.4 Single application of biostimulants for plant growth promotion

5.4.1 Introduction

In recent years, bio-chemical substances with stimulatory effect on plant growth came into focus of researchers and practitioners. Usage of biostimulants is an emerging method with possible drastic impact on the intensification of agricultural crop production. Quantities of biostimulants needed to achieve significant impact on crop production are in general rather in the micro- than in the macro-scale. This reduces infrastructure limitations of rural areas to a minor issue, while low financial costs as well as needed rudimentary equipment may be feasible also for small-scale farmers. Adding to that, some biostimulants can be produced on-site, from specially cultivated plants, integrated in the farm layout.

In order to strengthen the proposed agricultural system of this study in regards of economic feasibility and high productivity of operation within the first season of implementation, it is tested, if chosen biostimulants of *Moringa* leaf extract, *Azolla* extract, glycerol spray and salicylic acid spray can significantly increase the fresh biomass production of Pigeon pea plants, hence can contribute to the intensification of agricultural production in rural areas.

5.4.2 Materials and methods: preparation of substrate, planting units, biostimulants and statistics

In preliminary experiments, it was found, that chosen biostimulants did not perform well in unfertilized substrate (data not shown). Therefore, during establishment of experimental pots, growing substrates were treated with the combined application of BioC, BoneC and CM as in the first experiment. Accordingly, the growth substrate of each pot was amended with 7.8 g BioC and CM each and 4.7 g BoneC.

Biostimulants were prepared as follows: *Moringa* leaf extract was freshly prepared directly before application. 200 g of fresh leaves of well-watered *Moringa oleifera* trees grown in the compound of Catholic Church, Arba Minch, Ethiopia, were harvested and grounded in a mortar with 100 ml bottled water. The paste then was blended by a mixer with additional 900 ml of bottled water. The extract then was filtered by a textile. This extract was considered as *Moringa* leaf extract concentration 3, with 9 % liquid *Moringa* concentrate (M3). For the second concentration with 6 % liquid *Moringa* leaf concentrate, 300 g of concentration 3 was diluted with 150 ml bottled water (M2). For concentration 1 with 3 % liquid *Moringa* leaf concentrate, 300 g of concentration 3 was diluted with 600 ml of bottled water (M1).

The same procedure was used to prepare *Azolla* extract, directly prior to application. Wet *Azolla* was harvested and drained by gravity in a sieve for 2 minutes. 200 g of Azolla was mortared with 100 ml of bottled water. The paste was then blended adding 900 ml bottled water. The received solution was filtered by a textile and considered as concentration 3 with 9 % liquid *Azolla* concentrate (A3). Equal to *Moringa* leaf extract, the *Azolla* extract concentrations 2 and 1 containing 6 % and 3 % liquid *Azolla* concentrate were prepared by dilution of concentration 3 with 300 g (A2) and 600 ml (A1) of bottled water, respectively.

For glycerol spray, 2.5, 5 and 10 g glycerol (99.7 %, Naissance, UK) was mixed with 1 l of bottled water, receiving glycerol solutions with concentration 1 = 2.5 $g*l^{-1}$ (w/v) (G1), concentration 2 = 5 $g*l^{-1}$ (w/v) (G2) and concentration 3 = 10 $g*l^{-1}$ (w/v) (G3).

SA spray was prepared in three concentrations from dissolving 1.38 g SA (99.6 %, BiOrigins, UK) in 1 l heated, bottled water (80 °C). This solution with a concentration of 10^{-2} $M*l^{-1}$ (w/v) was considered as concentration 3 (S3). 100 ml of this solution was further diluted with 900 ml of heated, bottled water for concentration 2 with 10^{-3} $M*l^{-1}$ (w/v) (S2). Concentration 1 was derived from mixing 10 ml of concentration 3 with 990 ml heated, bottled water to receive a solution with 10^{-4} $M*l^{-1}$ (w/v) SA (S1).

To summarize, four different biostimulants were tested, each in 3 different concentrations: *Moringa* leaf extract (M1, 3 %; M2, 6 %; M3, 9 %), *Azolla* extract (A1, 3 %; A2, 6 %; A3, 9 %), glycerol spray (G1, 2.5 $g*l^{-1}$; G2, 5.0 $g*l^{-1}$; G3, 10.0 $g*l^{-1}$) and SA spray (S1, 10^{-4} $M*l^{-1}$; S2, 10^{-3} $M*l^{-1}$; S3, 10^{-2} $M*l^{-1}$).

For application, 60 ml of the respective solution was sprayed uniformly with a hand-pressurized, 1-litre spray bottle (Würth, Germany) to the receiving experimental unit, with each plant receiving around 2.5 ml of solution. Foliage spraying was conducted only at sunset, starting around 6 p.m. local time.

During the experiment, plants were treated twice with the respective biostimulant by foliage spraying, in two weeks interval, starting 14 days after the removal of excess seedlings from the pot (at 24 DAS) and again at 38 DAS. Two weeks later (52 DAS) the plants were harvested and examined.

Four replicates per treatment were used, with each replicate (experimental unit) containing 12 single observations. Total number of observation pots was 624, of which three failed to grow or sustain plants (one in each treatment of M1, G1 and A2).

Explorative data analysis by histogram and normal plots as well as Shapiro-Wilk test ($p < 0.001$) showed violation of normality distribution by fresh mass residues. Variances were found to be equal between all groups (Levene test; $p > 0.05$). Visual box-plot analysis indicated no outliners. Considering the central limit theorem of approximate normality distribution for high number of test objects, almost same group size and generally robust ANOVA procedure as well insignificant blocking factor, data were subjected to one-way between subject ANOVA without block interaction terms (Lüpsen, 2019).

5.4.3 Results

Data are mean ± standard error, unless stated otherwise. The statistical analysis revealed no significant treatment effect of any of the biostimulants tested (F (12,605) = 0.599; p = 0.843; table not shown). The fresh biomass production of the untreated control (4.47 ± 1.80 g/plant) did not differ significantly from any other treatment, neither the lowest yield of A3 (4.34 ± 2.00) nor the highest of G3 (4.78 ± 2.01) (see Table 11 and Figure 19).

Table 11: Fresh biomass of Pigeon pea plants (g) by single application of biostimulants *Moringa* leaf extract (M), *Azolla* extract (A), Glycerol spray (G) or Salicylic acid spray (S). Shown are the mean values ± SD for all treatments, including control (Con).

Level of concentration	Fresh biomass (g/plant) Treatment Con	M	A	G	S
1	4.47 ± *1.80*	4.38 ± *1.74*	4.31 ± *1.90*	4.14 ± *1.89*	4.48 ± *1.70*
2	-	4.60 ± *1.84*	4.23 ± *1.84*	4.72 ± *1.96*	4.51 ± *1.86*
3	-	4.34 ± *1.89*	4.06 ± *2.00*	4.78 ± *2.01*	4.44 ± *2.06*

a. Coefficient of variability cv = 0.0386

b. Standard error of the means s = 0.075

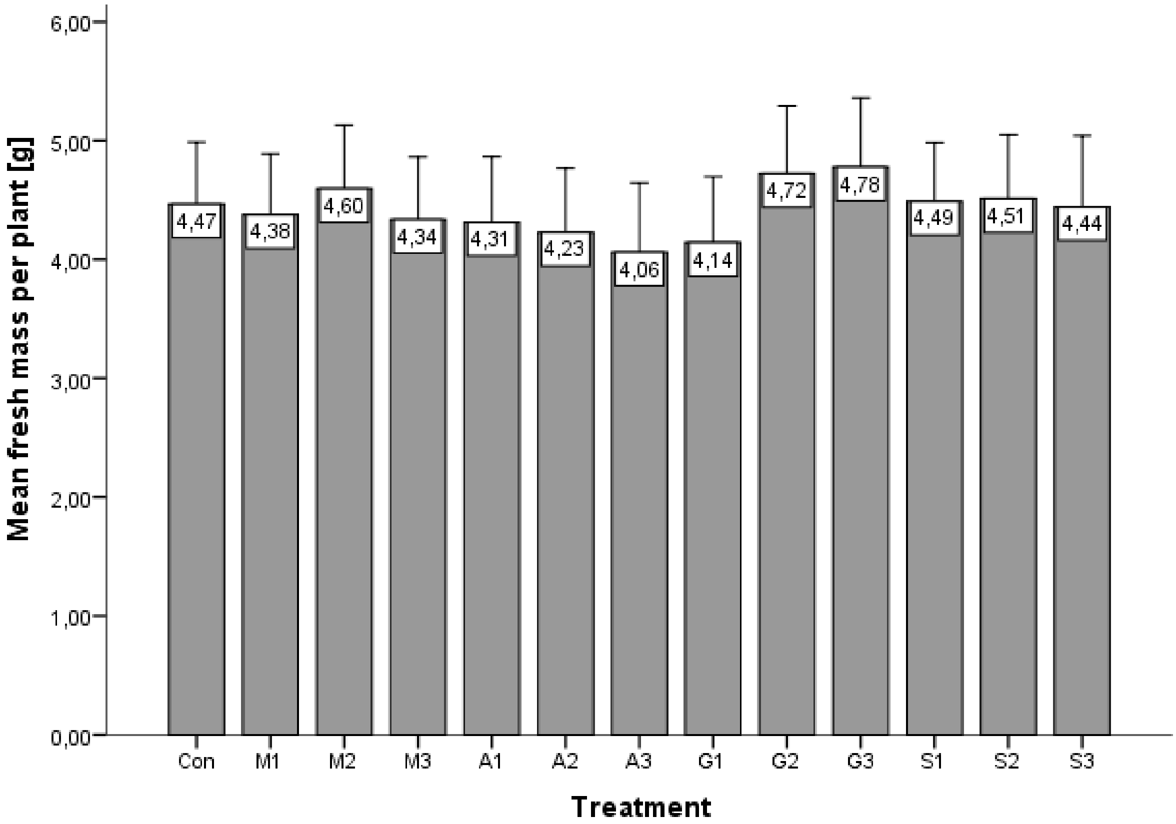

Figure 19: Mean fresh mass per Pigeon pea plants [g] by application of plant additives of either *Moringa* Leaf Extract (M), *Azolla* extract (A), Glycerol spray (G) or Salicylic Acid spray (S), each in three levels of concentration (1, 2, 3), or no additive application but water spray serving as control (Con). All plants were fertilized wit bone char, bio char and cow manure in experimental setup stage. Error bares: 95 % CI.

5.4.4 Discussion and conclusion

The results indicate, that under the experimental conditions, biostimulants did not show any statistically significant effect on plant growth of Pigeon pea, in contrast to findings in literature and results from pre-experimental trials (data not shown). This may be due to non-optimal growing conditions of heat stress during the experiment (average noon temperature 30.91 °C, no shading) or lack of availability of certain essential micro nutrients, especially needed to facilitate effects of improved plant physiology. Also, properties of biostimulants may be chosen not optimal in preparation method, concentration and application rate, at least for the selected test plant Pigeon pea. Plants grew reasonably well in comparison to equally amended plants of the combined application of BioC, BoneC and CM in the soil amendment experiment, with a total mean fresh mass per plant of 4.42 g between all groups. Therefore, it is considered here, that although the fertilization of plants was successful in the biostimulant experiment, the environmental stress for plants in early growing stages was too high in order to utilize any potential effect of the administered biostimulants. Further, usage of biostimulants may be susceptible for non-optimal growing conditions of plants and may be facilitated by fully developed root growth and improved soil formation to actual bear capacities for elevated uptake of macro and micro nutrition and water, which likely was not the case in this short-duration experiment. Also, fully established mycorrhizal colonization of treated plants could facilitate effects of biostimulants, as several authors showed synergistic effects of both (Abdallah et al., 2017; Garg and Bharti, 2018; Gryndler et al., 2005; Rouphael et al., 2017; Zhang et al., 2018). Surely, future research under greenhouse and field conditions should properly evaluate the potential of biostimulants in crop production in the proposed agricultural system. The performance of biostimulants under various soil conditions and levels of abiotic stress should be tested, both before and during encountering.

5.5 Conclusion: Experiments

BoneC can be an effective alternative fertilizer material, when compared to CM as the common standard fertilizer in small-scale subsistence farming in rural areas. P use efficiency of livestock remains can be substantial increase, leading to increased rates of available fertilizer quantities based on their P content, from recycled waste materials. Agricultural production could be enhanced.

BioC did not improve plant growth significantly, at least over the short experiment period. However, to utilize effects described in literature, BioC may become fully active only over multiple seasons as the soil gradually improves in structure and stable SOM content.

Under heat stressed experiment conditions, biostimulants did not show positive or negative effects on plant growth. Described effects of enhanced resistance to abiotic stress like drought could not be observed here. However, biostimulants may be able to elicit described effects but in a priming function, not relief function, as effects are based on priming effects of enhanced root growth and strengthened immune system of the plant, prior to encountering the stress. Biostimulants may be more sufficient for successful application as elicitors of priming effects and plant growth stimulation in already partly or fully established agricultural systems, with improved soils rich in stable SOM, able to supply high quantities of water and nutrients needed in the short-term.

6 Case study: Watershed Lake Chamo, Arba Minch, Ethiopia

6.1 Analyse of current situation

Ethiopia is a landlocked country in Eastern Africa, with the Rift Valley at its centre, stretching from northern Afar region to southern Ethiopia, diverting the Ethiopian highlands into eastern and western plateaus by tectonic and volcanic activity. The actual rift is 40 to 60 km in width, creating a valley, in which most of Ethiopian lakes are located. Located in the Gamo Gofa Zone of the Southern Nations, Nationalities and People's Regional State (SNNPRS) and partly Oromia Regional State, Lake Abaya and Lake Chamo are the biggest lakes of the Rift Valley and source of important ecosystem services, such as water renewal, climate stabilization and food and energy production (see Figure 20).

Figure 20: View above the two lakes Abaya (left) and Chamo (right) in the Rift Valley of Ethiopia. Hisch (2019).

Ecological status of Lake Abaya and Lake Chamo were severely impaired in the last decades due to high sediment loads and nutrient entry from soil erosion processes, caused by deforestation and non-adapted agricultural practices in the mountain areas of the corresponding watersheds with high slope gradients, as well as increased usage of inorganic fertilizer materials in agricultural production of surrounding farm land (Eshetu, 2018; Utaile and Sulaiman, 2016).

The ecosystem of Lake Abaya already is considered as irreversibly damaged, as water quality was diminished in general over the last decades, while in specific, water turbidity through suspended clay particles is highly elevated, deteriorating the proliferation of phytoplankton by hindered photosynthesis as the start of the aquatic food chain (Eshetu, 2018).

With current rates of sediment loads entering Lake Chamo, a similar fate as Lake Abaya is expected in the near future, with shrinking water surface area due to sedimentation, a broken food chain reducing amount and diversity of fish and other wildlife, disruption of the regional water cycles and reduction of climate stabilization effects (Eshetu, 2018).

A collapse of the local water cycles due to ongoing siltation and hence turning water surfaces into land area reducing evaporation from Lake Abaya and Chamo would lead to a drastic change of the regional climate, disfavouring agricultural production and hence, human settlement.

With Gamo Gofa zone as the major dessert banana production centre for the national market of Ethiopia, not only the livelihoods of local people in Lake Chamo watershed are at stake, but also the national supply of the most important fruit, almost considerable as a staple food in the diet of Ethiopians, further diminishing fragile food security of the country (Ambisa et al., 2019).

6.2 Baseline data

Lake Chamo covers a total area of 295.36 km² with a rather small total watershed area of 1199.87 km², mainly located western of the lake, with slopes between 0 and 5 % in the wetlands near the lake, while mountain areas can have very steep slopes above 35 % (see Figure 21 and Figure 22). Mean annual precipitation of the watershed was recorded at 1133 mm, with highest values of 2006 mm in the mountain areas and lowest values of below 600 mm in the plain wetlands near the lake. Average mean temperature in the region is recoded at 21.9 °Celsius. Typical soil types in the area are nitisol, acrisol, luvisol and vertisol, which partly show high susceptibility to erosion due to low tree cover and only sparse vegetation, especially on sloping farmland in mountain areas (see Figure 23). Accordingly, delta areas are increasing in size, turning water surface area into land area (see Figure 22) (Eshetu, 2018).

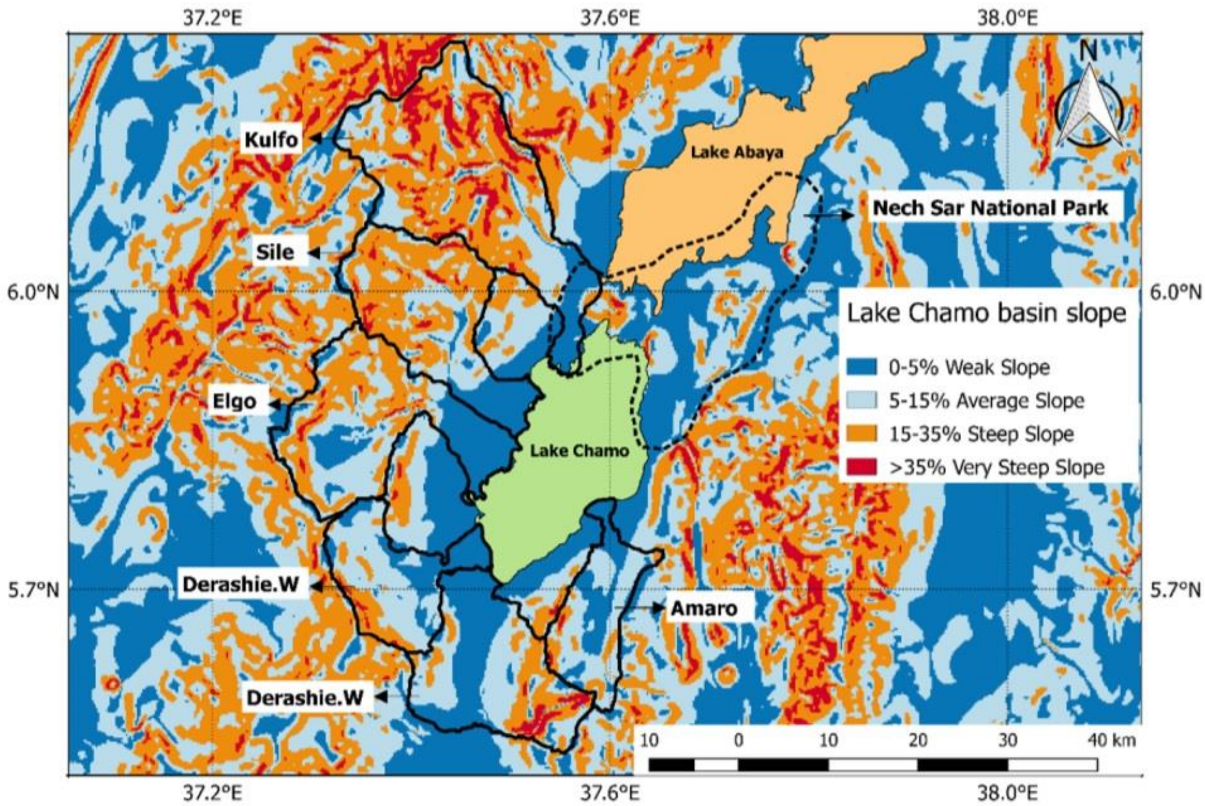

Figure 21: Slopes of Lake Chamo basin, from Eshetu (2018), p. 26.

6.3 Proposed system for commercial banana growers in Lake Chamo watershed

Banana cultivation in Lake Chamo watershed area in Arba Minch Zuria is mainly based on monocropping banana plants in dense manner (3 x 3 m distance between plants), in fields close to the shoreline of the lake, mostly within the wetlands of Elgo and Sile water catchment west of Lake Chamo. Surface runoff and drainage water from the mountain areas is diverted regularly through soil channels

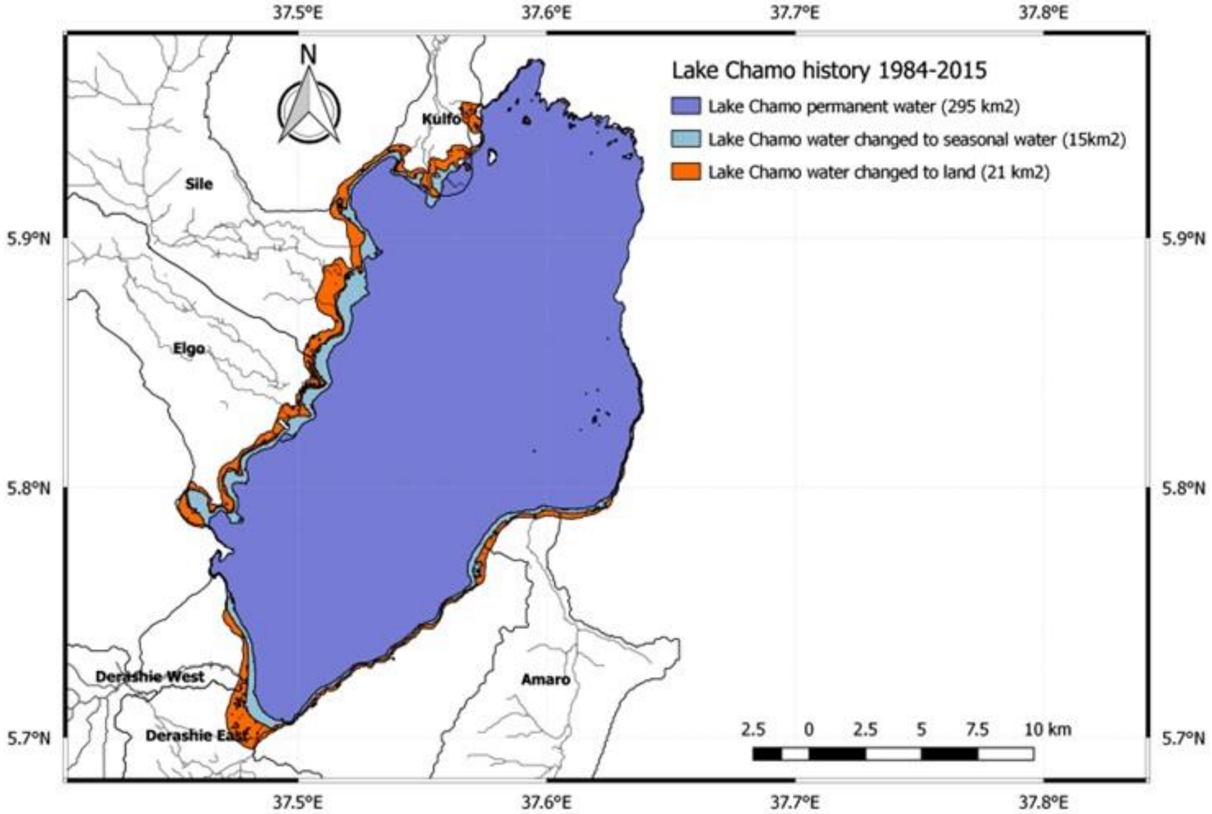

Figure 22: The water history of Lake Chamo from 1984-2015, Eshetu (2018), p. 32.

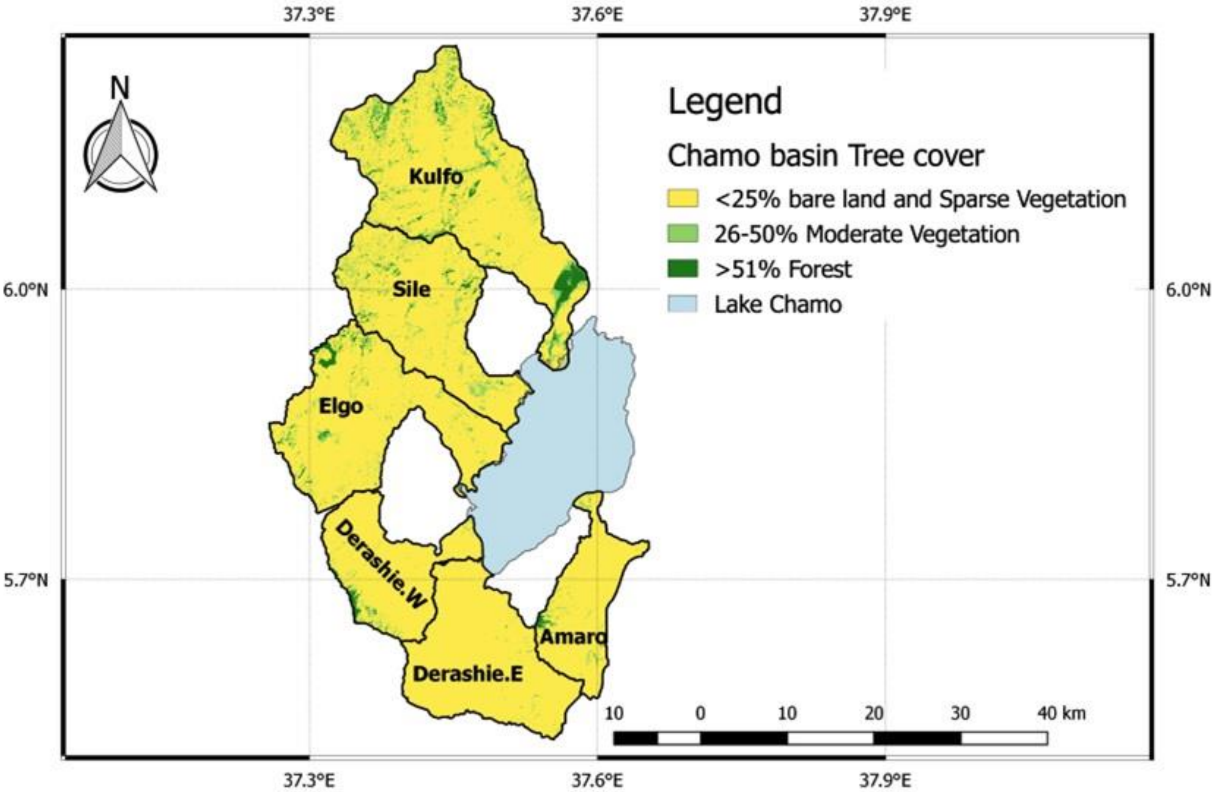

Figure 23: Vegetation cover map of Lake Chamo basin, Eshetu (2018), p. 31.

for irrigation purpose. Lake water is not utilized for irrigation purpose at it shows high pH and high electric conductivity, despite being rich in nutrients (Utaile and Sulaiman, 2016). Usage of conventional fertilizer is rather uncommon, while organic fertilizers are lacking in quantity and quality (personal communication with Endrias Alto, Arba Minch Town Municipality).

Precipitation in the wetlands close to the lake is only around 600 mm/a, so access to water is majorly dependent on discharge from the mountain areas (Eshetu, 2018). However, as water retention, infiltration and groundwater recharge are declining in those areas, runoff water carrying high loads of sediments is only available passing through the wetlands for short periods directly after rain events and may cause damage to the plantations by flash floods, which all in conclusion, may drastically reduce yield potential in affected banana farms (personal communication with Eliyas Guffiso, local farmer).

6.3.1 Soil amendments for commercial banana farms

BioC from woody waste materials (from farm residues or utility plants) and BoneC from animal bones from the slaughterhouses of the nearby cities should be produced and utilized to improve soil structure and fertility of the banana farms. As soil amendments are of organic origin, banana farmers can improve soil nutrient status (especially of P) and soil life, without detrimental effects of the environment (e.g. transport of water-soluble fertilizer materials into the corresponding water body of Lake Chamo). Farmers can shift to organic production, which could net them higher market prices for organic products.

Those soil amendments, offering additional soil habitats, can improve the diversity and quantity of soil life, especially of fungi and bacteria (Jin, 2010; Lehmann et al., 2011; Steiner et al., 2008a, 2007a). To assure reestablishment of beneficial, natural soil biology in formerly mono-cropped banana stands, receiving soils should be additionally inoculated with infectious material of PGPF and PGPB by soil amendment with natural forest soil from areas of the close vicinity.

AM fungi can improve resistance to attack of pathogenic fungi or nematodes, while potential plant damage can be lessened by enhancing plant nutrient and water status, antibiotic production and occupation of suiting infection sites of root surfaces (Anene and Declerck, 2016; Koffi et al., 2013). Also, as soils close to the shoreline of Lake Chamo were shown to be slightly saline, AM fungi may also contribute to coping of banana plants with abiotic stress of salinity and drought (Aroca et al., 2007; Evelin et al., 2012). P from BoneC can be efficiently utilized by AM fungi and cooperative solubilizing bacteria and improve P status of banana, which again might increase yield levels.

Also, specific fungi and bacteria may be cultured and added in the inoculate, such as *Pseudomonas fluorescens*, to further strengthen resistance and repellence capacity of banana plants against detrimental nematodes like *Radopholus similis* (Aalten et al., 1998; Saravanan et al., 2004), which would be especially effective in symbiosis with AM fungi (Siasou et al., 2009).

6.3.2 Plant system for commercial banana farms

General soil nutrient status should be improved by intercropping of banana with leguminous companion plants, which also may contribute to the prevention or repellence of detrimental pest and pathogens. As the burrowing nematode is a major pest for banana plants, non-host plant species should be included in the cropping of banana (Chitamba et al., 2014). Cowpea (*Vigna unguiculata*) and sunn hemp (*Crotalaria juncea)* were found to be efficient in reducing the activity and quantity of *Radopholus similis* population, when intercropped with banana (Chitamba et al., 2014), especially when also AM fungi were nurtured in the same growing substrate (Anene and Declerck, 2016). However, as *Crotalaria* spp. is shade-sensitive, an adapted agroforestry with *Sesbania* spp. and

Moringa stenopetala and *oleifera* with *Crotalaria* spp. in the shade-free interspace should be integrated in the banana farm layout, in alternating rows of east-west direction. Here, *Sesbania* spp. and *Moringa* spp. are cultivated for the production of green manure and pruned regularly to prevent dense canopy forming. BioC can be produced over the mid- to long-term from the produced woody biomass.

As *Crotalaria juncea* is not cultivated in direct vicinity to bananas due to sensitivity to shade, the plants should be regularly cut and used as green manure for banana. The allelochemical alkaloid monocrotaline-pyrrolizidine of *Crotalaria* spp., mainly responsible for suppression of nematode proliferation and weed germination (Anene and Declerck, 2016; Thoden et al., 2009), was found to remain active 16 days after harvest in leaves (Skinner et al., 2012). Hence, by using *Crotalaria* spp. cuttings as green manure, its anti-nematode effects could be utilized without direct intercropping, but this hypothesis should be tested in future research. However, germination of seeds and growth of plants co-cultivated with *Crotalaria* spp. was found to be decreased, so further studies are needed to evaluate functioning of the proposed system.

As a direct intercropped plant, *Desmodium* spp. may be suitable for planting next to banana, as the leguminous plant is moderately tolerant to shade. It may also support the resistance to biotic stress as a highly mycotrophic plant, utilized as suppressor of *Striga* weed and pests other than *Radopholus similis* (Pickett et al., 2014).

The floating plant *Azolla* spp. should be cultivated in the irrigation channels to produce N rich green manure or forage for poultry. As side effects, *Azolla* can reduce evaporation and reduce mosquito breeding in open water bodies, while the water channels can serve as a natural boundary for a holistic planned grazing system (Bao-lin, 1988; Diara and Van Hove, 1984; Mwingira et al., 2009) (see Figure 24).

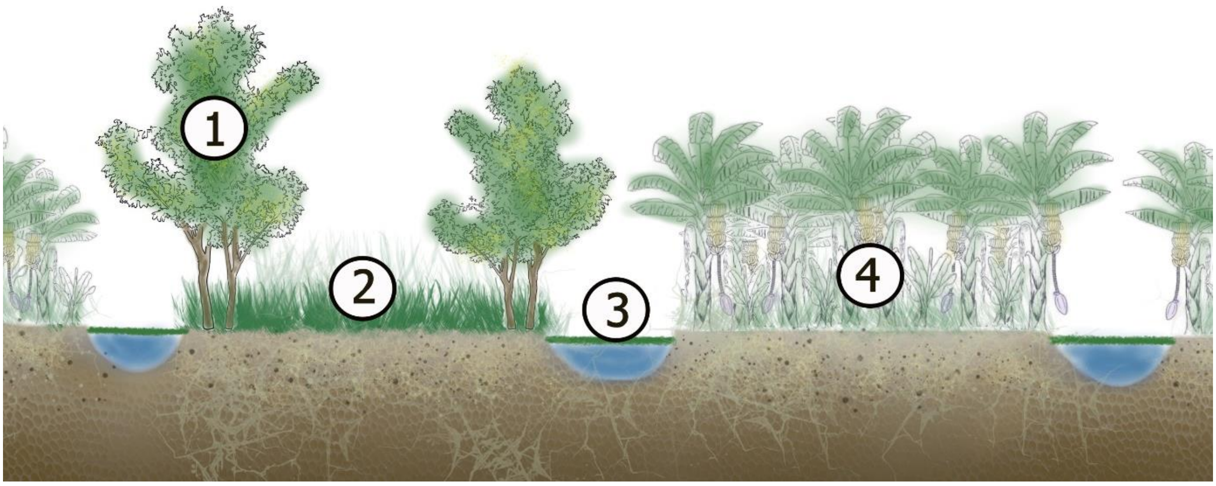

Figure 24: Schematic depiction of planting system for commercial banana cultivation in Lake Chamo watershed. *Sesbania sesban* and *Moringa* spp. trees rows (1) are placed in east-west direction to avoid shade for green manure production of *Crotalaria* spp. (2). *Azolla* spp. (3), cultivated on the surface of the water irrigation channels, is partly shaded by canopy of trees and banana plants (4), which are intercropped with shade-tolerant *Desmodium* spp. and *Vigna* spp. for biological N_2 fixation and establishment of mycorrhizal fungi networks. Wibbing (2020).

6.3.3 Plant management and livestock for commercial banana farms

Banana plants can be treated with regular spraying of biostimulants, e.g. *Moringa* leaf or *Azolla* extract from plant material cultivated on-site or compost tea from self-produced compost. A broad variety of biostimulants presented in this study should be tested under field conditions in the banana plantations to evaluate beneficial treatment, as they may enhance activity of AM fungi, which again may benefit plant nutrient supply and resistance to abiotic and biotic stress.

Silvopastoral livestock management can be adapted in the form of chicken, ranging through the banana fields, separated by the irrigation channels. They can graze on the intercrops of banana (e.g. *Desmodium* spp.), the residues of the green manure and cultivated *Azolla* from the channels. By doing so, they partly work in the green manure in the soil and may also reduce additional, detrimental pest insects by foraging, leaving their manure as fertilizer. Shaded by the banana plants, they may be protected from preying raptor birds, but may be endangered by other wildlife, such as snakes.

6.3.4 Conclusion: Adapted system for commercial banana farms

Changing environmental conditions will have drastic effects on the agricultural production in Lake Chamo region over the next decades, of fishermen as well as small-scale and commercial farmers. The latter produce the majority of national supply of dessert banana, the most important fruit in Ethiopia, almost a staple in people’s diet. When deterioration of Lake Chamo is continuing as in the last decades, its survival is clearly at stake. Furthermore, with Lake Chamo in a deteriorating state, drastic changes for the Lake Chamo watershed are to be expected, with higher temperatures and less precipitation in all the region, threatening crucial production of banana and the livelihood of many people.

To secure production of banana in the region, several adaptations of the holistic system for productive restoration of land and water resources presented in this study were compiled, from soil amendment with BoneC and BioC, soil inoculation with natural soil microorganisms, to intercropped, silvopastoral agroforestry planting system including local tree species and floating plants with manifold purposes and adapted livestock management of chickens. Also, biostimulants may enhance productivity of the agricultural production at a very low cost to benefit ratio. A special focus was placed on the threat of burrowing nematode *Radopholus similis*, which can severely affect banana yields. Here, by intercropping with repellent plants and nurturing of rich soil life, resistance to biotic stress could be drastically improved. In the proposed system, majorly, locally available waste resources are utilized or self-produced on-site, so investment costs are considered low and affordable for farmers, while local nutrient cycles can partly be closed.

However, the severe issue of flash floods from high rates of surface runoff and erosion during erratic rain events in the mountain areas of Lake Chamo watershed remains unsolved and out of control of banana farmers near the lake shoreline. To prevent destruction and siltation in the banana plantations and improve prevention of erosion, water retention and soil fertility in the mountain areas, also recommendations for small-scale subsistence farmers in the Lake Chamo watershed region are presented in the following.

6.4 Proposed system for small-scale subsistence farmers in Lake Chamo watershed

Small-scale farmers in the Lake Chamo watershed face rising difficulties in their daily operation. Rain fall patterns shift and become more and more unpredictable, while overall soil fertility and hence agricultural production is declining (personal communication with Eliyas Guffiso, local farmer). Malnutrition by quantity and especially quality is common, as seasonal diet is based mostly on carbohydrates, lacking protein, fats (especially omega-3 fatty acids), vitamins and minerals, as the major fraction of high value agricultural products such as animal products or fruits and vegetables are sold in the local markets for little financial gain.

Additional challenging for the small-scale farmers are high rates of surface runoff and following severe soil erosion within their farms with high slope gradients by deteriorating soil structure and fertility as well as lack of vegetative cover by extended fallow periods, removal of crop residues and deforestation. As groundwater recharge by lack of retention and infiltration is low, access to domestic water from wells is more and more difficult, while the excess water is causing floodings and damage in the down slopes near the lake (personal communication with Eliyas Guffiso, local farmer).

6.4.1 Extension of Rain Water Harvesting measures for small-scale farmers

Soil erosion is a serious issue for small-scale farmers on sloping terrain within the mountain areas of Lake Chamo watershed area. By altering natural forest area into agriculture farming space, the local water cycle comes more and more out of balance, as precipitation water is barely retained and infiltrated in the dominant agricultural systems due to a lack of vegetative soil cover, especially in the beginning of the rainy season, after months of fallow period. Surface runoff is forming and causes severe soil erosion, washing off the top layers of soil, which often contains the largest fraction of organic matter. By these processes, not only water needed for sufficient cultivation of crops is lost, but also fertile soil, which then causes floodings downstream and sedimentation of Lake Chamo. A lose-lose situation for both, small scale subsistence farmers and banana farmers.

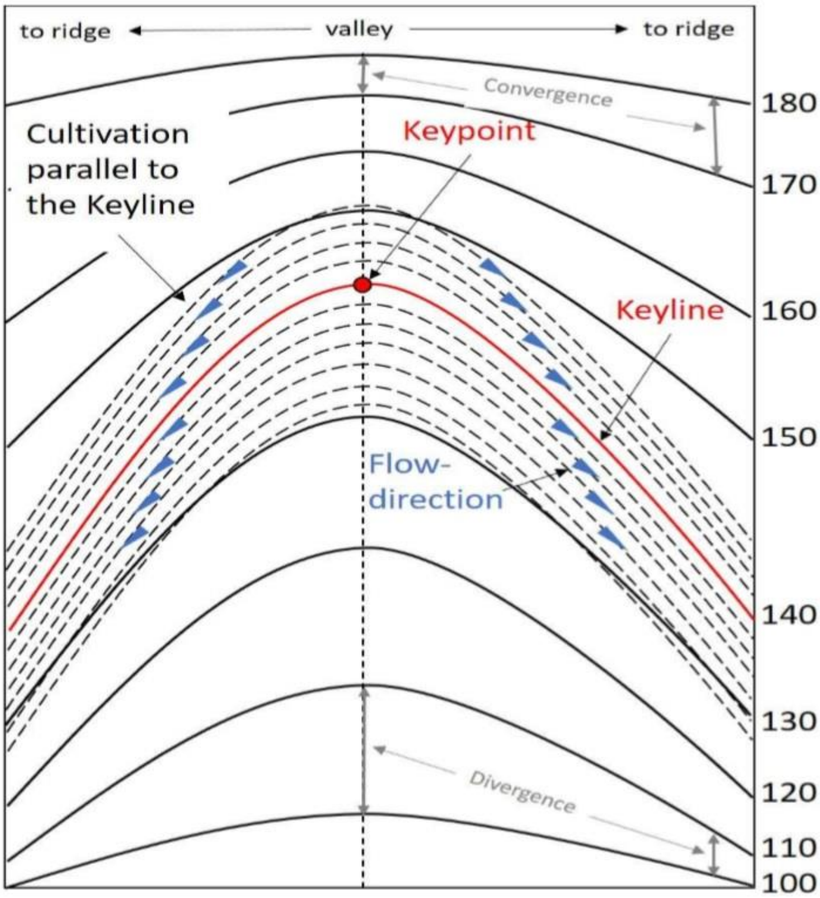

Figure 25: Keyline design of a primary valley for cultivation of dry ridges by water diversion, from Möller (2017), p. 36.

Accordingly, and partly already in place, measures of Rain Water Harvesting should be expanded in the mountain areas of Lake Chamo watershed, integrated in the planting system to generate symbiotic effects. Examples of suitable RWH techniques can be found in the surrounding communities in the area and mostly are based on stone terracing and trenches. However, additionally, contour lines should be implemented, supported by adapted plant selection to stabilize established trenches.

Here, the technique of Keyline Design managing on-site water flow dynamics could be utilized. By Keyline design, total infiltration and therefore water usage efficiency of a given sloping area can be drastically improved by hydration of distal dry ridges, while soil erosion and gully formation processes in primary gullies or valleys of the area can be lessened (Yeomans and Yeomans, 2008).

For implementation, the Keypoint of the area is identified as the transition point of convex to concave topography within the water accumulating primary valley. From this Keypoint as the highest elevation, a Keyline trench or furrows with a slight downward gradient towards the corresponding ridge is constructed, roughly following the contour lines of the area. Following, parallel to the Keyline, starting above and below the Keypoint in the primary valley, "off-contour" trenches or furrows can be constructed, which divert water from its natural accumulation point in the primary valley to the distal ridges, as their end point at the ridge is lower in elevation than the starting point at the valley (see Figure 25). Dry soil volumes of the ridges, otherwise mostly inactive in water storage, can be activated for water infiltration, and thus can be utilized for reforestation or agricultural activity.

Stone bunds should be considered wherever stones are available in the near vicinity, while Checkdams should be integrated for gully control. In areas with lesser slopes, swales can be constructed to enable diversion of water towards trees and bushes, which then after full establishment, can access deeper groundwater levels and distribute water for surrounding, interlinked annual plants by the formation of CMN. Here, earth moon bunds should be constructed in farms, where access to suitable stones is limited. Retained water can tremendously improve plant production in many situations. Examples of Rain Water Harvesting technologies, established and proven in the area, can be found in Möller (2017) (see Figure 26,Figure 27 and Figure 28).

When precipitation water can be retained and infiltrated in the mountain areas of the watershed, large water volumes, generated in short time period of erratic rain fall, can be partly buffered over time and will slowly, but continuously move down towards the banana plantation areas, where flash floods can be prevented.

Figure 26: Earth moon bunds retaining water during rain event in Arba Minch, Ethiopia. Wibbing (2017).

Figure 27: Contour line trenches retaining water during a rain event in the Slope Farming Project site, Arba Minch, Ethiopia. Wibbing (2017).

Figure 28: Intercropped vegetation in the Slope Farming Project Site, Arba Minch, Ethiopia, benefitting from prior implementation of contour line trenches. Wibbing (2017).

6.4.2 Soil amendments for small-scale farmers

To further increase the water storage capacity and therefore the yield potential of the agricultural soils of the small-scale farmers, special attention should be given to enhanced rates of stable SOM formation. For this purpose, soil amendments could be produced on-site, by the farmers, from organic waste residues of their farm and their household. This could include wood ash and char residues from cooking or BoneC or ash from animal bones of the household or community. As access to resources often is a limiting factor, more efficient usage wherever possible needs urgent consideration. An example is the usage of pyrolysis stoves for cooking or heating, which are run with firewood or agricultural residues, but produce BioC as a by-product. Low-cost solutions are at hand and simple to construct, even with local materials like clay.

Beneficial soil microorganisms should also be re-introduced in soils under conventional cultivation to increase quantity and diversity of soil life, especially of AM fungi and Sebacinales. Here, healthy soil from natural forest areas should be collected and spread in the fields, which then are cropped with highly mycotrophic, perennial plants.

Human excreta collected in an earth dug toilet can be composted with kitchen wastes and ashes from the fireplace and should be utilized for production of non-food plants such as forage or wood trees.

6.4.3 Plant system for small-scale farmers

Said beneficial soil microorganisms should be nurtured permanently by cultivation of perennial plants of an adapted agroforestry system. Leguminous trees and bushes ensure establishment and maintenance of CMN and facilitate nutrient and water supply to flat rooting intercropped plants from deep soil layers and biological symbiotic N_2 fixation. Aligned along the contour lines of the slopes or along the terraces, these perennial plants ensure stability of the soil by root formation and support retention and infiltration of precipitation water, while wind velocities can be reduced. By a dense canopy and live or dead vegetation cover all year round, splash erosion is largely reduced as well as evaporation from bare soil. Soil temperatures are balanced and strong UV radiation is prevented. Moisture level of the soil is enhanced, as well as soil life activity, again benefitting nutrient cycling and soil formation (for example see Figure 29).

Figure 29: Small-scale subsistence farm with intercropped field of cabbage, taro, maize and sugar cane in Humbo area, Ethiopia. Consultation by Terepeza Development Association Humbo-Damat Woyde Climate Change Resilient Livelihood Project. Wibbing (2017).

Specially selected trees and plants can be used to produce food, forage or woody material, on- and off-season, next to crops, pasture and green manure growing in the interspace of the tree rows. Suitable plant selection should be highly diverse and synergistic and may include, among others, *Moringa* spp., *Leucaena leucocephala*, *Gliricidia sepium*, *Faidherbia albida*, *Sesbania sesban* and *Sesbania grandiflora*, *Calliandra calothyrsus*, *Erythrina* spp., *Albizia lebbeck*, *Acacia* spp., *Terminalia brownie* and *Ziziphus mucronata*. Perennial and annual intercropping plants may include common staple crops like wheat, Teff, maize, potatoes, sweet potatoes, yam, cassava, taro, false banana, vegetables of carrots, cabbage, tomatoes, onions, garlic, ginger, peppers and fruits of papaya, banana, passionfruit, avocado, guava, apples, pears, plums and sugar cane. Yielding companion plants, among others, may include vetiver grass (*Chrysopogon zizanioides*), Bermuda grass (*Cynodon dactylon*), perennial peanut (*Arachis glabrata*) and beans and peas of different species, like *Lablab purpureus*, *Cajanus cajan* or *Canavalia gladiate.* Utility and forage plants should be grown with multiple benefits, such as Napier grass (*Pennisetum purpureum*) and *Desmodium* spp. arranged in a push-and-pull system or lowland bamboo on the edge of the farm as an element of fencing, windbreak and soil stabilization, while producing material for housing construction, furniture building or BioC production.

Cultivation should be performed according to principles of Regenerative or Conservation Agriculture. Reduced tillage operation facilitates rapid formation of stable SOM. Other conservation projects in the region show, that soil structure, fertility and water storage capacity can be tremendously increased, while need for weeding and tillage are continuously reduced (e.g. Terepeza Development Association Humbo-Damat Woyde Climate Change Resilient Livelihood Project). Yields can be increased with less input and become more reliable despite changing environmental conditions (see Figure 30).

Figure 30: Practiced conservation agriculture with intercropping and mulching of maize, beans and pigeon pea in Humbo area, Ethiopia. Consultation by Terepeza Development Association Humbo-Damat Woyde Climate Change Resilient Livelihood Project. Wibbing (2017).

6.4.4 Plant management and livestock for small-scale farmers

During cultivation, whenever needed, the canopy of the trees should be trimmed for forage or mulch and to also allow efficient growth of understory plants. After harvest, crop residues should remain on-site, as mulching material for protecting soil moisture levels and to feed emerging soil life.

If possible, livestock should be included in the farm operation. Chicken can be used to reduce soil borne pests from manure application in fields. Detrimental practices of uncontrolled open grazing should be stopped in favour of holistic planned grazing to increase efficiency of livestock management of cattle, goat or sheep and to retain nutrients in manure on-farm for closed nutrient cycles.

6.4.5 Conclusion: Adapted system for small-scale farmers

Low agricultural productivity of small-scale subsistence farmers in rural areas of developing countries has many reasons. One is often the high deficiency of plant available nutrients in agricultural soils and the lack of suiting fertilizer materials, restricted in accessibility for most resource-poor farmers in rural areas without functioning infrastructure and developed markets. Bare soil with high clay content and low soil fertility with lack of organic matter as often to be witnessed in Ethiopia is not supportive in retention and infiltration of water, hence water status of soil often is poor, but rate of erosion is high. By combination of locally available soil amendments often considered as wastes, an adapted planting system with specially selected plants and management practices of Regenerative Agriculture, fertile soil can be formed, which then can again improve yields in quantity, quality and reliability despite changing environmental conditions. However, as rain events become more erratic, a top priority should be the prevention of surface runoff formation in the mountain areas of Lake Chamo watershed by increased retention and infiltration through physical measures of RWH. Here, individual farmers on-farm and as well as communities off-farm in public spaces are required to establish or extend RWH measures, wherever needed and possible. Parallel to the enhanced retention and infiltration by RWH

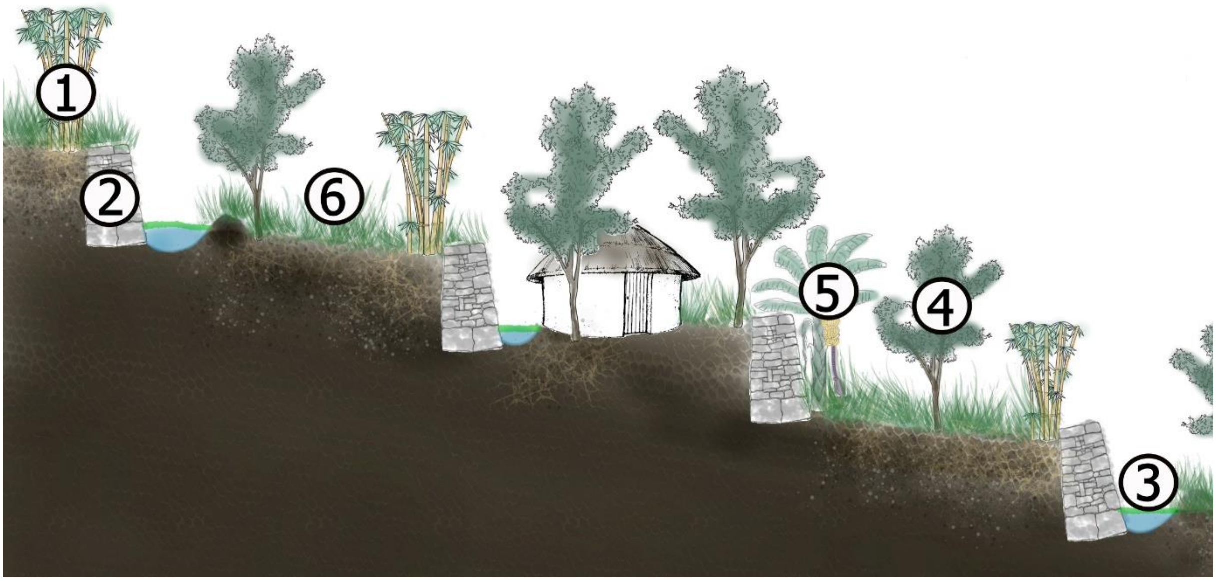

Figure 31: Schematic depiction of small-scale farm in mountain area of Lake Chamo watershed. Bamboo (1) serves as a boundary plant, stabilising the stone terrace (2), which is constructed along the contour line of the slope. Behind the stone bund, a contour trench (3) with Keyline design is dug to retain water on-site for irrigation and *Azolla* spp. cultivation. Trees (4) are planted along the contour line in rows, creating shade in the interspace, benefitting cultivation of fruits, vegetable and pasture grasses (5,6). Wibbing (2020).

measures, water storage capacity of soils is to be increased. Here, the nurturing of soil formation processes by addition of selected soil amendments, inoculation of soil forming microorganisms and adapted management of perennial vegetation cover (including mulching, crop residue management, agroforestry systems) become of vital importance (see Figure 31).

As a “by-product” of these activities, fertile soil with improving water status can be formed, which is able to enhance crop production quantity- and quality-wise and therefore can improve the livelihood of small-scale subsistence farmers in mountain areas of Lake Chamo watershed.

6.5 Conclusion: Holistic approach for restoration of Lake Chamo watershed

While people in rural or urban areas of Lake Chamo watershed follow diverse business or livelihood models, they all share the fundamental dependency on the various ecosystem services of the lake. The maintenance or re-establishment of those fundamental ecosystem services is of upmost importance, on a local, but also regional and national level, as the major fraction of the national banana supply is produced here. With ongoing deterioration of Lake Chamo, the livelihood of the local people is highly threatened, as well as the already fragile food security of the whole country.

In this case study, the holistic approach developed in this study based on productive restoration of land and water resources was adapted to local needs of small-scale subsistence farmers as well as banana cultivars in Lake Chamo watershed area, both linked by their dependence on the lake. By the adapted system, enhancement of yield levels may be reachable, while stable SOM in soils can be built up, supporting retention and infiltration of water by its large water storage volume, ultimately leading to reduced rates of surface runoff and hence erosion, which otherwise would further diminish water quality of Lake Chamo.

Specific recommendations for immediate action were given to strengthen local production with locally available resources and plants, with beneficial effects on the condition of Lake Chamo and overall ecosystem of its watershed. Successful projects in the same region show, that tremendous improvement can be made in the short- and mid-term in regards of stable SOM formation, enhancement and stabilization of yields and sustainment of livelihoods. When the same is done on a large scale, all around Lake Chamo watershed, its fundamental ecosystem functions for all inhabitants of the region may be secured.

7 Conclusion

With tremendous issues of ongoing soil degradation, deforestation, changing environmental conditions of climate change, current low levels of agricultural production and a decline in availability of P fertilizer materials, the systemic agricultural supply power of SSA is likely to decrease gradually in the next decades. A rapidly growing population on the other side though increases the supply demand in affected regions, but also globally. The disbalance, created by these opposing trends, may lead to an imminent collapse of food and water security of large parts of the SSA population and needs strong response from humankind by intensification of agricultural production in SSA, while restoring degraded environmental resources of land and water.

Conventional agricultural production methods relaying on synthetic fertilizer of N and P seem unfit to meet the demands and requirements as well as the resource limitations of extensive, small-scale subsistence farmers as the major group of food producers in SSA. Alternative approaches of agricultural management and materials will be required to facilitate needed enhancement of agricultural productivity of those farmers as the backbone of food security in SSA. Such a holistic, multi-layered system was developed in this conceptual study, based on comprehensive literature review.

Next to various beneficial effects on plant growth, AM fungi can become essential in nutrient and water supply for plants in challenging soil conditions of the target region. Their ability to utilize insoluble P fertilizer like BoneC or fixed soil P in general marks the fundamental mechanism to partly eliminate and neutralize the major issue and drawback of high P fixation capacity of soils. Here, the P acquirement and transport system of soil P are changed fundamentally, from passive water dissolution and diffusion, towards active scavenging, solubilization and transport by AM fungi and helper microorganisms. The necessity of P diffusion towards plant roots may be bypassed in mycorrhizal soils, as solubilized P ions are rapidly transported within fungal mycelium, protected from the risk of refixation in P unsaturated soils. Therefore, sufficient fertilizer efficiency also from insoluble, alternative P fertilizer materials like BoneC can be reached in P fixing soil conditions, when P acquisition of plants is mediated by AM fungi. Concluding, in a holistic approach for productive restoration of degraded soils in SSA, the promotion of AM fungi and their complementary soil biology should be considered as the essential key element and therefore of highest priority.

Hence, to increase the activity of AM fungi and to further enhance formation rates and stability of stable SOM formation as well as rates of plant production, soil amendment with BioC and micro nutrients are considered further key elements of the holistic agricultural system, along principles of regenerative and conservation agriculture with adapted measures of agroforestry, SCI, holistic planned grazing and certain biocontrol measures. All these diverse methods are utilized to facilitate establishment and maintenance of active soil life and stable SOM formation and are fostered by specific plant selection and high biodiversity.

Also, biostimulatory substances are considered as plant additives, to increase plant performance in established, robust systems for elevated biomass production, which again enables higher rates of soil C sequestration, following, higher rates of soil life activity, stable SOM formation and so on.

In the conducted experiments the usage of BoneC was proven to serve as an effective, additional fertilizer material for *Cajanus cajan* as an important crop plant in southern Ethiopia, which can contribute to needed intensification of agricultural production in rural areas of SSA. BioC singly cannot serve as a suitable fertilizer over the short-term, but again in combination with BoneC and CM, yields can be increased in comparison to current production methods.

Biostimulants however showed susceptibility to abiotic stress factors of heat, as no positive or negative plant response was observable. Here, the full establishment of all components of the proposed system including plant establishment and stable SOM formation may allow the utilization of biostimulatory plant additives in a second step for potential enhancement of biomass production as reported in literature.

In a case study the holistic system was adapted to local conditions and needs of both commercial banana farmers and small-scale subsistence farmers in Lake Chamo watershed in southern Ethiopia. Practical recommendations for a shift in the local agricultural practice for productive soil restoration and were given, benefitting agricultural output, resilience to abiotic and biotic stress, while utilizing local waste resources. When applied on a large scale within all the watershed, irreversible deterioration of Lake Chamo could be prevented. Its impaired environmental state may be even reversed, to again improve its stabilizing effect on the local micro climate and turn it once again in a reliable source for water and food for all the region.

In this thesis, a holistic agricultural system was specifically designed to enable the usage of water-insoluble BoneC as an alternative fertilizer material for small-scale subsistence farmers in rural areas of developing countries, producible locally, from a so far untapped waste resource with very limited equipment and only basic knowledge needed. By promotion of AM fungi and soil life in general, this P containing material and - maybe more importantly - fixed soil P reserves in general are efficiently utilized for plant supply and sufficient fertilizer recovery rates in nutrient deficient soils with high P fixation capacity are reachable. Regenerative agricultural systems, based on promotion rather than diminishing of AM fungi as the mediating key element of microbial soil activity, will gain more track over the next decades, as rising issues of climate change, soil degradation, drought events and low agricultural output in SSA can be counteracted synergistically and simultaneously, predominantly by the formation of stable SOM. Concurrently, the dependency on fossil, costly and potentially harmful conventional production goods like fertilizer or pesticides is drastically reduced, but locally available waste materials are utilized. A needed holistic system combining feasible measures for productive restoration of degraded soils and intensification of agricultural production was presented in this study, which can enhance food supply in areas facing drastic population growth and environmental changes.

However, considering the large pool of unavailable, fixed P reserves in many regions around the world, and the relatively small contribution of exogenous P fertilizer material in agricultural production in comparison, it becomes obvious, that in future efforts to utilize soil P reserves should be increased substantially.

Here, BoneC may be a suitable low-cost, widely available material to kickstart re-establishment of perennial vegetation, which then again can re-establish and nurture beneficial soil life. Finally, over the mid to long term, soil P reserves can be accessed as discussed in this thesis, contributing to enhanced agricultural production by improved P supply to crops. Applied but fixed soil P from intensive fertilization can be mined for plant supply after all and thus can be recovered and reintroduced into the P cycle. Therefore, the proposed system could also become important for conventional agriculture in industrialized countries within this century, eventually. The P cycle itself should be closed by then through fully implemented resource-oriented sanitation systems and full recycling of agricultural wastes, to terminally solve the global issues of fossil P in specific, and soil degradation in general.

Appendices

Appendix A

Calculation P recovery from beef cow remains of bones and manure

Table A.1: P remains in cow manure over lifetime

Age*	Life stage of cow	Daily weight gain	Weight at birth	Weight at end of year	Weight average over year	relative P_2O_5 content in manure per 1000kg LW**	absolute P_2O_5 content in manure	absolute P conent in manure***
year	-	kg	kg	kg	kg	kg/y	kg/y	kg/y
1	Calf	0.50	35.00	217.50	126.25	73.00	9.22	4.01
2	finishing	1.00	-	582.50	400.00	39.00	15.60	6.78
							Sum in kg P	10.785

* = common age of beef cows at slaughtering in Ethiopia is 2 years

** = http://mvtl.com/_static/web/assets/media/pdf/nutrient_tables.pdf, latest access 30.03.2020

Table A.2: P remains in BoneC from cow bones

Fraction of Cow LW / BoneC	Percent of cow LW	Calculation and comment
LW*	100	
HCW*	50	50 % LW = HCW**
CCW*	49.5	99 % HCW = CCW**
Bones	14.85	17 to 32 % CCW = Bones**; 30 % for skinny cows as common in Ethiopia
BoneC	3.861	26 % Bones = BoneC; 26 % mass yield by slow pyrolysis of unrendered bones at 750 °C for 45 min, see Zweetsloot et al., 2014
P content in BoneC	0.423	10.95 % total P content of BoneC from undrendered bones pyrolsed at 750 °C for 45 min, see Zweetsloot et al., 2014
Absolute P content in BoneC in kg, from bones of 2-year-old cow with 582.5 kg LW***	2.464	

* = LW: live weight; HCW: hot carcass weight; CCW: cold carcass weight

** = http://www.omafra.gov.on.ca/english/livestock/beef/facts/05-075.htm, latest access 30.03.2020

** = see Table A.1

Table A.3: Absolute and share of P remains in manure and BoneC over lifetime of cow

P fraction	P remains over lifetime in [kg]	Share of total P remains over lifetime in [%]
P in manure*	10.785	0.814
P in BoneC*	2.464	0.186

* = for 2 year old kg beef cow with 582.5 kg LW; see Table A.1 and A.2

Appendix B

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