Bioaugmentation and biostimulation of a South African coal-based Technosol as a mine rehabilitation strategy

by

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ABSTRACT

Annually, over 70 million tonnes of material are added to coal waste dumps in South Africa. To alleviate the environmental and social burdens of unsightly mine waste dumps, improved management of existing discarded materials and new wastes, and fertile soils for land rehabilitation are essential. To achieve successful mine closure and restore derelict land, sustainable mine rehabilitation schemes aligned with circular economy principles should be prioritized. A proposed method for effective and controlled resource management based on waste valorisation involves re-purposing the major coal ash fraction of coal waste, provided it has been classified as benign in terms of acidification, salinisation and metal deportment, to fabricate a soil (Technosol) by amending it with a suitable organic waste source to provide both soil structure and nutrient availability and establishing an appropriate microbial consortium. This study investigated the improvement of Technosol fertility through biostimulation and bioaugmentation. By introducing a diverse microbial community, typically associated with healthy soils and initially absent in mine waste, the establishment of self-sustaining fertile topsoils to rehabilitate disturbed mining areas is accelerated.

The Technosol used in this investigation was fabricated from ultrafine coal tailings amended with malt residue from a local brewery. The fabricated soil was inoculated with a commercially available soil inoculum (EM Pro-Soil) and amended with different dosages of malt residue (0%, 2.5%, 5% and 7% w/w). *Eragrostis tef* (teff) plants were grown in each of the treatments, including a non-inoculated control. Seasonal teff growth was mimicked by performing a second trial of plant growth studies. Soil health, quality and fertility were determined from assessment of soil physical, chemical, and biological properties that inferred Technosol feasibility for implementation.

Bioaugmentation increased nutrient availability for plant growth through organic material degradation. From plant-microbe interactions, soil inoculation resulted in increased microbial biomass and metabolic activity compared to non-inoculated Technosols. Bioaugmentation and biostimulation with more than 2.5 wt.% malt residue (MR) reduced metal(loid) solubility. Technosol microbiome diversity following inoculation depended on applied amendment dosage and was positively influenced by alkaline soil pH, lowered salinity, water permeation, and vegetational growth. Soil pH was regulated biogenically through MR application as biostimulant for microbial respiration. Teff biomass production and inflorescence were favoured in warmer conditions as a result of accelerated microbial activities and photosynthesis. The effect of microbial inoculation was more evident during the second plant growth trial with higher bacterial and archaeal abundances. Here we evaluated the succession of the microbial community by qPCR analysis of 16S rRNA V3 to V4 regions. Following vegetation and inoculation, abundances of nirK gene encoding nitrite reductase denitrification enzymes were augmented in Technosols fabricated with higher dosages of MR, suggesting enhanced nitrogen uptake. Vegetation in inoculated Technosols amended with 5 wt.% MR assisted remediation of soil iron and sulfur to suitable levels, reduced leaching, favoured seedling emergence with temporal effects, and showed the greatest potential for nitrogen cycling from nifH gene abundances that correlated to enhanced phosphorus uptake. The results supported the feasibility of implementing Technosols as topsoils to reduce socio-economic and

environmental impacts of coal mine related processes, by targeting the Sustainable Development Goals (SDGs) 9, 11, and 15.

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Anything and everything is possible when you're rooted in faith.

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GLOSSARY OF TERMS

Abbreviations

ACC	1-Aminocyclopropane-1-Carboxylate
AMF	Arbuscular Mycorrhizal Fungi
AP	Acid Potential
ARD	Acid Rock Drainage
ASTM	American Society for Testing and Materials
AWC	Available Water Capacity
BNF	Biological Nitrogen Fixation
CHNS	Carbon, Hydrogen, Nitrogen, Sulfur
CEC	Cation Exchange Capacity
CMM	Coal Mine Methane
CW	Coal Waste
DNA	Deoxyribonucleic Acid
dsDNA	Double Stranded DNA
EC	Electrical Conductivity
ECe	Electrical Conductivity of Saturated Paste
ECEC	Effective Cation Exchange Capacity
EcM	Ectomycorrhizal Fungi
EIA	Environmental Impact Assessment
EMF	Ectomycorrhizal Fungal
EMP	Environmental Management Plan
ESP	Exchangeable Sodium Percentage
FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organization
FC	Field Capacity
FDA	Fluorescein Diacetate
gDNA	Genomic DNA
I	Inoculated
IAA	Indole-3-Acetic Acid
ID	Identification
ISRIC	International Soil Reference and Information Centre
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
MR	Malt Residue
MWRS	Municipal Waste Removal System
NCBI	National Centre for Biotechnology Information
NI	Non-inoculated
NNP	Net Neutralisation Potential
NP	Neutralisation Potential
NS	Native Soil
OC	Organic Content
OM	Organic Material
PGPR	Plant Growth Promoting Rhizobacteria
PLFA	Phospholipid Fatty Acid
PVC	Polyvinyl Chloride
qPCR	Quantitative Polymerase Chain Reactor
RoM	Run off Mine
rRNA	Ribosomal Ribonucleic Acid
SDG	Sustainable Development Goal
SIR	Substrate Induced Respiration

SMI	Soil Microbial Inoculum
SOM	Soil Organic Matter
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TN	Total Nitrogen
WISE	World Inventory of Soil Emission Potentials
WHC	Water Holding Capacity

CHAPTER 1

1 INTRODUCTION

1.1 Project Background

The economic benefits from coal beneficiation are evident especially in South Africa which has large reserves of this natural resource; however, with over 70 million tonnes of material added to coal waste dumps in South Africa only in 2016 (South African Coal Roadmap Steering Committee, 2013), it becomes increasingly important to manage the existing waste materials and the disposal of new wastes to alleviate its environmental and social burden. Coal mining and related unit operations cause dust emissions, loss of arable land, acid rock drainage, and large volumes of waste rock (Zornoza, et al., 2017; Miller & Kohlhase, 2010; Weiler, et al., 2018; Sekhohola-Dlamini, et al., 2022). Nonetheless, it also offers the potential for repurposing of associated materials to other uses consistent with the circular economy principle.

The continuously developing coal mining industry and contested nature of coal have resulted in a high demand for robust waste management strategies, monitoring systems, and sustainable rehabilitation schemes to address their well-known long term impacts (Hattingh, et al., 2018). Furthermore, the development of mine rehabilitation strategies involves economic, social, environmental, political and sustainability principles and does therefore not have a one-glove-fits-all solution. Circular economy solutions are needed to adjust value chains for sustainable development and to treat the legacy coal waste.

Soil health is a global concern. Richter (2007) suggested that the future of the natural environment is dependent on soil health. Soil support biophysical and biogeochemical functions between the atmosphere, hydrosphere, lithosphere and biosphere. The health, quality and fertility of this non-renewable source are interlinked. While post-mining activities are dependent on the agricultural potential of the rehabilitated mine land, mining activities are typically deleterious to soil availability and quality (Cowan, et al., 2016). Recently, the potential use of coal waste (CW) originating from the collieries in the Mpumalanga area in South Africa, amended with organic materials to fabricate soil (Technosol) has been demonstrated; this can be used to restore degraded mine sites to their desired land capability. This may facilitate achieving mine closure, whilst reducing the volume of waste disposed and eliminating financial burdens associated with conventional mine rehabilitation requiring natural soils (Deeb, et al., 2017; Firpo, et al., 2014; Sekhohola-Dlamini, et al., 2022; Amaral Filho, et al., 2020).

Valorising coal waste to fabricate a soil suited for rehabilitation aligns with the circular economy principle, describing an economic system based on the cyclic use of resources within a process (Lebre, et al., 2017). Currently, ultrafine coal tailings have no economic value, although rich in carbon and sulfur suited to nutrient sequestration (Weiler, et al., 2018). Amendment materials are waste products from local processes e.g., municipal deposits, refineries, wastewater treatment systems or vegetable processing plants (Herran Fernandez, et al., 2016; Jordan, et al., 2017; Macia, et al., 2014). It holds specific value (structural ameliorants and source of nutrients) for fabricated soils (Herran Fernandez, et al., 2016).

A functioning soil microbiome is intrinsic to soil development and sustaining plant growth (Atlas & Bartha, 1998). Plant-microbe interactions evolve within the soil matrix to support ion mobilisation, organic matter decomposition, pollutant degradation, metal translocation, carbon sequestration, nitrogen cycling, phosphate availability and photosynthesis amongst other functions (Tiwari, et al., 2021). Soil microbial ecology is influenced by biotic and abiotic factors (Valarini, et al., 2003). Figure 1 illustrates the intricate and interdependent relationships between soil, plants and microorganisms in maintaining soil health, quality and fertility.

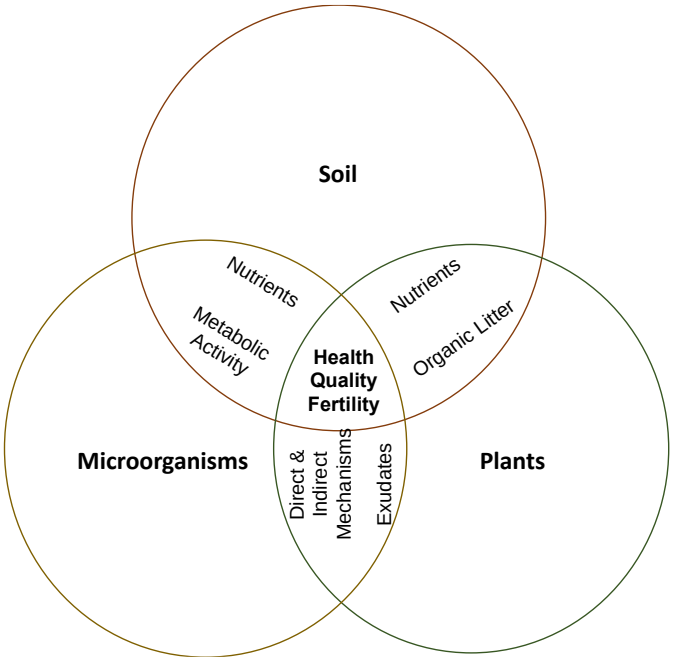


Figure 1: Interdependence of soil health, quality and fertility (as described by Atlas & Bartha, 1998).

The diverse native microbial communities associated with self-sustaining soils are initially absent in mine waste derived soils (Sansupa, et al., 2021). For a self-sufficient Technosol to perform as a topsoil and ensure effective rehabilitation of the disturbed mine land, it must provide a suitable environment for plant growth with high quality yields, and fulfil biogeochemical transformations without environmental risks (Nortcliff, et al., 2012). Thus, the balance between soil structure, nutrients and microbial communities must be obtained. Consequently, biostimulation and bioaugmentation are necessary in mine waste-based Technosols (Adams, et al., 2015).

Previously conducted research presented on the feasibility of fabricating a soil from coal tailings (Amaral Filho, et al., 2020) with different amendments for plant growth. However, further research is required to evaluate the effects of amendment dosages on plant growth and soil fertility, Technosol performance with seasonal effects, the function and development of the Technosol microbiome after bioaugmentation and biostimulation, and the benefit and feasibility of including bioaugmentation on amended coal-based Technosols.

1.2 Project Scope

The quality and physical structure of a Technosol is determined by the parental material, amendments and soil microbiome present or established within the fabricated soil. This study focused on the introduction of an active microbial consortium to facilitate the succession of the Technosol for improved plant growth performance. This was investigated by assessing the ability of a bioaugmented coal-based Technosol to sustain itself and through conducting plant life cycle experiments with a parental material amendment in various concentrations.

Technosols in this investigation were fabricated from ultrafine coal tailings amended with various ratios of malt residue from a local brewery as an example amendment, and bioaugmented with a commercially available inoculum. Amaral Filho et al. (2018) showed that the malt residue added nutrients, organic material and structural improvement that is necessary to sustain plant growth in coal derived Technosols. Weiler et al. (2020) described enhanced plant growth, soil nutrient levels and microbial biomass following inoculation of these Technosols with the mixed culture inoculum. Teff, indigenous to Mpumalanga (Truter, 2007) and commonly used in mine restoration (Amaral Filho, et al., 2020), were grown in each of the treatments, with a non-inoculated control. Seasonal teff growth was mimicked by performing a second trial of plant growth studies. Molecular techniques were employed to profile change in microbiomes in bioaugmented Technosol with teff growth and amendment dosages. The results were used to develop financial estimates for considering the feasibility and potential of implementing biostimulated and bioaugmented coal-based Technosols as self-sustaining topsoils.

1.3 Thesis Structure

The thesis is structured in six main sections as summarized in Figure 2. The first chapter introduces the investigation, provides an overview of the problem, research aims and scope. Chapter two critically reviews literature on related work in terms of findings and knowledge gaps. From this, the project hypotheses were formulated along with key questions to address the research problem. Chapter 3 details the research approach and methodology, materials and apparatus required to achieve the project objectives and to test the hypothesis developed from literature reviewing. The results and discussions thereof follow in Chapter 4, while financial estimates based on findings in this chapter were made in Chapter 5 to consider the feasibility and implications of scaling up. The final chapter integrates project findings with the significance thereof, addresses the hypotheses and discusses recommendations for further research.

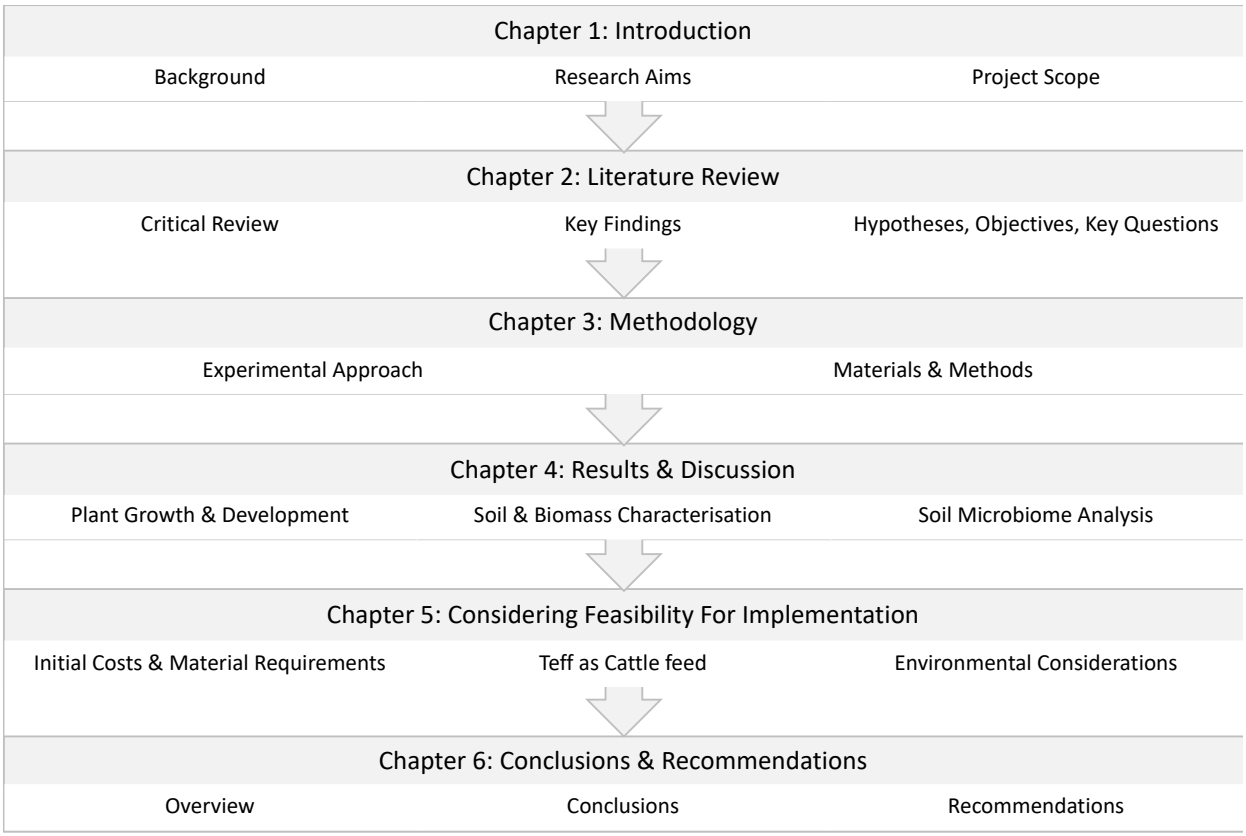


Figure 2: Flow diagram showing thesis structure.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Coal Mining in South Africa: Background, Challenges, and Opportunities

Globally, coal remains a vital energy source, supplying almost 29% of the total energy requirements for steel production, power generation, and cement manufacturing industries (Hughes, et al., 2019). Coal practices, mine rehabilitation strategies and the significance thereof in South Africa are discussed.

2.1.1 *Coal Mining Practices in South Africa*

The economic benefits from coal mining are evident especially in developing countries such as South Africa which has large reserves (15-55 billion tonnes) of this natural resource (Eberhard, 2011). South Africa has the largest global coal export terminal (Richards Bay Coal Terminal, Kwazulu-Natal Province), which along with the abundance of coal reserves, motivates the continuous advancement of local collieries (Eberhard, 2011). South African coalfields are primarily located in eMalahleni and Ermelo in the Mpumalanga Province, with recent exploration in the Limpopo Province (Sekhohola-Dlamini, et al., 2022). The national estimated production in 2017 reached 252.3 million tonnes; listing South Africa as a top 10 global coal producer (Hughes, et al., 2019), meeting 77% of the local energy demands (Chamber of mines of South Africa, 2018). Here, coal mining provides employment to 17% of the South African workforce with each miner linked to many different livelihoods. From 2010, the eMalahleni community fully depended on coal mining and was one of four towns to produce 67% of the nation's value added from coal (Makgetla & Patel, 2021). Although renewable and green technologies are gaining popularity with a strong negativity with regards to coal mining in the shift to the low carbon economy, the market for coal mining in South Africa is unlikely to rapidly conclude. Yet, it does signal the need to improve its environmental footprint. Coal processing strategies are constantly improved to prolong the lifespan thereof through optimisation of production systems to reduce waste and exploitation of new reserves (Hughes, et al., 2019).

Coal mining may take place either as opencast mining aboveground or as underground mining, based on the specific geographical conditions. The aboveground (or opencast) methods are classified into open pit or strip mining. Where, open pit mining differs from strip mining as the top-surface material is relocated for overburden material and not replaced in a nearby excavated section (Hattingh, et al., 2018). Thereby, resulting in large quantities of excavated rock dumps that are exposed to weathering, altering the landcover and hydrology (Weyer, et al., 2019). The majority of coal processing at eMalahleni is open pit mines.

The market specification or grade for the coal market depends on its use which extends past electricity generation. South African coal grades are categorized in Table 1 based on physical and chemical characteristics. Grades A, B, C and D commonly refer to metallurgical, synfuel, thermal and export coal (refer to Figure 3).

Table 1: South African coal market specifications (Steyn & Minnitt, 2010).

Characteristic	Grade A	Grade B	Grade C	Grade D

Ash (%)	15	16	18	21
Sulfur (%)	1	1	1	1.5
Calorific value (MJ/kg)	> 27.5	> 26.5	> 25.5	> 24.5

The majority of RoM is 'washed' or 'screened' to remove associated impurities and achieve market specifications (refer to Figure 3). Figure 3 illustrates the different coal products, specifically the large export market as well as the large volumes of waste produced during processing and beneficiation. Coal beneficiation methods are most often based on wet physical processes that produce a fine slurry and a solid waste stream. The slurry (tailings) is sent into tailing ponds and the coarse coal discards is typically discarded into stockpiles (Hattingh, et al., 2018). Coal from tailings is recovered through froth flotation or similar methods. However, generally only 0.5% (v/v) of produced coal waste (CW) in a mine is reused in this manner (Fecko, et al., 2013). In Figure 3, more than 22% of the South African RoM coal is discarded to waste dumps, with each beneficiation step contributing to this fraction. Consequently, sustainable methods are needed to reduce the volume of waste generated.

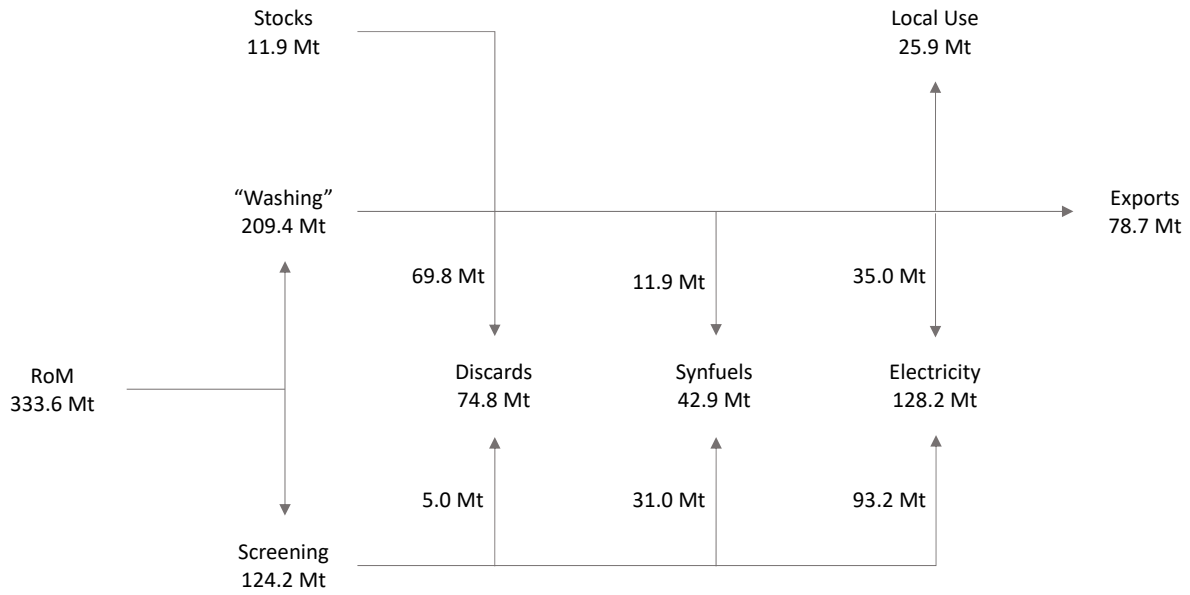


Figure 3: Run-off mine coal processing in South Africa (adapted from South African Coal Roadmap Steering Committee (2013).

With over 70 million tonnes of CW material annually added to dumps in South Africa (South African Coal Roadmap Steering Committee, 2013), the management of existing waste materials and the disposal of new wastes become paramount for sustainable development (Potgieter & Truter, 2010). The management of CW offers potential for retrieval of coal values as well as re-purposing of associated materials to other uses consistent with the circular economy. In regions such as eMalahleni, where several local collieries approach the end of their productive lives or are being phased out in favour of renewable energy, the locus of production is shifting away from traditional linear coal processing. However, CW can be seen as a multi-product resource that can provide economic and social opportunities, and environmental benefits.

2.2 Environmental Impact of Coal Mining in a South African Context

South African legislation demands mining rehabilitation for mine closure and to reduce mining related deleterious environmental impacts (Weyer, et al., 2019). The negative effects of coal mining on the biophysical environment include loss of biodiversity (terrestrial, aquatic and soil biota), land degradation, changes in local geomorphology, air pollution, acid rock drainage (ARD), diminishing of resources, and modifications in soil quality, structure and fertility (Zornoza, et al., 2017; Miller & Kohlhase, 2010; Weiler, et al., 2018; Sekhohola-Dlamini, et al., 2022). In preparation of coal processing, native topsoil is removed to stockpiles where, for decades, the dumps are left unmaintained and compromised by weathering resulting in arid soil (Cowan, et al., 2016). The mined land and dumped waste have long term and immediate effects on its surrounds, shown in Figure 4. Stockpiles and associated contaminants directly alter the surrounding water sources, fauna and flora, whereas, particulate matter from unsightly construction directly modifies air quality. Surface water reacts with the pyritic content within the waste causing ARD that seeps into adjacent water resources, finally polluting the groundwater (Zornoza, et al., 2017). The supply of potable water from rivers and dams in areas of South African coalfields are directly influenced by mining activities. Consequently, the used coal mine land is attenuated to an unfertile, acidic, nutrient deficient, microbially inactive and structurally poor state (Badenhorst, et al., 2018). These impacts, along with social issues such as the health concerns related to both underground and surface coal mining, are often argued to outweigh the economic value of coal.

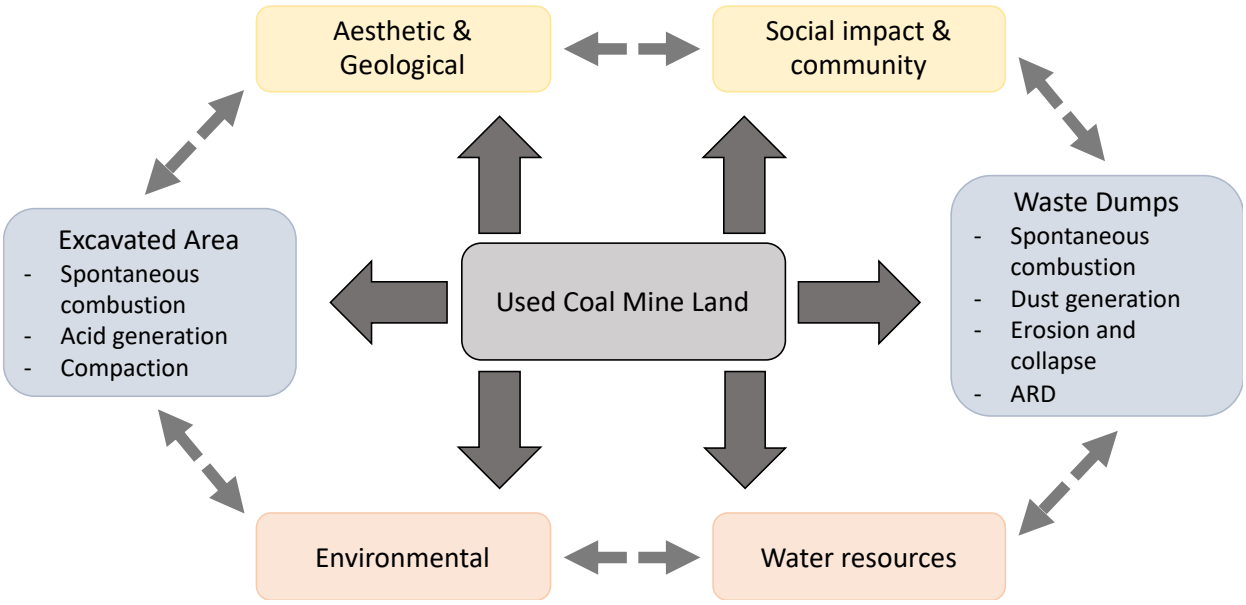


Figure 4: Representation of immediate, direct (solid arrows) and long term, indirect effects of coal mining, stockpiles and CW dumps. Adapted from Sekhohola-Dlamini et al. (2022).

Commonly, topsoil (50-100 cm layer) with lime, fertiliser and annual or perennial grass seeds are imported and deposited on the exposed CW and mined land (Cowan, et al., 2016; Sekhohola-Dlamini, et al., 2022). As native soils are rendered unfertile, high overhead costs are also associated with the topsoils due to sourcing, excavation and transportation thereof (Cowan, et al., 2016). Covering or masking with

topsoil aims to restore soil carbon loss and bio-stimulate indigenous microorganisms (Badenhorst, et al., 2018). However, plant growth and soil fertility are rarely sustained due to low cation exchange capability, limited organic matter, and a lack of essential plant nutrients (N, P, K, etc.) (Sekhohola-Dlamini, et al., 2022; Badenhorst, et al., 2018). Additionally, degradation of the carbonaceous CW is unachieved and healthy soil microbiomes are unobtained. This strategy is not sustainable.

Increasingly, studies are being conducted on reducing the impact of coal processing through valorisation and re-purposing of CW. As waste disposal and ARD treatment facilities are costly and the duration of ARD generation long-term, preventive approaches are preferred. This means risk removal by either using new approaches for final disposal to prevent oxidation of sulfidic minerals or removal of sulfide prior to disposal (Galatto et al., 2007; Kazadi Mbamba et al., 2012; Kotsiopoulos and Harrison, 2017; Machado and Schneider, 2008; Soares et al., 2009), or through developing zero waste strategies in downstream processing. Zero waste strategies include both recovering lower volume products of value and re-purposing the major coal ash fraction, the latter for either fabricated soils (Amaral Filho et al., 2016; Firpo et al., 2015; Weiler et al., 2018) or construction materials (Argane et al., 2015; Santos et al., 2013).

2.2.1 *Mine Rehabilitation*

Legislated compliance acts, such as the National Environmental Management Act no.107 of 1998 (NEMA), regulate South African mining related activities in attempt to minimise negative environmental and social effects. The first land rehabilitation guideline for coal surface mining was published by the Chamber of Mines in 1981 and updated in to the Mineral and Petroleum Resources Development Act (MPRDA) no. 28 in 2002. Various amendments have been made to MPRDA and guidelines published, such as including public participation (amendment in 2008).

It is desirable for the impacts of coal mining processes on the environment to be minimized through the successful implementation of sustainable mine rehabilitation schemes. Mining rehabilitation is defined as the process through which degraded land is restored to productive land through anthropogenic and natural solutions (Cessford, 2004). The development of mine rehabilitation strategies involves political, economic, social, and environmental factors. Implementation is dependent on these dynamic aspects and is further complicated by the conflicting ideas from the stakeholders involved, delayed licensing, international pressure and resource mismanagement (Cessford, 2004). It often results in minimum and unsatisfactory regulatory compliance. However, the incorporation of sustainability principles into policies and legislation has increased local and national awareness. Sustainable development has become central to mining processes and is now being incorporated into mine rehabilitation and closure procedures while mines are still operational (Hattingh, et al., 2018).

Soil stockpiling, soil replacement, soil amelioration, and revegetation with topsoils are common mine rehabilitation strategies that aim to transform the derelict land (Hattingh, et al., 2018). However, these schemes are not performed for a prolonged period to restore all soil properties such as soil water permeability (Abraha, et al., 2019). The unfertile soil conditions in mined land prevent plant roots from establishing (Karaca, et al., 2018). Thus, additional topsoils with revegetation, or phytocapping, is used to rehabilitate deteriorated mine land by restoring moisture, decreasing the bioavailability of heavy

metals, and improving evapotranspiration (Karaca, et al., 2018; Lamb, et al., 2014; Hrynkiewicz, et al., 2018). It is focused on plant growth specifically in soils covering waste dumps to reduce the volume of water percolating and reacting with accumulated waste within the soils, thereby mitigating the effects of ARD (Lamb, et al., 2014) and associated metal mobilisation. Rehabilitation through revegetation has been shown to effectively reduce the amount of greenhouse gases emitted (less than 1500 tons of equivalent carbon dioxide per year) by CW dumps (Cook & Lloyd, 2012).

Phytoremediation is interlinked with revegetation. It focuses on restoring soil productivity through metal mobilization and extraction by plant growth. Endemic plant species are used to re-create a natural habitat for local fauna and flora. The alternative is high biomass species that easily grow in extreme conditions and offer the potential to process into plant-based products. The plants should ideally be adaptable to poor soil and harsh weather conditions (Navarro-Cano, et al., 2018). Plants native to the mining area are often most suited due to their ability to grow in the specific climatic conditions which make them more likely to succeed. Phytoremediation and revegetation are often used interchangeably when considering rehabilitation of coal mined land. The strategies are rooted in three scientific fields: plant physiology, pedology, and microbiology (Hrynkiewicz, et al., 2018). Their success is distinctly influenced by the appropriate selection of plant species (Navarro-Cano, et al., 2018), amendment materials (compost, biochar, etc.) to native and topsoils, and biotic soil properties (Sekhohola-Dlamini, et al., 2022). A continuous issue in revegetation is whether the selected plant species and associated microorganisms can establish, withstand and sustain the pressures associated with seasonal changes, and if the soil nutrient content is sufficient.

Bioremediation is a technology developed to degrade, minimize, or transform pollutants in natural environments through biological activity. It is commonly used in wastewater industries and has only recently been incorporated into hazardous waste management systems. The process of bioremediation is dependent on the ability of the selected microorganisms to rapidly acclimate and perform specific metabolic activities that target contaminants. The viability thereof is determined by the pollutant concentration and bioavailability, nutrient availability, and environmental conditions (moisture content, pH, temperature, oxygen levels, etc.) for promoting the metabolic functions, growth, and proliferation of the microbial cultures. Recent research has shown the feasibility of treating mining waste through microbial activity (Sekhohola & Cowan, 2017; Zornoza, et al., 2017). Recently, a patented bioremediation technique, Fungcoal, has been initiated to convert high ash content coal discard to humic substances (used in chemical and agricultural industries) through enforcing plant-fungal symbiosis with C4-perrenial grasses and coal-degrading fungi (arbuscular mycorrhizal fungi and saprophytic ectomycorrhizal fungi) (Sekhohola-Dlamini, et al., 2022). The success of this technology is limited by its strong dependence on establishing a vegetation cover with appropriate plant species and on the quality of the CW. Thus, the rehabilitation strategy is focused only on the biocatalysts within the rhizosphere (to eventually produce a soil-like substrate), disregarding overall Technosol quality for sustained rehabilitation with multiple suitable plant species.

Bio-stimulation is an extension of bioremediation through optimising the environmental conditions by applying limiting nutrients such as carbon, oxygen, nitrogen, and phosphorus to stimulate existing

microorganisms (Adams, et al., 2015). Bioremediation and bio-stimulation have been tested in several applications and although the biological approach to remediation in the mining sector seems promising since they are directly related to soil quality, it is yet to be established. This project investigated vegetational growth in biostimulated-CW with exogeneous microorganisms and if the fabricated soil is capable of reducing the metal(loid) content.

2.2.2 *Achieving Low Environmental Impact Mine Closure*

Mine closure is the final stage in the mining lifecycle. It can only be reached once rehabilitation of the land site during the decommissioning and post-mining stages is classified as successful and complete (Hattingh, et al., 2018). At this point, the land can be commissioned to a new owner. When achieving this final goal, the land complies with the criteria set in all three pillars of sustainable development (environmental, social and economic). The overarching aim of rehabilitation strategies is thus, to restore the mine site to provide a land that is financially viable for investment whilst simultaneously having the capacity to be prosperous for environmental and social development. However, site relinquishment is rarely achieved due to inadequate or failure of administered remediation, rehabilitation or regeneration schemes of the mine land and mining waste. The ineffectiveness of current mine remediation strategies is evident when considering statistics concerning greenhouse gas emissions from mining waste dumps (Cook & Lloyd, 2012). The sand covered dumps continue to burn at rates similar to unrehabilitated waste dumps. Therefore, alternative mine rehabilitation methods are required that are implemented from initial land disturbance until the decommission phase of the mine site (Hattingh, et al., 2018).

Restoration of degraded post-mine sites is dependent on the landscape needs (Hattingh, et al., 2018). In 2018, a site-specific rehabilitation framework was proposed by the Land Rehabilitation Guidelines for Surface Coal Mines. It outlines an iterative process consisting of four stages: 1) planning, 2) implementing, 3) monitoring, and 4) refining, correcting, and re-planning. The Environmental Impact Assessments (EIAs) examine the environmental risks associated with coal mine development. Legislated Environmental Management Plans (EMPs) for mines are based on risk mitigation. Both these tools provide guidance for, and bring attention to, the economic, social and environmental value of waste management systems. Waste management schemes developed from EIAs and EMPs should prioritize sustainable development to minimize deleterious environmental impacts. However, in practice the environmental assessment tools are often reported unsuccessful in ensuring compliance, governance, and implementation due to limited technical and scientific information, a lack of willingness to regulate the proposed mitigation strategies, and its inability to facilitate on-site monitoring programs (Eberhard, 2011; Morgan, 2012).

Consequently, waste management does not have a one-glove-fits-all solution, and ineffective regulation contributes to the issue of current mine closure strategies. It is unlikely that an insular, rigid and ‘added-on’ technology will be able to solve this problem; however, using innovative, proactive, and flexible technologies in conjunction with one another may hold multiple benefits. This encompassing approach is a characteristic of the circular economy principle for sustainable mine waste management systems.

2.3 Circular Economy Principle in the Mining Sector

The increased awareness of sustainability in mine development has shifted the focus from linear economy principled waste treatment and waste disposal schemes such as ARD treatment facilities (Taha, et al., 2017) to preventative methods for controlled resource management and effective waste management with mitigated impacts on the environment (Kinnunen & Kaksonen, 2019). These methods are based on zero waste strategies and waste valorisation which form part of the circular economy principle.

The circular economy principle is based on an economic framework where waste and additional by-products are placed back into a process for the cyclic use of materials in a system (Lebre, et al., 2017). It identifies strongly with industrial ecology through cradle-to-grave use of materials (Jelinski, et al., 1992). Reusing and repurposing the discards are cost-effective approaches to waste management and it simultaneously encourages sustainability principles. When successfully implemented, the volume of primary resources used and/or overall volume of waste generated in the processes are significantly reduced (Amaral Filho, et al., 2020; Lebre, et al., 2017). Therefore, the cyclical use of materials prolongs the lifetime of resources to improve the process economy and resource efficiency.

In industries such as food and beverage and wastewater, sustainable development motivates for implementation of the circular economy principle to reduce the number of initial resources brought into a specific system. In the mining industry, the focus is on minimizing the volume of waste and emissions produced, maximising the recovery of the primary product (in this case, coal) and using waste resources to replace other primary resources such as stone or sand. Mining waste is often disregarded since the value of the material contained in the final product overshadows the material losses in the product value supply chain. However, the circular economy demands changes in the value chain and thereby a different perspective on process resources. When implemented at mines, minerals would be excavated without exceeding environmental limits and resources would be utilized to their full potential (Lebre, et al., 2017). Further, the decreasing grade of many mining resources is highlighting the value of the primary product in residual waste materials, especially historic materials.

Repurposing the mining waste aligns with the United Nations' Sustainable Development Goals (SDGs), and has potential to contribute to achieving SDG 8, 9, 11, 12, 15 and 17, by closing the loop and creating jobs, by utilising otherwise wasted resources, by halting and reversing land degradation and by promoting the development and implementation of environmentally sound technologies (Department of Statistics South Africa, 2019). Multiple investigations on valorising mining waste from open-pit and underground coal mines have been performed, and demonstrated its feasibility. Publications by Amaral Filho et al. (2020) and Weiler et al. (2020) investigated desulfurised coal waste amended with organic material to fabricate Technosols that can be used as a sustainable topsoil and as a cattle feed source, respectively. Kinnunen & Kaksonen (2019) confirmed that research on methods for integrating the circular economy principle in the mining sector globally through waste valorisation, is lacking. However, it corroborates research by Weiler et al. (2020) and Amaral Filho et al. (2020) by suggesting additional applications to reuse tailings in 3D printing and as a mineral matrix in geopolymers (Kinnunen & Kaksonen, 2019). Taha et al. (2017) demonstrated this by developing a feasible process for producing eco-friendly fired bricks

from coal tailings and residual coal from the mineralized waste. Potgieter & Truter (2010) reused class F fly ash from a South African colliery combined with chicken manure to rehabilitate the post-mined land, and reported that higher shoot biomass was achieved using the alternative amendments compared to lime and chemical fertilisers. Thus, fabricating Technosols from coal processing and other waste sources is an example of using the circular economy principle to produce a value-added product (Macia, et al., 2014).

2.4 Technosols

2.4.1 *Design Considerations*

The World Reference Base (WRB) for Soil Resources classify Technosols as engineered soils with parental materials (primary waste sources) derived from anthropogenic activities and pedogenesis influenced by their technical origin. Pedogenesis includes chemical, physical and biological processes that play a role during soil formation. Technosols are designed on the principle of waste valorisation to reduce the volume of waste accumulated and to enhance rehabilitation of degraded land through accelerated formation of a fertile soil (Weiler, et al., 2018; Zornoza, et al., 2017). Therefore, Technosols form part of the circular economy principle (Macia, et al., 2014).

To create a soil-like substrate, Technosols are fabricated according to specific design considerations to ensure the desired outcomes such as sustaining plant growth as a topsoil. Technosols are comprised of parental material and amendments. To enhance the fertility and functionality, bio-stimulation through amendments are required to provide the nutritional needs and impact structure based on the pre-defined purpose. To increase soil performance and effectiveness, microbial communities associated with healthy soils are often added through bioaugmentation techniques.

For a technology or a process to be classified as feasible, it must comply with predetermined criteria. It must be simple, efficient, economically viable and environmentally friendly (when valuing the principle of sustainability). Implementation costs for Technosols are dominated by raw material and maintenance costs. Multiple studies have been performed on the technical feasibility of Technosols as a tool for waste management. Firpo et al. (2014) established a framework for constructing fabricated soils from coal mining waste amended with biosolids and steel slag. Novo et al. (2013), Hernan Fernandez et al. (2016), Sekhohola et al. (2017), and Amaral Filho et al. (2020) directly investigated the feasibility of using mining or urban waste amended Technosols to sustain plant growth without negative socio-environmental impacts. In most studies, plant growth experiments were performed as a measure of its potential. These experiments were performed under controlled conditions. The variations in parental material amendments and differences in plant growth experiments and characterization methods all led to results that support the feasibility of using a Technosol as a waste management tool. However, these studies do not explore the fabricated soil microbial communities and their activities nor on plant growth in Technosols as a function of ambient (subject to seasonal variation) conditions.

2.4.2 *Parental Materials*

Technosols are not only constructed from coal mining waste but also from degraded soils originating from mining activities and the excavation of rock. Commonly used primary waste sources include fly ash, biosolids (sewage sludge), ashes, and landfill waste (IUSS Working Group WRB, 2015). Research also suggest that Technosols can be implemented in various geographical settings. Deeb et al. (2017) focused on weathered limestone from the Parisian basin. Macia et al. (2014) investigated marine dredged material, whereas Jordan et al. (2017) focused on limestone quarries. Hernan Fernandez et al. (2016) fabricated a Technosol primarily from demolition waste from a construction treatment plant in Gardelegui (Spain), and Neina et al. (2016) focused on tantalite mining in Rwanda. The various parental materials used in the studies are in accord with the WRB definition of Technosols. None of these studies provide justification or procedures for the Technosol's fabrication. However, all investigations presented favourable results for plant growth and phyto-degradation. This is an indication of the scope and vast applicability of Technosols. Yet, Technosol research is in its infancy and has been focused on investigating which Technosol make-up (ratios of waste, native soil, and amendment materials) will result in a fertile and economically viable soil.

The properties of a soil are strongly influenced by the origin and the physical and chemical characteristics of the parental materials. Therefore, initial feasibility studies must be performed to evaluate the potential of using a specific waste source to generate a value-added soil-like substrate. Coal deposits are often associated with silicates and sulfur-containing minerals. To minimise the possible negative impacts of these fractions on the endemic environment, CW are commonly desulfurised prior to fabricating Technosols. Ultrafine coal slurry from the eMalahleni colliery was processed in a two-stage desulfurisation system to produce a low sulfur CW that was used to fabricate the Technosol by Amaral Filho et al. (2020). Weiler et al. (2018) collected coal mine discard from collieries in the Barro Branco seam in Brazil. It was subjected to dense medium separation to split the sulfide-rich and carbon-rich fractions of the waste. Comminution of the parental waste material may also form part of the preparation for Technosol fabrication. Coarse solid CW particles are ground (in a jaw or mill crusher) and sieved to obtain the desired particle diameter that resembles native soil, usually to a particle size diameter below 2 mm (Amaral Filho, et al., 2020; Deeb, et al., 2017; Weiler, et al., 2020). Coal flotation tailings are carbonaceous, slightly alkaline and currently have no economic value, thus also making it a suitable primary waste source for value-added Technosols (Amaral Filho, et al., 2020).

2.4.3 *Amendment Materials*

Primary waste materials cannot be used independently due to poor water permeability (Macia, et al., 2014), imbalanced pH (Firpo, et al., 2014), and a lack of nutrients and soil structure (Amaral Filho, et al., 2020; Weiler, et al., 2020). Hence, the addition of amendments to parental materials in Technosols plays a significant role in ameliorating quality, structure, and fertility of the fabricated soil (Herran Fernandez, et al., 2016). Properties such as acidity adjustment, physical structure improvement (water holding capacity or porosity), alkalescent source, microbial augmentation, organic matter, and nutrients (e.g., Ca, Mg, P, K, and N) provision are important when choosing which parental material amendments to incorporate into Technosols (Firpo, et al., 2014; Jordan, et al., 2017; Novo, et al., 2013; Badenhorst, et al.,

2018). Availability and proximity of the amendment materials to the construction and provisional implementation site also play a role in the selection process of appropriate sources and amounts.

Various waste sources can be used as amendments. Weiler et al. (2018) amended a Technosol with rice husk ash (physical structure ameliorant), sewage sludge (source of organic matter) and steel slag (neutralisation agent and source of micronutrients) that were all available near the mine site. Research by Weiler et al. (2020), Amaral Filho et al. (2020), and Sekhohola et al. (2017) incorporated various ratios of either native topsoil, compost, anaerobic digester sludge, or MR to ameliorate CW-derived Technosols. Additionally, Sekhohola et al. (2017) inoculated the Technosols with a fungal culture. The ratios in which these amendments were added ranged between 2.5% and 5% w/w (combined). The research all reported positive results for Technosol fertility (metal content was remediated to below toxic levels, and Ca, Mg and S nutrient levels were sufficient to support plant growth). Santos et al. (2019) demonstrated that the addition of 3% of organic matter to a Technosol constructed from sulfide-rich mining waste produces alkaline soil conditions, which are advantageous for plant growth of *Lavendula pedunculata* (French lavender) and *Cistus ladanifer* (gum rockrose) at long-term (three years) controlled conditions. This supports the theoretical basis on which amendments are incorporated into anthropogenic soils.

Amendments such as leaf litter and animal manure have been incorporated into arable soils for centuries (Potgieter & Truter, 2010). In recent years, compost has become a popular amendment to industrial and agricultural soils, and to Technosols. It is generally added in a specific ratio to soil, to maintain or produce a soil with a circumneutral pH and an organic matter content of 2% (Weiler, et al., 2018). Hernan Fernandez et al. (2016) suggests that compost is a better suited amendment for Technosols compared to sewage sludge which is often associated with a high heavy metal content. Based on the net neutralisation potential (NNP), the ratio of compost additions can be determined. NNP is the difference between the neutralisation potential (NP) and acid potential (AP) of a substrate. Negative NNP values are an indication of potential for acidification. In the previously mentioned papers, none of the authors justify the ratios in which the amendment materials were added, except for Weiler et al. (2018). Amaral Filho et al. (2020) suggested that the high ash content, low phosphate content, and low organic carbon content of compost resulted in it functioning as a secondary soil source, whereas anaerobic digester sludge and MR had a greater contribution to nutrient amendment in the Technosol. Therefore, it is possible to hypothesize that when amendments are used in conjunction with one another it would produce a more fertile soil (high plant biomass) and potentially a self-sufficient soil since certain amendment types have a higher content of accessible organic matter.

Research by Amaral Filho et al. (2020), Deeb et al. (2017), and Weiler et al. (2018, 2020) concur that the beneficial effects of various amendments to Technosol quality include increased microbial community activities, improved soil structure and aggregations, augmented nutrient cycles (through sulfur speciation), and better plant growth. The research is supported by numerous studies (Firpo, et al., 2014; Neina, et al., 2016; Abraha, et al., 2019) and provides justification for the use of amendments in engineered soils. Nonetheless, the period for which amendments have beneficial effects in Technosols remains unclear as does the change in quality of the Technosol with time. For this, the application rates and dosages of amendments must be investigated over several plant growth cycles.

2.4.4 *Bioaugmentation*

Microorganisms are essential to soil health and plant growth. Research has shown that rehabilitation strategies are often unsuccessful when using asynchronous approaches of either applied nutrients or an inoculum. Hence, for sustained rehabilitation, a simultaneous approach consisting of bio-stimulation and bioaugmentation is required (Adams, et al., 2015). The diverse native microbial communities associated with self-sustaining soils are initially absent in coal-based Technosols (Sun, et al., 2019; Sansupa, et al., 2021). Therefore, by implementing the proposed combination of parental material amendments with exogenous microbial communities, it will establish a stable microbial population that is able to sustain itself (Zornoza, et al., 2017).

Bioaugmentation is defined as the process through which a specific environment is supplied with an external source of specialized microorganisms to augment pollutant biodegradation, replace the indigenous microbial communities (Karpouzas & Singh, 2010), or to improve plant development (environmental diversity, root growth and metal mobilization) (Hrynkiewicz, et al., 2018). The microorganisms are selected for their capability to perform a specific metabolic function in an environment. In agriculture it has been used as an effective bioremediation tool (Da Silva & Alvarez, 2010). Valarani et al. (2003) showed that degradative processes, especially the humification of fresh organic material (e.g., plant leaf litter), were improved when integrating exogeneous microorganisms into soils. According to Hrynkiewicz et al. (2018) inoculation of rhizobacteria in heavy metal contaminated soils enhanced the efficiency of phyto-remediation through improved plant growth *Brassica* spp. (mustard) and metal mobilization.

A different form of bioaugmentation exists that is not based on the establishment of microbial communities rather focused on catalysing biodegradation otherwise restricted due to unfavourable external conditions such as high acidity, toxic pollutants, low nutrient levels, inability to sufficiently retain water, and competitive native microorganisms. For this reason, additional sources of nutrients and physical ameliorants are also applied to the specific environment (e.g., soil) to ensure successful bioaugmentation (Da Silva & Alvarez, 2010). Another technique with significant promise is the use of genetic elements as an inoculum to transfer genes with the desired catabolic potential to native bacteria. This minimizes the previously identified risk of external conditions affecting the exogenous microorganisms' performance (Da Silva & Alvarez, 2010). Genetically modified organisms (GMOs) are acclaimed in bioaugmentation technology. The widespread use of GMOs is a testament to their success; however, the long-term negative impacts remain ill-defined. In agriculture, it could cause the loss of biodiversity from modifications to the microbiome structure through extrinsic gene transfer to the local microorganisms.

Advantages of bioaugmentation in Technosols include rapid acclimation of microorganisms and enhanced degradation of priority pollutants (Da Silva & Alvarez, 2010). Zornoza et al. (2017) reported that the addition of extrinsic microorganisms to Technosols resulted in soil carbon sequestration. The metabolic activity of the microorganisms resulted in calcite precipitation and the degradation of inorganic carbon. This study, along with Sunsupa et al. (2021) and Valarani et al. (2003), motivate the application of mixed cultures au lieu of single bacterial species to ensure prolonged benefit from initial

bioaugmentation and sustained rehabilitation. The mixed culture inoculation strategy promotes microbial diversity and microbial biomass production, whereas single strains, or pure cultures, are primarily used when targeting a specific contaminant.

2.4.5 *Advantages and Disadvantages*

The value of Technosols extends past the mining industry into metropolitan and industrial areas as various sources of waste materials can be used. Research on Technosols also suggests multiple applications thereof. Weiler et al. (2020) showed that when alfalfa (*Medicago sativa*) is grown in Technosols constructed from CW amended with compost, it is a feasible cattle feed. Amaral Filho et al. (2020) successfully investigated the feasibility of integrating Technosols in collieries for the restoration of degraded mine land. According to Amaral Filho, the waste is valorised and the volume of natural soil required for mining rehabilitation is significantly reduced. Hernan Fernandez et al. (2014) suggest that phytoremediation through Technosols formulated from demolition waste, steel slag and compost is a suitable solution for the lack of natural soil in vacant municipal areas. This is corroborated by Zornoza et al. (2017), Sekhohola et al. (2017) and Weiler et al. (2018).

Indirect advantages of mine land reclamation include carbon and sulfur sequestration (Weiler, et al., 2018). The use of various waste sources as parental materials and amendments is beneficial because it is available in large quantities and at low cost often neighbouring the mines. It is safe for land application and ensures the reuse of organic matter that would be otherwise wasted (Santos, et al., 2019). Vegetation in CW-based soils helps reduce water loss and any associated detrimental environmental effects (Hrynkiewicz, et al., 2018; Karaca, et al., 2018). Small pores and cracks in the soil surface facilitate oxygen diffusion to the rhizosphere to support biogeochemical processes and plant growth (Cervantes, et al., 2011). Socio-economic and environmental impacts of coal mine related processes are also minimized by:

1. Reducing the amount of topsoil required for coal mine rehabilitation (Amaral Filho, et al., 2020; Sekhohola-Dlamini, et al., 2022).
2. Reducing the volume of CW disposed in dumps (Weiler, et al., 2020).
3. Reducing surface and groundwater contamination (Jordan, et al., 2017).

Technosols are developed based on the sustainable development framework that calls for a circular economy. The challenges of implementing this in the mining industry are linked to the factors inhibiting the development of new value chains (Kinnunen & Kaksonen, 2019). Kinnunen & Kaksonen (2019) suggest that the valorisation of mining waste needs to be a primary development aim long before the site relinquishment phase. This aligns with the Land Rehabilitation Guidelines for Surface Coal Mines (2018) advocating for the concurrent implementation of mine rehabilitation strategies and excavation processes. Therefore, mine restoration and site relinquishment plans involving Technosols must be developed in the feasibility study of the mining project.

Primary disadvantages of Technosols are associated with uncertainty and implementation. Different methods for coal beneficiation lead to variation in waste streams between mines. Thus, opportunities for waste valorisation deviate between mines. Technosol fabrication becomes site-specific thereby limiting quick and simple implementation schemes (Kinnunen & Kaksonen, 2019). The value of implementing

waste valorisation systems into existing mining processes is unknown to stakeholders (Kinnunen & Kaksonen, 2019). The functionality and the contribution of Technosols to the mining sector, to the surrounding environment, to communities, and to future potential investors must first be assessed and justified. Novo et al. (2013) and Firpo et al. (2014) concur that the long-term behaviour of Technosols must be investigated prior to the implementation thereof. Such studies must include assessments of appropriate plant species based on the specific types of parental materials as the success of Technosols is dependent on good vegetational growth (Santos, et al., 2019). Leaching in mine waste-based soils release environmentally sensitive elements to the surrounding area (flora, groundwater, river networks, etc.) causing salt accumulation, nutrient removal, metal toxicity and ARD (Izquierdo & Querol, 2012; Asrari, 2014). Coal discards are associated with high Fe and S contents, therefore, the associated metal mobility and ARD generation are of concern. Investigations on the long-term effects of initial and periodic applications of amendments have not yet been performed. Some amendments may require regular applications and increase the cost of sustaining Technosols as a mine rehabilitation scheme. However, Santos (2019) suggests that when plant species with an economic value (such as *Lavandula pedunculata*) are cultivated in Technosols, the income generated from the plant biomass production could offset the implementation cost.

2.5 Achieving a Self-Sustaining Topsoil

Pedology is the scientific field of soil research discerned in the 18th century. The research has expanded significantly over the last century to include the influence of the Anthropocene age on pedogenesis (Antisari, et al., 2014). Accelerated rates of urbanization and pollution, and the expansion and continuous development of technology, agricultural practices, industrial production systems and mining processes alter the affected soil's quality, fertility and structure (Richter, 2007). Thus, fertility, quality and physical structure of a soil are multifaceted and interlinked aspects. For Technosols to function as a self-sustaining topsoil, the engineered soils must fulfil primary soil functions and prolong a low ecological risk. Additionally, the concentration levels of major elements (carbon, nitrogen, phosphorus, etc.), minerals, and trace elements (e.g., boron and molybdenum) must be balanced and below hazardous levels (Firpo, et al., 2014). When the characteristics for a specific Technosol and its design goal are accurately defined through technological application and scientific knowledge, the Technosol should be self-sustaining by functioning as a circular economy tool.

2.5.1 *Pedogenesis*

Pedogenesis is the mechanisms (chemical, physical and biological processes) that play a role during soil formation from a parental material (minerals, rocks, organic materials or artefacts). A soil can be defined as an active process and response structure (Sauer, 2015), that operates as an open thermodynamic system with energy and material inputs and outputs, biophysical translocations (e.g., dispersion, diffusion, nutrient absorption and decomposition), biogeochemical cycles, and biomass production (Osmond, 1961). This system interacts with multiple interfaces such as the atmosphere, hydrosphere, lithosphere and biosphere. The pedogenesis of any soil is dependent on five interlinked factors: climate, time, relief (topography), parental material and indigenous microbial activity (Osmond, 1961). The

following equation was developed by Jenny (1941) to describe a soil (denoted as s) as a function of the five soil-formation factors:

$$s = f(cl, t, r, p, o \dots) \quad [1]$$

Where, *cl* represents the climate, *t* is time, *r* is relief, *p* represents parental material, *o* is organisms and […] is the unidentified factors that may need to be considered.

The formation factors determine the chemical, physical, mineralogical and morphological characteristics of a soil (Da Silva Rego, et al., 2016; Sauer, 2015); thus, the quality, structure and fertility or agricultural potential of the soil. Soil distribution is influenced by the topography and lithography of the specific area. Consequently, understanding a soil's formation processes and characteristics is key in accurately determining its agricultural potential. The rate of change in soil properties is influenced by these factors that concurrently change with anthropogenic pollutants. Soil chrono-sequence studies are used to evaluate the rates of processes that play a role in pedogenesis. (Sauer, 2015)

The challenge is to accurately quantify the effect or contribution of each of these formation factors, especially the biological activities, on pedogenesis. The assessment of microbial community activity and diversity is complicated by complex microbiome function, diverse and dynamic structure, seasonal influences and spatial variation. Analysing fabricated soils is often used in research to investigate the role of microbial activities and parental materials on soil constitution and purpose (Deeb, et al., 2017).

2.5.2 *Main Factors Influencing Soil Structure, Quality and Fertility*

Good soil health implies effective and undisturbed soil functions. Important soil functions are biomass production, water and nutrient filtration, nutrient accumulation and stabilization (Bonfante, et al., 2019; Nortcliff, et al., 2012), soil aggregation, pollutant degradation and energy transformations (Suzuki, et al., 2005). Various approaches have been developed to analyse soil quality. Assessing and maintaining soil fertility require understanding of soil pedogenesis, soil characteristics and requirements, of the relationship between pedology and hydrology, and of the genesis and development of erosional cycles (Richter, 2007; Valarini, et al., 2003). In literature, a synonymity between soil quality, soil fertility and soil structure exists. It prompts the equal consideration of all soil properties (chemical, physical and biological) for more effective and thorough indications of soil ecosystem functions (Mills & Fey, 2004; Valarini, et al., 2003). This interpretation of soil characterisation is corroborated by Deeb et al. (2017) who examined soil aggregation through the interactive effects of compost, plants and earthworms. The research highlighted the significance of evaluating soil quality through both organic matter content and soil biota.

The pedoderm is the top nutrient rich surface layer of a soil. Abiotic and biotic factors that negatively influence soil quality, first alter the nutrient, humus and moisture content of the pedoderm (Mills & Fey, 2004). Thus, any attempts in maintaining soil quality, should be aimed at conserving the structure and function of the pedoderm. Soil disruption as a result of mining or agricultural activities, results in reduced plant leaf and root biomass, and exposed soil organic material (SOM) that reacts with atmospheric oxygen, decreasing microbial activities and significant reductions in nitrogen levels. Abiotic factors that

modify soil biota, vegetation, electrolyte concentration and nutrient content inherently effect a soil's structural stability (Mills & Fey, 2004). Consequently, limiting a soil's ability to effectively retain moisture and nutrients, and advancing erodibility. In some instances, it results in crusting; a phenomenon described by reduced or collapsed soil porosity when soil aggregates are continuously exposed (Mills & Fey, 2004).

Parental materials and amendments in Technosols are selected for specific characteristics (for example an alkaline pH) to achieve soil fertility. Zornoza et al. (2017) reported that an alkaline Technosol pH minimizes heavy metal (such as aluminium) toxicity through absorption or coprecipitation of insoluble ferric oxyhydroxides with free metal ions. Amaral Filho et al. (2020) concluded that pH levels above 7 in Technosol substrates supported plant growth and reduced the likelihood of aluminium toxicity (problematic at pH below 5.5). This was supported by Botta (2015) who identified aluminium solubility in acidic soils. Botta achieved improved plant growth, microbial activity, nutrient availability and pollutant degradation in slight alkaline soil conditions. Sun et al. (2019) reported pH as the primary environmental variable affecting soil microbiome structure by using a Random Forest ensemble model. The research highlighted the effect of soil pH on the quality and fertility of Technosols; thus, the value of selecting appropriate amendment type(s) and dosage(s).

The influence of earthworms on soil formation and quality is widely published. Earthworms promote soil particle aggregation (Mills & Fey, 2004). Satchell (1955) described the value of earthworm activities in soil through increased macro-porosity, enhanced soil aeration and drainage, and improved soil moisture infiltration. Deeb at al. (2017) showed that earthworms can significantly improve soil aggregates stability, especially in Technosols, and that the known benefits of compost are only observed in the presence of plants or earthworms.

A set of soil parameters was developed for Southern African soil by employing a 1:2M Soil and Terrain Database (SOTERSAF version 1.0) and auxiliary soil profiles that are held in the International Soil Reference and Information Centre – World Inventory of Soil Emission Potentials (ISRIC-WISE) database. It provides valuable information for when conducting biophysical assessments, agricultural process modelling and simulation, ecological zoning and environmental change evaluation (Batjes, 2004). The parameters are listed in Table 2.

Table 2: Soil Parameters as defined by ISRIC Report (2004).

Parameter	Unit
Organic Carbon	mg/kg (ppm)
Total Nitrogen	mg/kg (ppm)
pH	
CEC_{soil}	cmol(+)/kg
CEC_{clay}	cmol(+)/kg
Base saturation	% CEC_{soil}
Effective Cation Exchange capacity (ECEC)	Defined in terms of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and exchangeable H^{+} and Al^{3+}
Aluminium saturation	% ECEC
Calcium carbonate content	%

Gypsum content	%
Exchangeable sodium percentage (ESP)	%
Electrical conductivity (EC)	dS/m
Bulk density	Dry weight per unit volume soil
Coarse fragments (> 2 mm)	Volume %
Sand	Mass %
Silt	Mass %
Clay	Mass %
Available water capacity (AWC)	mm/m, from -33 to -1500 kPa; % w/v

Parameters must be within national legislative limits (Botta, 2015) based on the specific land use (Herran Fernandez, et al., 2016). Prior to Technosol fabrication, amendments and parental materials are characterised based on these and additional parameters. The level of complexity of the analyses is directly dependent on the objectives of the research. Characterisation of the substrates (soil-like mixtures) is also required. The results (such as the heavy metals concentrations) are often more attenuated compared to the separate materials (Herran Fernandez, et al., 2016), as a consequence of the ameliorating effects of amendments to parental materials in Technosols.

Weiler et al. (2018) conducted plant growth experiments with CW amended-Technosols. Results for fertility parameters showed that a high cation exchange capacity (CEC) is favourable in circumneutral soil pH as it facilitated cation cycling for uptake by plants (Botta, 2015). The high CEC minimizes adverse losses associated with leaching. When considering plant nutrients and ions necessary for plant growth, low CEC corresponds to a soil with low organic matter content. Whereas, basic cations result in higher CEC indexes that improve soil fertility (Weiler, et al., 2018; Botta, 2015). Pollution indexes are practical tools for defining the suitability of Technosols for specific applications, and for evaluating pedogenesis of Technosols (Herran Fernandez, et al., 2016). To assess environmental soundness of Technosols, ecological risk parameters such as the Risk Index (RI) are commonly utilised. A RI value below 150 indicates low risk for heavy metal pollution (Herran Fernandez, et al., 2016).

Organic matter (OM) content (%), electrical conductivity (EC; dS/m), and phosphorus, potassium, sulfur concentrations (ppm) are useful to evaluate chemical soil properties (Botta, 2015). Microbial activity has been used as a primary indicator of soil fertility and quality. Valarini et al. (2003) investigated initial and integrated health of a clay loam soil through biological activities of exopolysaccharides and from phosphatase and esterase enzymes. Physical and chemical characteristics of the soil samples were also analysed to support the microbiological fertility parameters. Valarini suggested that biological indicators provide more detailed understanding of soil ecosystem functions compared to other soil quality parameters. Dangi et al. (2011) concur and suggest that the progress of land rehabilitation strategies must be assessed through studying the soil microbiota diversity. In this investigation, soil fertility is evaluated through microbiological activity before and after plant growth. Additional parameters, such as pH and water retention are measured throughout the plant growth experiments to provide a well-rounded profile of soil health.

Seed germination, microbial proliferation, water percolation, and shoot and root growth provide a framework for physical soil properties (Botta, 2015). Soil aggregation (natural porous compounds that form between soil particles of sand, silt, clay and OM) is a soil parameter used to analyse soil quality and its ability to perform primary soil functions. Additionally, soil bulk density, soil porosity, water holding capacity (WHC) and soil texture are commonly characterised when analysing soil physical properties. According to the review on the declining soil quality in South Africa by Mills & Fey (2004), WHC has been the single most popular indicator of soil quality due to the simplified techniques of measuring soil water infiltration rates. However, its effectiveness as a tool for developing management strategies for SOM content is questioned as WHC is not directly proportional to SOM content (Mills & Fey, 2004). Characterising water retention through field capacity before plant growth ensures for accurate irrigation to avoid water loss through boundless percolation, and nutrient and metal(loid) leaching beyond the rhizosphere. Additionally, run-off water from CW-based soils containing sulfidic minerals may generate ARD (Mjonono, et al., 2019). Water retention alters with plant root development and amendment application (Jordan, et al., 2017). The WHC (%) for a Technosol with a South African coal waste to native soil ratio (CW/NS) of 3:1 in the study by Amaral Filho et al. (2020), was more than 10% higher than that of a Technosol constructed from the same ratio of CW and NS by Sekhohola et al. (2017). This is a direct result of the addition of 2% (w/w) MR which acted as a physical ameliorant.

Chemical properties detail the soil nutrient contents that fluctuate with climate conditions, land use and plant growth. SOM is frequently used as an indicator of soil quality (Mills & Fey, 2004; Valarini, et al., 2003) and is pertinent for developing fertile Technosols (Novo, et al., 2013; Herran Fernandez, et al., 2016). It plays a key role in a soil's ability to perform geochemical cycles and to percolate water (Botta, 2015; Weiler, et al., 2018). SOM is an indication of the biologically active components (such as bacteria, earthworms and fungi) and a representation of the concentration of organic nutrients available in a soil (Botta, 2015). It is mainly characterised based on concentrations of carbon (C), nitrogen (N), sulfur (S) and phosphorus (P) within soil (Weiler, et al., 2018). These nutrients are used during vegetational growth and are the first to deplete in agricultural soils.

Soil macronutrients are: nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K), and are important for determining arability for cattle feed production (Badenhorst, et al., 2018). The important micronutrients or trace elements are: copper (Cu), boron (B), zinc (Zn), manganese (Mn), sulfur (S), iron (Fe) and chromium (Cr), that are usually present in lower concentrations (Botta, 2015). Macro- and micronutrient levels are measured to determine if concentrations are above hazardous levels as established by local authorities. The table below lists the standard plant nutrients that are present as cations or anions in most soils.

Table 3: Chemical names, symbols and their nutrient ions present in soil. (Botta, 2015)

Chemical Name	Chemical Symbol	Ion form necessary for plant growth
Aluminium	Al	Al^{3+} (also present in other forms)
Boron	Bo	$H2BO^{3}$-, HBO_3^{2-} (also present in other forms)
Calcium	Ca	Ca^{2+}
Chlorine	Cl	Cl^{-}

Copper	Cu	Cu^{2+}
Hydrogen	H	H^{+}
Hydroxyl	OH	OH^{-}
Iron	Fe	Fe^{2+} (also present in other forms)
Magnesium	Mg	Mg^{2+}
Manganese	Mn	Mn^{2+}
Molybdenum	Mo	MoO_4^{2-}
Nitrogen	N	NO^{3-}, NH^{4+} (also present in other forms)
Potassium	K	K^{+}
Phosphorus	P	H_2PO^{4-} (also present in other forms)
Sodium	Na	Na^{+}
Sulfur	S	SO_4^{2-}
Zinc	Zn	Zn^{2+}

Soil phosphorus (P) facilitates energy transfer during photosynthesis through ATP, which is used for microbial proliferation, plant growth and seed production. Sufficient soil P levels ensure root development, soil agglomeration and quickened plant maturity cycles (Muindi, 2019). Along with phosphorus, zinc (Zn) is a key nutrient for chlorophyll production and radicle (primary root during seed germination) development (Badenhorst, et al., 2018). However, exposure to too high zinc concentrations (above 100 ppm) could result in metal toxicity, thereby inhibiting iron translocation, causing cell disruption and preventing plant root development (Gyana Rout, 2009). Potassium (K) is essential for mitochondrial metabolism, root stimulation, resistance to pestilence, hastened maturity, enhanced inflorescence and biomass yields (Rawat, et al., 2016). Whereas, calcium (Ca) is important for microbial cell development, nitrogen for protein synthesis, and magnesium for chlorophyll production (Badenhorst, et al., 2018). In studies where fabricated soils were amended with sewage sludge, phosphorus and OM concentrations increased, whereas increased calcium, magnesium, manganese and boron levels emanated from incorporating slag into Technosols (Weiler, et al., 2018). Carbon/nitrogen (C:N) levels tend towards equilibrium following the OM decomposition rate (Valarini, et al., 2003). Reductions in N prompts a loss of C, which induces a decrease in SOM. Optimum C:N ratios enable protein synthesis and enhance biological transformations that are necessary for seed germination and plant growth.

Metal contents in soil profiles are essential for evaluating soil toxicity and acidity potentials that influence plant growth and a soil's ability to defer erosion and acid leaching. Metal concentrations in soils are largely related to weathering and the specific parental materials (Da Silva Rego, et al., 2016). To prevent hazardous levels of soil metal(loid) concentrations, local authorities set out national standards as part of the Waste Act (2014) for soil quality. The table below summarizes the maximum screening values for important metal(loid)s in South African soils. It was used for comparison and to infer the feasibility of coal-based Technosols in this study.

Table 4: National Standards soil quality (National Environmental Management: Waste Act, 2014).

Symbol	Unit	Maximum Soil Screening value for all land uses
As	mg/kg	5.8
Cd	mg/kg	7.5
Cr (III)	mg/kg	46000

Cr (IV)	mg/kg	6.5
Co	mg/kg	300
Cu	mg/kg	16
Pb	mg/kg	20
Mn	mg/kg	740
Hg	mg/kg	0.93
Ni	mg/kg	91
V	mg/kg	150
Zn	mg/kg	240
Sulfates	mg/kg	4000

Additional significant characterisation methods for soil chemical properties include pH, total organic carbon (TOC), sulfur speciation, redox potential, and electrical conductivity. Soil TOC is often used to evaluate the decomposition rate of plant material. A low TOC could be from low levels of available plant biomass within the soil and intermittent precipitation (Da Silva Rego, et al., 2016). Sulfide, sulfate and pyritic contents are important to determine ARD potential, especially in mine waste-based Technosols. A slow rate of pyrite oxidation is desirable since chemical and biological reactions transform sulfur species to compounds that are accessible and beneficial for plant growth and microbial proliferation (Weiler, et al., 2018). *Acidithiobacillus* spp., *Leptospirillum* spp., and *Ferroplasma* spp. are known to catalyse pyrite bio-oxidation (Dong, et al., 2020).

The change in redox potential (Eh) of a soil with time refers to potential substrate reduction or oxidisation. High redox values (above 800 mV) are diagnostic of an aerobic environment (Husson, 2013). Husson (2013) suggested that Eh when used along with pH, is a powerful tool to evaluate agronomic conditions necessary for plant growth. Photosynthesis, nitrogen fixation, proteo- and lipo-synthesis are endothermic reduction reactions involving electron transport. The energy released when the synthesized products are oxidised, is used by microbial and plant cells. However, the energy potential of the biotic cells is dependent on the reducing power generated by mitochondria during the Krebs cycle. When these reactions take place, the redox kinetics change and influence the thermodynamics of the predominant reactions. Thus, Eh directly influences soil microbial activity, diversity and development. Aerobic microbes such as *Azotobacter* sp. proliferate well in moderate Eh conditions. Fungi prefer anaerobic reducing conditions with Eh values above 250 mV, whereas bacterial abundances are dominant in highly reducing conditions corresponding to Eh values of 0 mV and lower (Husson, 2013). Yet, redox fluxes show high variability to space and time, especially that of aerobic soils (Husson, 2013). Subsequently, interpretations for soil health and quality are difficult to formulate solely from Eh results.

Useful measures of soil biological properties are microbial biomass, basal soil respiration, earthworm populations, decomposition of OM and activities related to various soil enzymes (Suzuki, et al., 2005; Thiele-Bruhn, et al., 2020; Ezeokoli, et al., 2020). Ezeokoli et al. (2020) addressed the dilemma of limited information on microbial community dynamics within reclaimed mine land soils, by investigating bacterial functions and structures of three post-coal mining reclamation soils. It was observed that physicochemical soil characteristics such as pH, sodium and calcium levels positively influenced bacterial communities. Thereby, underlining microbial communities' sensitivity to environmental variables and the

usefulness of microbiota as a bioindicator. This interconnectivity between soil properties is apparent when looking at the role of soil pH, and the influence thereof on soil processes and characteristics. The figure below illustrates the links between soil pH and biogeochemical properties.

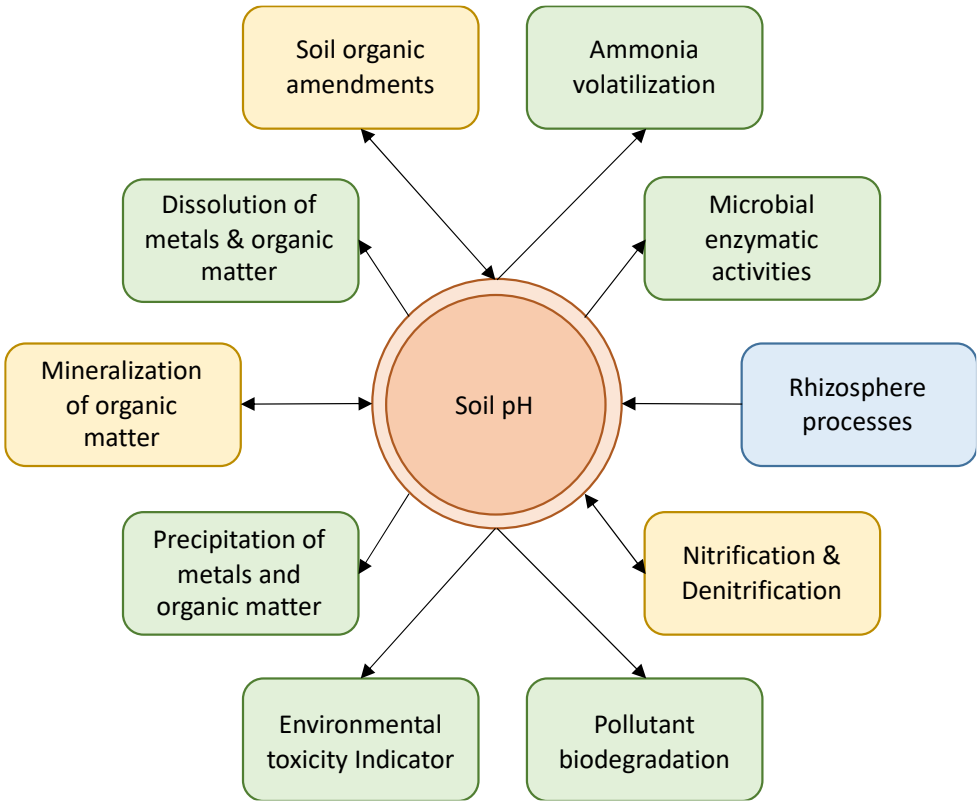


Figure 5: Biogeochemical processes as a function of soil pH. Adapted from Neina (2019).

Soil pH alters through nutrient cycling (e.g., calcium, phosphorus, sodium and magnesium) and carbon dioxide leaching into soil, where, protons and aluminium ions then regulate pH levels.

2.6 Soil Microbiology

The soil microbiome is at the centre of nutrient cycling and plant development, and plays a pivotal part in all ecosystems (Tiwari, et al., 2021). The plethora and heterogeneity of soil microbiomes globally are evident when looking at the diversification of microorganisms within each environment. The majority of soil microbes is bacteria and fungi, followed by archaea, protists and viruses (Tiwari, et al., 2021). Yet, between microbial communities, there resides large variation in functional (gene) and structural diversity of soil microorganisms primarily due to environmental factors (Margerison, et al., 2020). Thereby, emphasizing the pertinence of studying the fabricated soil microbiome to optimise Technosol health and quality.

2.6.1 *Soil Microbiome Structure*

Mechanisms through which soil microorganisms regulate and facilitate microbiome functions are influenced by community structure. Core species (such as nitrifiers and methanogens) are defined as groups of organisms that influence processes (e.g., nitrification) in specific ecosystem functions (Suzuki, et al., 2005). For plant growth and specifically root proliferation, important microbes are arbuscular

mycorrhizal fungi (AMF) (Thiele-Bruhn, et al., 2020), ectomycorrhizal fungi (EcM) (Sekhohola-Dlamini, et al., 2022), and rhizobacteria (Thavamani, et al., 2017) which are unique to and abundant in the rhizosphere.

Bacteria that colonize plant roots are categorised as plant growth promoting rhizobacteria (PGPR) (Margerison, et al., 2020). The structure of PGPR communities is regulated by plant roots' exudates (Tiwari, et al., 2021). Endophytic microorganisms such as Actinobacteria, Proteobacteria, and Acidobacteria inhabit plant organs, predominantly plant roots, where, they facilitate bio-geochemical cycling and improve plant tolerance to environmental stress. The diversity, distribution, and roles of soil protists are intrinsic to a soil microbiome. They include all heterotrophic (nutrition obtained from external organic carbon sources) and phototrophic (nutrition obtained from organic compounds self-synthesized using sunlight) eukaryotes except for plants, animals and fungi (Geisen, et al., 2018). Thavamani et al. (2017) and Thiele-Bruhn et al. (2020) discussed the negative impacts that mining processes have on the diversity of AMF and saprotrophic communities responsible for nutrient and metal accumulation. Thus, PGPR inoculation of mine waste-based Technosols is needed to augment microbial proliferation for sustained plant growth in used mine land rehabilitation strategies. Soil protists can form mutualistic or parasitic relationships with plants. The pathogenic and predatory (phototrophic) forms alter microbial communities' structure by feeding on bacteria and fungi within soils, and/or becoming plant pests (Margerison, et al., 2020). Heterotrophic protists increase soil TOC (Geisen, et al., 2018). Therefore, they are often used as bioindicators for soil toxicity and health. Although abundant within soils, literature on their diversity and structure lacks (Geisen, 2015; Margerison, et al., 2020).

2.6.2 *Soil Microbiome Function*

Biologically active soil facilitates biophysical transformations and contaminant degradation (Botta, 2015). The microbes interact with plants to decompose mineral complexes and disturb organic layers that mobilize previously inert nutrient materials (phytostabilization) (Osmond, 1961; Zornoza, et al., 2017). Microbiome functions include carbon and nitrogen sequestration (shown in Figure 6), precipitation of metals by bacterial and root surfaces and secretions, decomposition of OM, nutrient cycling, plant root development, and pollutant degradation (Novo, et al., 2013; Margerison, et al., 2020; Sansupa, et al., 2021). Microorganisms, especially mesophilic eukaryotes, archaea and bacteria, have greater capacity for bio-oxidation of various materials at temperatures just above room temperature (Ntougias, et al., 2006). Figure 6 illustrates the interactions between soil microbes and plants to facilitate cycling of carbon and nitrogen (life-sustaining elements) in ecosystems.

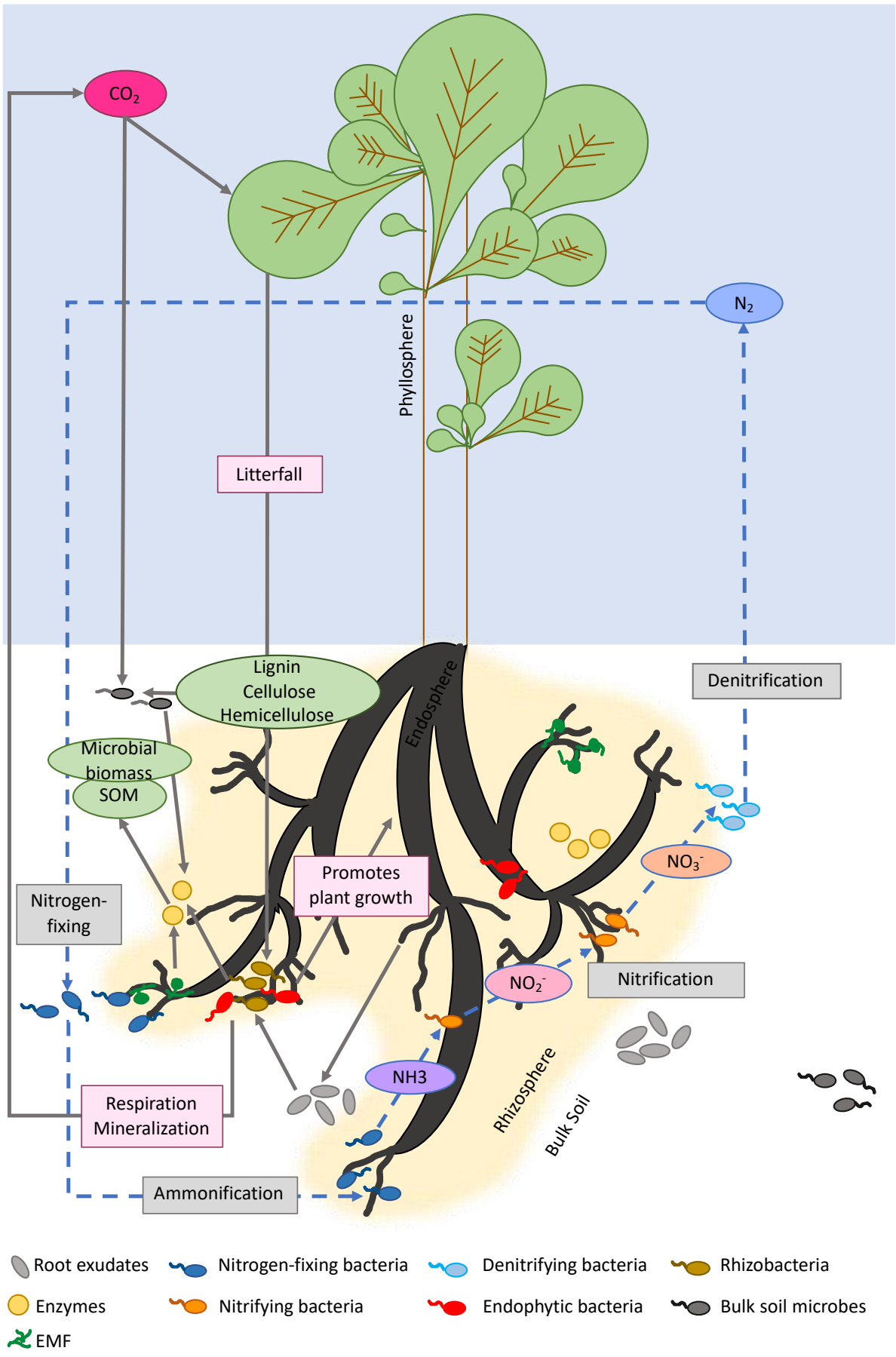


Figure 6: Plant-microbial interactions for the cycling of carbon and nitrogen. (Own work)

Nutrient cycling, particularly carbon, is regulated by microbial enzymes that breakdown complex forms of nutrients in root exudates (metabolites containing carbohydrates, amino acids and lipids) and leaf litter (lignin, cellulose and hemicellulose) into soluble, mobile forms that are translocated into plant organs or form part of SOM (Tiwari, et al., 2021). Soil microbes release carbon dioxide through respiration which increases soil carbon content (necessary for microbial activity), enhances extraction of heavy metals, and improves soil fertility and plant growth. Thereby, creating a carbon sink and facilitating carbon cycling within the ecosystem.

Nitrogen is essential for soil quality and plant productivity (Moreau, et al., 2019). To fix atmospheric dinitrogen for augmented nitrogen availability to plants and eukaryotes, diazotrophic microorganisms (bacteria and archaea) are needed. The microbes perform biological nitrogen fixation (BNF) by forming asym- and symbiotic relationships with plants. Some plant species assimilate more nitrates than ammonia (Hirsch & Mauchline, 2015). Gaseous nitrogen is reduced into ammonium by nitrogen fixing soil bacteria or archaea. Sterngren et al. (2015) observed that nitrogen fixation through archaea, rather than nitrifying bacteria, is more important in grasslands with poor quality soils compared to fertile soils. When a symbiotic relationship between plants and N-fixing bacteria is absent, cyanobacteria (free-living, oxygenic photosynthetic bacteria) are responsible for the soil nitrogen input. Following ammonification, nitrifying bacteria (e.g. cyanobacteria, Azospirillum spp., *Nitrobacter* spp., Herbaspirillum spp., *Nitrosomonas* spp.) and archaea oxidise ammonia (reduced form of atmospheric nitrogen) into nitrites, then to nitrates (Margerison, et al., 2020). Whereafter, denitrifying bacteria transform nitrates back into nitrogen (denitrification) to complete the nitrogen cycle.

Additional nutrient cycling, such as phosphorus and sodium, is also regulated by soil microbes. AMF produce external mycelia to form a mycorrhizal network (Tiwari, et al., 2021). This enhances nutrient and water uptake within a soil matrix, by increasing a plant roots' sorption surface area (Margerison, et al., 2020). It also ensures spatial occupation where pathogens could have resided to reduce a plant's susceptibility. AMF proliferation is supported by photosynthates (carbon based product of photosynthesis) (Margerison, et al., 2020). Hence, AMF is pertinent to carbon cycling and creating a carbon sink within the soil microbiome. EcM communities synthesize extracellular enzymes for nutrient breakdown of complex organic substrates (Sekhohola-Dlamini, et al., 2022). Yet the rate of contaminant substrate breakdown and metal immobilization is dependent on soil quality. Thus, referring back to the challenges associated with the Fungcoal technique. PGPR facilitate phosphate solubilisation and synthesize phytohormones and siderophores pertinent to metal mobilization (Tiwari, et al., 2021).

Endophytes (microbes on plant roots), rhizobacteria and fungi promote plant development that indirectly enhance metal sequestration and precipitation prospects. Microbial-assisted plant development is done through three sets of mechanisms all facilitated by PGPR. These mechanisms are (i) nutrient cycling (nitrogen fixing, metal mobilization), (ii) environmental stress tolerance (pathogen attacks, salinity, pollution), and (iii) enzyme (1-aminocyclopropane-1-carboxylate deaminase or ACC deaminase) and phytohormone (indole-3-acetic acid or IAA) synthesis (Wood, et al., 2016). Phytohormones are signal molecules that play a key role in all plant growth processes; from embryo to reproductive development. Here, IAA is especially important in lateral root growth which correlates to available surface area for

water, minerals and metals uptake. Ethylene is a phytohormone used for metabolism and cell growth regulation. 1-Aminocyclopropane-1-carboxylic acid is hydrolysed into alpha-ketobutyric acid and ammonia (carbon and nitrogen source for microbes) by ACC deaminase to reduce and regulate ethylene levels. The presence of metals in soils are often linked to an overproduction of ethylene, which has an inhibitory effect on plant growth. Therefore, bioaugmentation with PGPR and strains that produce IAA and ACC deaminase, will promote root and shoot growth and indirectly benefit phytoextraction (Hrynkiewicz, et al., 2018). Figure 7 graphically summarizes the function and structure of a soil microbiome for microbial-assisted plant development through nutrient and metal translocation.

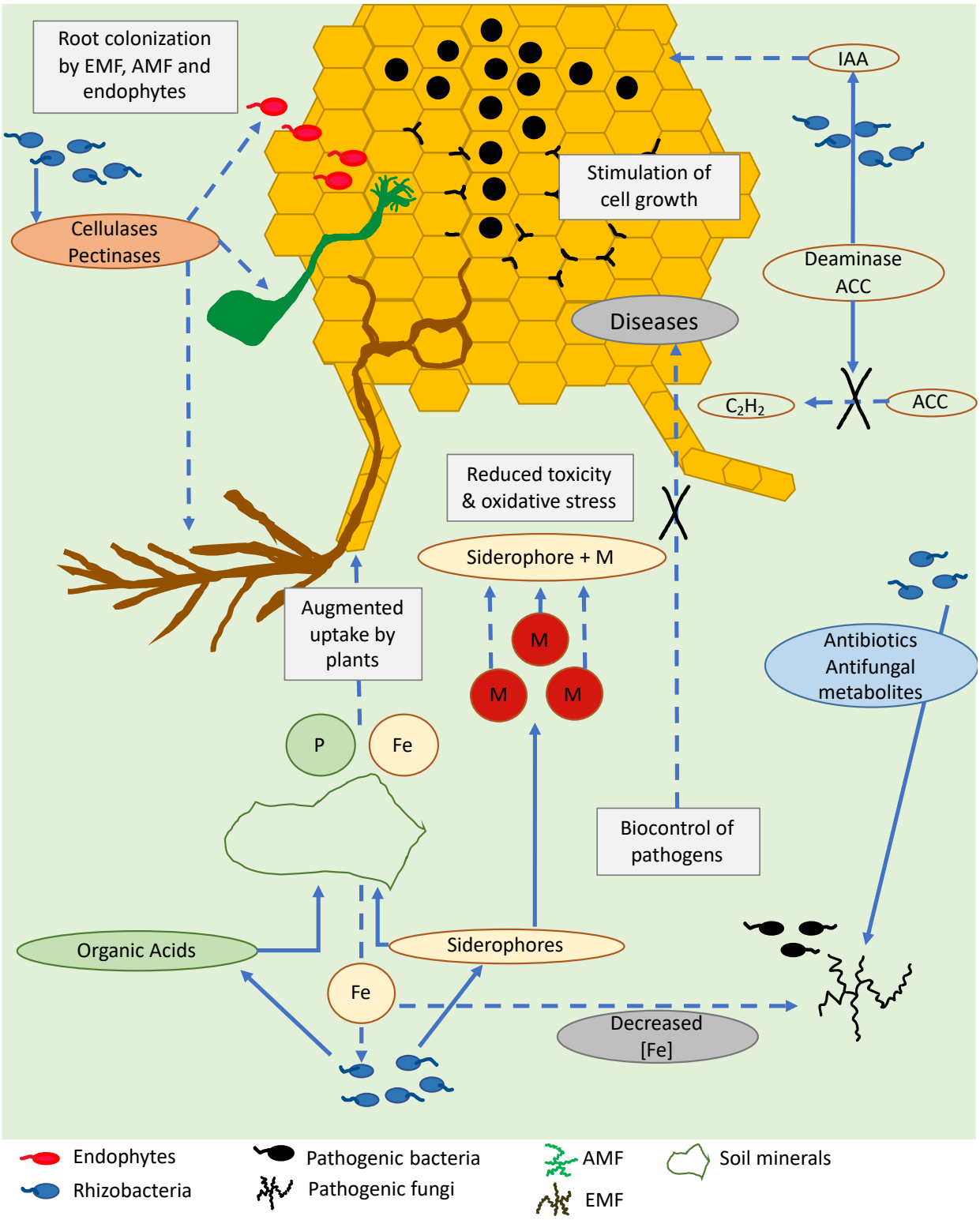


Figure 7: Plant-microbe interactions for nutrient uptake and metal mobilisation. Adapted from Hrynkiewicz et al (2018). Where, ACC is 1-aminocyclopropane-1-carboxylic acid, Fe is iron, IAA is indole 3 acetic acid, M is metal, and P is phosphorus.

To maximise coal-based Technosol health, the engineered soils can be inoculated with microorganisms such as *Bacillus, Enterobacter, Rhizobium and Pseudomonas* that are capable of translocating metals and

support plant growth (Wood, et al., 2016; Sun, et al., 2019). To ensure proliferation of these microbes, the amendments to the Technosols must be optimised accordingly.

2.6.3 *Technosol Microbiome*

Dangi et al. (2011) identified gram-positive and gram-negative bacteria, eubacteria, fungi, AMF, and actinomycetes in reclaimed mine soils, while Thavamani et al. (2017) discussed the prolific nature of acidophiles (archaea and bacteria such as *Acidobacterium capsulatum*) in soils with acidification tendencies. It provided an overview of soil microbial diversity in mined sites. It included nitrospira (iron-oxidizing bacteria such as *Ferroplasma acidiphilum*), bacteroidetes (e.g., *Flavobacterium sp*.), proteabacteria (alpha-, beta- and gamma-proteabacteria such as *Acidithiobacillus spp*. and *Acidiphilium spp*.), actinobacteria (e.g., *Ferrimicrobium acidiphilum*), and firmicutes (iron-reducing and sulfur-oxidizing bacteria, such as *Sulfobacillus acidophilus* and *Alicyclobacillus pomorum*). However, microbial diversity in Technosols, especially that of CW-amended Technosols, is ill-defined (Sansupa, et al., 2021). Technosol microbial populations will vary from native mine soils, but microorganisms reported by Thavamani et al. (2017) provide a good framework to build upon when undertaking this investigation.

Microbial proliferation is a concern in metalliferous soils. Therefore, incorporating selected efficient microbes through a soil microbial inocula (SMI), could potentially result in microbial colonization and sustained rehabilitation of degraded mine soils. Moreira-Grez et al. (2019) and Thavamani et al. (2017) suggested an inoculum comprised of PGPR, nitrifiers and phosphate-solubilizing (such as *Pseudomonas* and *Azotobacter*), AMF, and phosphate- and potassium-solubilizing bacteria for soils fabricated from mining waste. Nonetheless, inoculum selection should be based on the Technosol materials and target pollutants.

Ezeokoli et al. (2020) determined that coal mine site soils had the lowest bacterial operational taxonomic unit (OTU) richness and diversity compared to control soils by analysing microbial enzyme activities and using 16S rRNA gene sequencing. Yet, of the classifiable OTUs, the most abundant phyla were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Planctomyces, and Verrumicrobia. And the dominant genera were *Acidobacter, Sphingomonas, Candidatus Udaeobacter, Acidothermus, Bacillus, Bradyrhizobium, Burkholderia-Cabelleronia-Paraburkholderia, Candidatus Xiphinematobacter,* and *Conexibacter* (Ezeokoli, et al., 2020). These species facilitate nutrient cycling, enhance environmental tolerance, support plant root growth and reduce plant susceptibility to pests (Ezeokoli, et al., 2020). *Candidatus Udaeobacter* is commonly found in nutrient deficient soils, subsequently making it an ideal biomarker species for observing soil quality over time. The study concluded that there is large bacterial genetic diversity within coal mine soils, but less community functional diversity. The results for the enzyme activities (urease, beta-glucosidase, acid and alkaline phosphatases) of coal mine soils were similar to values obtained for agricultural soils. It was also found that activities increased with soil age. Thereby, implying great value and potential for proliferation of microbial communities in CW-based fabricated soils. Ezeokoli et al. (2020) further motivates for the use of bioaugmentation in coal mine rehabilitation. Further research is needed to close the knowledge gap on the microbiome function and diversity in reclaimed soils at coal mines in the eMalahleni area.

Gupta et al. (2018) investigated the potential of Firmicutes as a remediation strategy for soils exposed to copper mine drainage in India. It validated the role of these microbes in metal reductive processes, specifically in their ability to reduce sulfate and iron. Thus, for mine rehabilitation, bioaugmentation within the phylum of Firmicutes with *Bacillus*, *Arthrobacter*, and *Streptomyces* species are recommended as these species are capable of thriving in various conditions, whilst facilitating necessary biogeochemical processes (Sansupa, et al., 2021). Sun et al. (2019) investigated the response of soil microorganisms in coal mine subsidised areas with vegetation. The dominant genera in these areas were *Nitrospira, Pseudomonas, Rhodoplanes, Bacillus, Candidatus, Streptomyces, Steroidobacter, Kaistobacter, Chryseobacterium*, and *Flavobacterium*. *Pseudomonas* and *Bacillus* are nitrogen cycling functional groups; hence, the bacteria would be vital in restoration schemes. The bacterial community composition in the study varied with SOM content, potassium, phosphorus, and with hydrolysable nitrogen levels. Whereas, the distribution of fungi was strongly influenced by soil pH. It emphasized the susceptibility of soil microbiome structure and function to biotic and abiotic factors.

2.6.4 *Methods Traditionally Used to Study Soil Microbiology*

Soil microbiome characterisation is complex in nature due to the soil property interdependence (Thiele-Bruhn, et al., 2020). Few techniques for microbiological indicators have been standardised, generating irregular results especially when non-standardised approaches are employed. Methods traditionally used to study the soil microbiota diversity are focused on identifying functional groups, determining species richness and tracking core species. Soil ecosystem functions differ between functional groups, and the relationship between genetic and functional diversity of these groups is complex with data often generating inconclusive results (Suzuki, et al., 2005). Techniques based on fatty acid analysis, or DNA and RNA analysis, are commonly used to study species richness. One of these methods, sequencing analysis of 16S ribosomal RNA (16S rRNA) genes for bacteria and archaea (Thavamani, et al., 2017), is also often used to characterise linkages between microbial groups. Analysis on 18S rRNA genes can produce data on protists. Although, these techniques are popular in microbiology, Thiele-Bruhn et al. (2020) question the reproducibility and reliability of such DNA and RNA extraction methods as the results are based on small samples of soil. Nonetheless, the dynamic aspects of these methods of analysis are preferred (Aira & Dominguez, 2010).

A multivariate analysis is effective for identifying the differences in soil biota between experimental and control substrates (Dangi, et al., 2011). Dangi et al. (2011) performed a phospholipid fatty acid (PLFA) analysis with the fumigation-extraction method to examine microbial community structure in reclaimed soils. Individual PLFA markers are used as fingerprints to identify and quantify different microbial groups. Total microbial biomass is then calculated as the total concentration of all fatty acids in each sample. Suzuki et al. (2005) suggested fatty acid methyl ester (FAME) as an alternative method for assessing soil biota structure. The research implies an advantage over PFLA, as the fatty acids can be sampled from neutral lipids, glycolipids and phospholipids to provide a more detailed characterisation of the soil microbiome. Other popular analysis techniques include substrate induced respiration, fumigation-incubation, ATP, and arginine ammonification analysis (Aira & Dominguez, 2010).

Botta (2015) suggests that an acidic pH leads to a decreased rate of microorganism proliferation; thus, reduced microbial activity. High concentrations of nitrogen and organic carbon denote active soil microbes (Dangi, et al., 2011; Herran Fernandez, et al., 2016). The microbial composition, function and diversity data must correlate to environmental assessment results to fully evaluate the impact of environmental and technical processes on soil biota (Thiele-Bruhn, et al., 2020).

Neina et al. (2016) evaluated the rise in ergosterol, amount of microbial biomass, and microbial decomposition of carbon and nitrogen in a Technosol to determine the properties directly linked to the effects of specific native amendments (farmyard manure and leaf litter of three local plant species). According to Baath (2001), the results for microbial biomass nitrogen (MBN), microbial biomass carbon (MBC) and ergosterol is a useful estimate for the fungal activity and to assess the microbial proliferation in a living soil. Microbial biomass as a measurement of microbial activity within a substrate, is an optimizable technique suited to various sample types. For soil samples, this method is based on measuring the first maximum carbon dioxide emission of the nutrient amended soil after an incubation period of six hours (Ananyeva, et al., 2011). Microbial respiration is induced by providing the substrate with a readily available nutrient and energy source (primarily glucose) in excess to catalyse microbial activity (Aira & Dominguez, 2010). The substrate induced respiration (SIR) is indicated as milligrams of carbon released per kilogram of soil (Boyrahmadi & Raiesi, 2018). The method for rhizospheric microbial biomass was optimised at an incubation temperature of 22°C; the mean temperature of topsoils in Western European during summer (Ananyeva, et al., 2011). Downfalls to this method are the redundancy and sensitivity to temporal effects. Even so, research by Neina et al. (2016) and Baath (2001) emphasized the use thereof in fabricated soil studies with multiple dynamic factors.

Hydrolytic enzymes from soil microbes are pertinent to OM breakdown, nutrient cycling and humic acid production (Sekhohola-Dlamini, et al., 2022). Fluorescein diacetate (FDA) analysis measures the hydrolysing abilities, thus; the decomposing abilities of the soil microbiota (Schumacher, et al., 2014; Adam & Duncan, 2001). Microbial membrane bound and free enzymes such as lipases, esterases and proteases hydrolyse FDA into fluorescein which can be detected through spectrophotometry or through measuring fluorescence (Schumacher, et al., 2014).

Microbial diversity within soil samples can be determined through the recognition technology; fluorescence in situ hybridisation (FISH). This method detects microorganisms using specific staining of complementary DNA/RNA strands, and fluorescent oligonucleotide probes. The targeted DNA is hybridized to the labelled DNA probe which contains a specific gene (in denatured single strand form) and originates from plasmic cosmids (Cui & Li, 2016). After hybridization, the target DNA can then be detected with fluorescent microscopy depending on the labelling method (such as PCR). This method is gene-specific and applicable to many different scenarios to identify the transcriptional gene dynamics and functions within biological processes, but the efficiency thereof is highly dependent on the labelling system (Cui & Li, 2016). In addition, background fluorescence from heterogeneous samples such as soil, influences the readings and is difficult to account for.

Thiele-Bruhn et al. (2020) advocate for the selective and repeatable metagenomics approach involving quantitative real-time PCR (qPCR). This approach examines microbial diversity during plant growth and geochemical transformations such as nitrogen sequestration, phosphorus transformation and pollutant degradation. qPCR correlate marker gene sequences (e.g., ammonium monooxygenase gene for quantifying nitrifying microorganisms) to the specific ecosystem functions and can therefore be used to assess soil water percolation and greenhouse gas emissions (Thiele-Bruhn, et al., 2020). The variable V3 to V4 region of the 16S rRNA gene is used as a barcode for determining bacterial sequences and communities through 16S rRNA sequencing. Community DNA is extracted from a substrate and the concentration of the extracted DNA is then quantified. This is a prerequisite to PCR amplification and when employed with it, these techniques can describe the metabolic activities as a potential of microbiome functions of the indigenous microorganisms (Gupta, et al., 2018). It involves the amplification by polymerase chain reaction (PCR) specifically targeted at the 16S rRNA gene. Whereafter the profiles are often displayed on agarose gels. In soil microbial work, universal primers are used to confirm the presence of bacterial species in the substrates (Sansupa, et al., 2021).

2.7 Plants and Their Functions in Mine Rehabilitation

2.7.1 *Plants for Mine Rehabilitation*

Herbaceous plant species are commonly used for initial rehabilitation of mining tailings. The thin leaves ensure high and stable transpiration rates, and the high growth rates associated with common grasses ensure a vegetative cover to capture soil moisture and minimize soil erosion (Karaca, et al., 2018). Li (2006) suggests that adaptable and rapidly growing grasses and legumes are most suited for mine rehabilitation. Research by Van Rensburg et al. (1998) concur that *Eragrostis tef* (Teff) and the perennial grasses, *Cynodon dactylon* (bermudagrass) and *Eragrostis curvula* (weeping lovegrass), show success in the rehabilitation of coal processing sites as these grasses have multiple uses and occurred with the highest frequency. According to Lamb et al. (2014), plants from the Poaceae family (grass family) such as *Dendrocalamus latiflorus* (bamboos) perform well in phytoremediation studies. Whereas, Zornozo et al. (2010) successfully conducted a phytostabilization study with *Lupinus albus L.* (white lupin) where the initial soil mercury content was significant. Navarro-Cano et al. (2018) suggest using nurse plants, such as *Osyris lanceolata* (African sandalwood) and *Atriplex halimus* (saltbush), that act as pioneering plants whilst promoting the establishment of other vegetation. Karaca et al. (2018) specify using multiple plant species on the same mining site since each plant reacts differently under environmental pressures and mimics a natural environment of mixed cultures.

2.7.2 *Eragrostis tef*

Teff is an annual cereal, part of the Poaceae (grass) family. It is primarily cultivated in lower parts of Africa for a wheat and sorghum alternative. Domestication of Teff occurred in Ethiopia in the 1800s during food scarcity whereafter the Botanical Gardens of Kew was responsible for the introduction of this 'ancient grain' to other parts of the world (Ketema, 1991; Dame, 2018). Currently, it is found throughout the world; a testament to its ecological diversity (Zhu, 2018).

There are numerous reasons why this grass is popular. It has the ability to grow in low and high moisture areas, yet is best adapted to areas that receive an annual rainfall of 600 mm (AGT Foods Africa Pty Ltd., 2017). Preferred growth conditions for teff are warm climates with temperatures above 18 °C, and well-drained soils with pH levels above 5 (AGT Foods Africa Pty Ltd., 2017). It is tolerant to saline soils. Teff is a low risk, self-pollinating plant capable of growing in harmony with other crops, and a good source of nutrition and high dietary fibre for both humans (used for whole-grain bread, pasta and beer making), horse, and cattle (straws used for animal feed) (Ketema, 1991; Zhu, 2018). Teff is gaining ground for its associated health benefits, and for its low-susceptibility to pestilence during storage (Dame, 2018). The teff seed (or grain) contains 13% protein, 8% fibre, and 2% fatty acids, as well as calcium (180 mg), magnesium (184 mg), iron (7.63 mg) and zinc (3.63 mg) (Gebremariam, et al., 2014). Recently, research on alternative uses for the wasted straws have been initiated. Chufo et al. (2015) successfully studied the potential of teff straws for biomethane production when pre-treated with sodium hydroxide. Gebremariam (2012) investigated its potential as a food and beverage product, with focus on using it in brewing processes or as a gluten-free alternative. The rapid growth rate of this pioneer species ensure land cover and repressed weed growth, aiding in erosion control. The root structures help stabilise soil aggregates and improve water retention.

Phenotypic characteristics of this plant include long, hair-like stems and panicles, with shallow, fibrous roots. During inflorescence, the spikelets (basic unit of grass flowers grouped into panicles) produce seeds that are less than one millimetre in diameter. The maturity period for teff grass is between 60 to 120 days, at which it reaches between 45 to 150 cm in height (Ketema, 1991). Inflorescence, and thus plant development, in teff is influenced by photoperiodism. Teff is classified as short day plants; therefore, the signal to flowering (or heading) is delayed when day length is increased longer than 10.5 hours (Van Delden, et al., 2011). A study on four different cultivars of teff found that when the day length exceeds 15 hours, the grains produced are often barren and the number of leaves are more. Depending on plant growth conditions, an average agricultural production ranges between 4 to 8 tons of dry plant matter per hectare per season (AGT Foods Africa Pty Ltd., 2017).

Teff is a prosperous grass in South Africa, commonly found in the eMalahleni area and often used in mine restoration schemes (Amaral Filho, et al., 2020). The choice for Teff in this study is corroborated by Karaca (2018), Li (2006), Lamb et al. (2014) and Van Rensburg et al. (1998) who reported on the use of grasses to support rehabilitation and phyto-management of mining tailings. Hu et al. (2001) observed that the soil microbiomes of grasslands, such as that of Teff, have a stable and significant carbon sink. Suggesting, that these ecosystems will be able to better withstand environmental stress and climate change compared to other vegetation types (Vicente-Serrano, et al., 2013).

2.7.3 *Mobilisation and Uptake of Metals into Plant Organs*

Rehabilitation by revegetation is dependent on plants and their affiliated microorganisms to immobilize and stabilize metal(loid)s within the soil, to facilitate metal sequestration into above ground plant biomass and to breakdown contaminants (Hrynkiewicz, et al., 2018; Wood, et al., 2016). The plant root structures and rhizobacteria help form soil aggregates, through decomposition of plant litter to produce

organic acids that bind pollutants or change soil pH levels. Finally, limiting metal mobilization within the soils as part of phytoextraction and stabilization. Plant secreted enzymes reduce elements to less toxic forms by performing biochemical (redox, sorption and precipitation) reactions (Hrynkiewicz, et al., 2018; Sun, et al., 2019).

Appropriate plant species have characteristics of rapid growth, high biomass generation, large root surface area, environmental tolerance, and the ability to accumulate metals. The process of metal mobilisation and uptake into plant organs involves the adsorption of metals onto root surface. Here, plant secretions, such as phytochelatins, amino acids, siderophores and metallothioneins reduce soil pH and increase ion mobility in the rhizosphere and facilitate the sorption reaction, where, bioavailable metal ions move via cell membranes to root cells into vascular cells of the xylem. Ligands such as histidine bind to the metal ions to allow extraction into plant stems and leaves for sequestration in vacuoles. (Hrynkiewicz, et al., 2018)

Metallophytes are plants native to the area that have already established their potential to adapt and tolerate the specific conditions with high soil metal(loid) concentrations (Alford, et al., 2010). In mining rehabilitation, the plant's sensitivity to pH and metal content of the soils must be considered when selecting appropriate metallophytes (Karaca, et al., 2018). Popular phytoremediating plants capable of extracting metals in levels of more than 50 times relative to the soil, are *Thlaspi caerulescens*, *Berkheya coddii* (especially for nickel extraction), and *Sedum plumbizincicola* (effective in cadmium and zinc polluted soils) (Hrynkiewicz, et al., 2018).

Microorganisms form a mutualistic symbiosis with the plants - the key to successful revegetation. When the aim is to reduce soil metal content, revegetation strategies must include phytoextraction (or phyto-sequestration) to mobilise metals into plant organs. This process is illustrated in Figure 8. The bioavailability of metals is increased by rhizobacteria through auto- and heterotrophic leaching and secretion of organic acids (citric, lactic, oxalix, malic, formic, etc.), biosurfactants and siderophores that enable dissolution of metalliferous compounds for direct phytoextraction through pH acidification. Following, sequestration of metals occurs in extracellular polymeric substances (EPS). The EPS are microbially synthesized polymers, creating a slime-like substance of organic compounds (e.g., proteins, lipids and DNA) which forms an ideal environment for biogeochemical reactions (Costa, et al., 2018) and easier metal uptake into plant organs.

Li et al. (2010) observed that the organic acids: formic, acetic, succinic, tartaric and oxalic acid, secreted by rhizobacteria (e.g., *Burkholderia cepacia*) extracted from Pb/Zn mines augmented the phytoextraction process of cadmium and zinc cations into *Sedum alfredii* (Asian perennial herb) plant organs. Recent research highlight the benefits of microbial biosurfactant-assisted phytoextraction through enhanced metal mobilisation (Liduino, et al., 2018; Liao, et al., 2016; Zhang, et al., 2010). These amphiphilic molecules (lipopeptides, sophorolipids, etc.) bind to metals to remove metal ions and increase the bioavailability within the soil matrix. In addition, microbes synthesize metal-binding ligands (siderophores); organic compounds with low molecular weights and a high affinity for iron homeostasis (Wood, et al., 2016). Siderophores are classified by their ligands into groups; phenolates, hydroxamates,

carboxylates, catecholates and mixed, and more than 500 siderophores have been identified. These compounds have an affinity for forming complexes with zinc, copper, cobalt, nickel, chromium, manganese and silver (Sharma, et al., 2018).

Altering the soil pH directly influences autotrophic metal leaching into plant organs. Soil microorganisms, in particular acidophilic bacteria such as *Thiobacillus thiooxidans*, can alter soil pH acidity through accumulating carbon dioxide as a by-product in microbial respiration, or with transmembrane pH homeostasis, where, hydrons are released by ATPase enzymes that catalyse phosphate hydrolysis. This leads to ion exchange to release metal cations from soil metalliferous compounds (Hrynkiewicz, et al., 2018). Figure 8 illustrates the direct microbial mechanisms involved in phytostabilization and phytoextraction which is necessary for mine land rehabilitation.

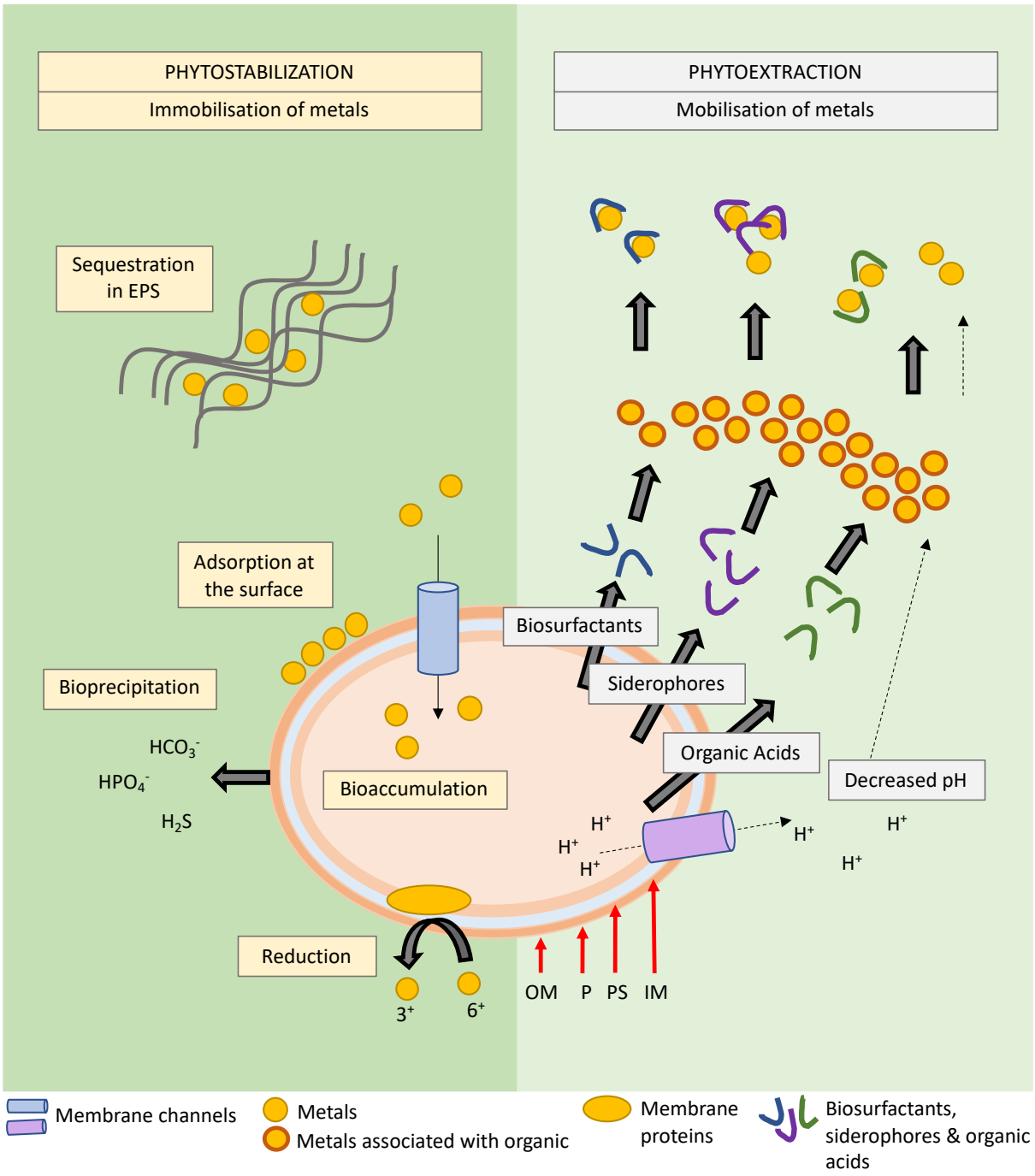


Figure 8: Mechanisms for phytostabilization and phytoextraction. Adapted from Hrynkiewicz et al. (2018). Where, OM is outer membrane, P is peptoglycan, PS is periplasmic space, and IM is inner membrane.

2.8 Research Problem Statement

Mining activities, as well as geochemical factors such as pH and climate changes, directly influence the soil microbiome function and structure. Mine rehabilitation strategies have been mostly unsuccessful as the focus has been solely on the addition of organic matter or chemical fertilisers to increase nutrient content and or increasing pH levels through the addition of chemical amendments. Most of these amendments have shown to be unsustainable. However, due to the complex nature of soil microbiota, little research has been done on the potential use and the effects of microorganisms on the rehabilitation of degraded mine land (Thavamani, et al., 2017). By applying a holistic approach, including assessment of the chemical, physical and biological properties, to study a Technosol through different seasonal growth trials of plant habitation. This is necessary to determine the feasibility of using bioaugmentation and biostmulation of Technosols as a rehabilitation tool for mine sites. Additionally, research on the plant-microbiome relationships needs to be performed to evaluate the development of self-sustaining Technosols. This study aimed to provide insight into the advantages associated with bioaugmentation and biostimulation of Technosols as a sustainable and cost-effective approach to mine rehabilitation.

2.9 Hypotheses

Due to the nature of mine wastes, the endemic microbial population present within these are expected to be limited or negligible. A 'healthy' topsoil is generally accepted to contain a diverse microbial community to facilitate the cycling of key nutrients (nitrogen, carbon, sulfur and phosphorus) within fertile, self-sustaining soils. The following hypotheses relate to the bioaugmentation of amended coal-based Technosols through the addition of a suitable microbial consortia, as inoculum, to increase the soil fertility and structure.

1. Bioaugmentation will increase the nutrient availability for plant growth through the accelerated degradation of organic material added as amendments.
2. Increased microbial activity is also expected to assist in the degradation or bioconversion of possible pollutants within the mine waste.
3. Bioaugmentation of Technosols are expected to result in a more diverse and metabolically active microbiome compared to non-inoculated Technosols.
4. Microbial proliferation following the bioaugmentation of Technosols would depend on the physical and chemical parameters of the material that are used in the Technosol's design.

2.10 Objectives

The main aim of the project was to evaluate the effect of the microbial community introduced into a fabricated soil generated from coal waste with plant growth.

Towards the realisation of this aim, the following objectives were proposed:

1. Investigate if bioaugmentation of a fabricated soil increased the soil fertility and structure, reduced metal(loid) solubility and enhanced plant growth.

2. Evaluate the influence of biostimulation through amendment dosages on the bioaugmentation process (microbial biomass, activity and abundance), soil structure and plant growth in a bioaugmented-Technosol.
3. Determine the abundance and change in soil microbiota within the bioaugmented-Technosols prior to and after plant growth.
4. Assess the feasibility of including bioaugmentation when preparing Technosols to sustain plant growth under endemic site conditions.
5. Evaluate the influence of climate variation on the ability of coal-based bioaugmented and biostimulated Technosols to support plant growth.
6. Consider the feasibility of utilising coal-based bioaugmented Technosols as topsoils to restore mine sites whilst minimising negative socio-economic effects.

2.11 Key Questions

The key questions posed are related to the analysis of the microbial community and activity, soil fertility and physical structure, and ability to sustain plant growth associated with bioaugmented-Technosols. To achieve the objectives set out above and realisation of the project aim, the following key questions were proposed:

1. Was soil fertility and structure increased, the metal(loid) solubility reduced, and plant growth enhanced in a bioaugmented-Technosol compared to the non-inoculated control?
2. How did the microbial abundances change with amendment dosages and plant growth in CW-based Technosols?
3. Did the inclusion of amendments as biostimulation to coal mine waste benefit soil fertility in conjunction with bioaugmentation?
4. Did an increased ratio of organic material as amendment enhance the microbial biomass, activity and abundance, improve the soil structure and support plant growth in a bioaugmented-Technosol?
5. What types of nutrient amendments are available close to the rehabilitation site and how do these potentially influence the microbial activity, soil structure and plant growth in a bioaugmented-Technosol?
6. Are bioaugmented and biostimulated-Technosols a feasible rehabilitation solution towards the formation of self-sustaining rehabilitated soil at coal mine sites?

2.12 Project Outline

To achieve the identified objectives and answer the key questions, a detailed project plan was developed. The milestones required to meet the aim of the study, are illustrated in Figure 9 below.

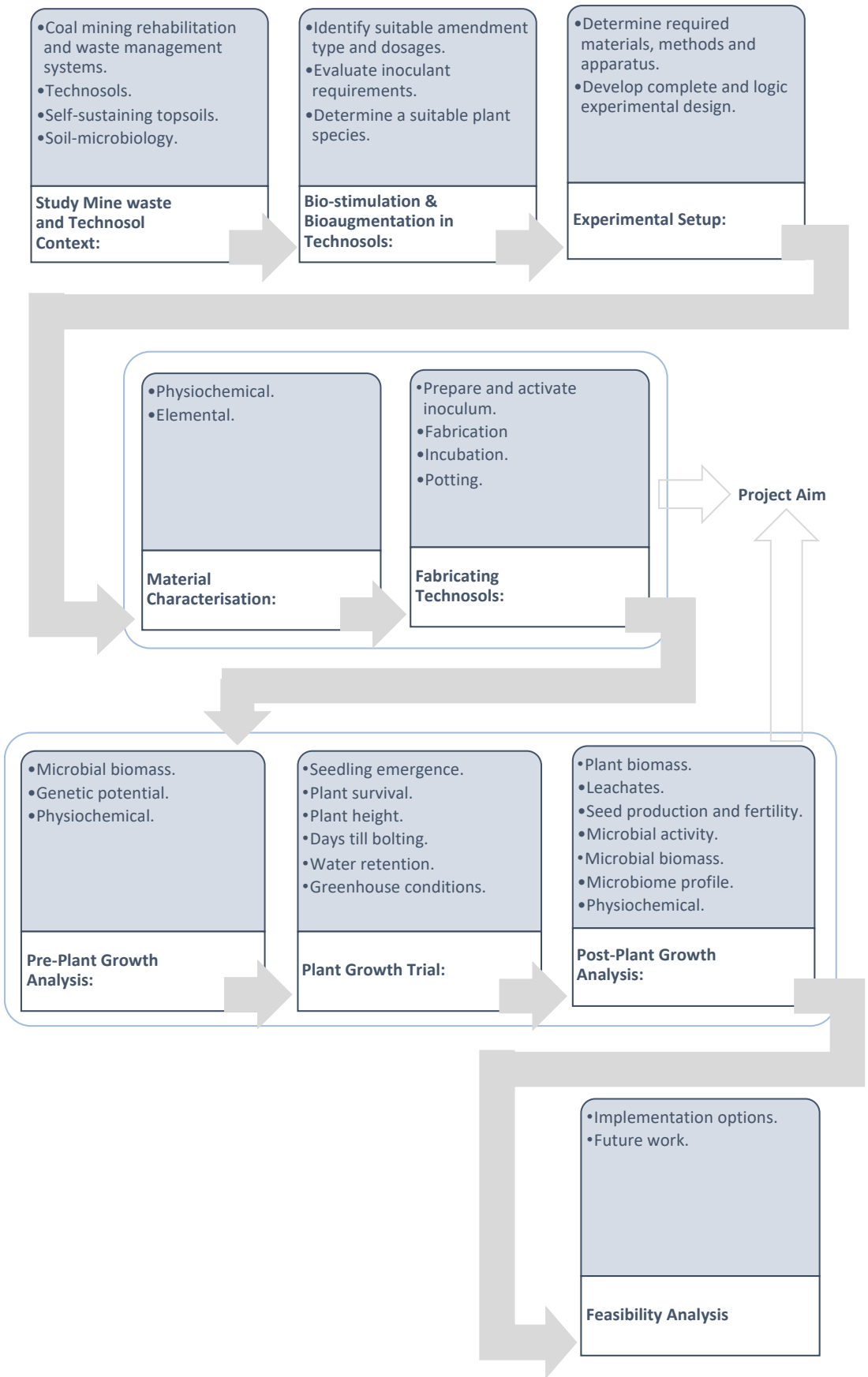


Figure 9: Flowchart of the research approach.

CHAPTER 3

3 METHODOLOGY

The methodology to answer the key questions and achieve the project aim identified in Chapter 2 is detailed in this chapter. The experimental plan was based on the work conducted by Weiler et al. (2020), Amaral-Filho et al. (2020) and Firpo et al. (2014).

3.1 Experimental Plan

Figure 10 summarizes the eight stages in the experimental plan that have been set out to achieve the project objectives detailed in section 2.10. The first stage is material selection, followed by the preparation thereof for characterisation which is the third step of the experimental plan. Subsequently, soils were fabricated based on procedures described by Weiler et al. (2020) for bioaugmented coal-based Technosols. Prior to conducting plant growth trials, the Technosols were characterised for physiochemical conditions and soil samples were collected for soil microbiome analysis. To investigate the potential of the Technosols to function as self-sustaining topsoils, two seasonal teff plant growth trials were performed in a greenhouse (sixth stage of the experimental plan) and pre-determined parameters were monitored throughout the trials. After plant biomass and soil were characterized accordingly, Technosol microbiomes were analysed and profiled using microbial techniques; FDA, SIR, DNA extraction and qPCR as shown in Figure 10.

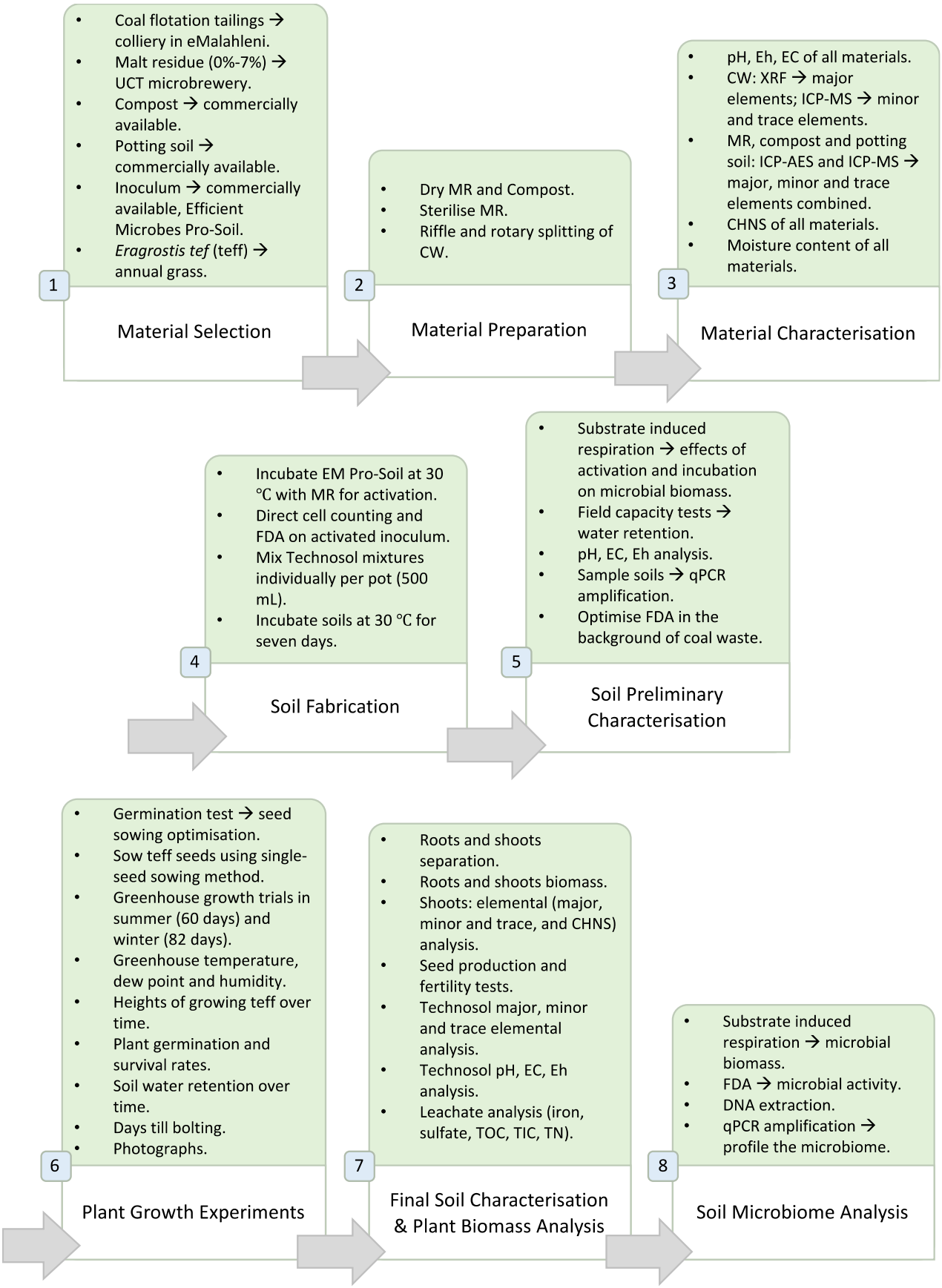


Figure 10: Experimental plan to achieve the project objectives.

3.2 Material Selection and Preparation

Material selection was based on previous Technosol studies that reported ameliorated soil structure and improved plant growth in fabricated soils with unmineralized OM, such as MR, compared to mineralized matter (e.g., compost). Additionally, the increasing costs and impact of commercially available chemical fertilisers and their leachates on the environment and soil bacterial communities (Lebrun, et al., 2021) motivate the use of alternative amendments (Bachman & Metzger, 2008). Bioaugmentation with a commercially available inoculum, Efficient Microorganisms Pro-Soil (EM Pro-Soil), showed favourable results for alfalfa growth and microbial biomass in MR amended CW (Weiler, et al., 2020).

Figure 11 depict the raw materials used in this study. Coal flotation tailings were collected from a local colliery in eMalahleni. Currently this material has no economic value and is disposed of in mechanical waste separation facilities (MWSF). MR (spent grain) was collected after the lautering process from South African Breweries in Cape Town. MR increases soil nutrient availability and water retention (Weiler, et al., 2020). To study the effect of MR addition on Technosol fertility and productivity and to consider various dosages of the amendments in correlation to the study by Weiler et al. (2018), ratios of 0%, 2.5%, 5%, and 7% (w/w) of MR were investigated. Compost and agricultural (potting) soil were used as controls. The compost and potting soil are both commercially available and was purchased from a local agriculture shop.

Coal tailings	Malt residue	Compost	Potting soil

Figure 11: Raw materials and controls used for Technosol fabrication and growth experiments.

The commercially available inoculum, EM Pro-Soil, performed the best in terms of supporting plant growth and augmenting soil macro- and micronutrients contents (Weiler, et al., 2020). The initial inoculum composition as defined by Efficient Microbes cc was; *Bacillus subtilis*, *Bifidobacterium animalis*, *Bi. Bifidum*, *Bi. Longum*, *Lactobacillus acidophilus*, *L. buchneri*, *L. bulgaricus*, *L. casei*, *L. delbrueckii*, *L. fermentum*, *L. plantarum*, *Lactococcus diacetylactis*, *Lactococcus lactis*, *Rhodopseudomonas palustris*, *R. sphaeroides*, *Sacchoromyces cerevisiae*, and *streptococcus thermophilus* (Efficient Microbes, 2006). Bacillus is a genus of bacteria often classified as PGPR (Margerison, et al., 2020), Lactobacillus promotes plant growth through organic matter decomposition (dos Santos, et al., 2020), and Rhodopseudomonas are known for their contribution in soil nitrogen cycling (Knowles, 1982).

Collected MR (2.5 kg) and compost (4 kg) were layered out onto separate large, flat trays, covered with net and placed in a shaded area in a UCT greenhouse to dry at 22 °C for 14 days. Using a spade, the material was regularly turned to ensure even drying. Following, dried MR was sterilized in batches of 250

g at 121 °C using a Hiclave HG-50 Upright Autoclave (Hirayama; Kasukabe, Saitama, Japan) to minimise microbial contamination and ensure microbial proliferation within the inoculum. MR particle size was suitable for soil fabrication and potting, and was used as is to reduce further processing costs if implemented at mine sites. CW representative samples (250 g) from a 40 kg collective sample were prepared using the standard protocol; ASTM D-2013, with riffle and rotatory sample splitters (ASTM D2013/D2013M, 2007). Agricultural potting soil required no processing nor additional preparation.

3.3 Material Characterisation

Coal tailings were characterized using a combination of XRF for major elements (Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti) and ICP-MS for minor and trace elements (Sc, V, Cr, Co, Ni, Cu, Zn, Rb, Sr, Y, Zr, Nb, Mo, Cs, Ba, La, Ce, Pr, Nd, Sn, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Pb, Th, U) to determine if the quantified values would result in engineered soils that are within national legislative limits for arable land (Botta, 2015). The major, minor and trace elements combined (Na, Mg, Ca, K, P, Si, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg, Pb) were analysed for MR, compost and potting soil by total digestion with ICP-AES and ICP-MS complete at SUN CAF. Total contents of carbon, hydrogen, nitrogen and sulfur were determined instrumentally using a Perkin Elmer 2400 CHNS Analyzer. The elemental contents of materials are characterised to determine applicability in vegetational growth and for environmental considerations (Herran Fernandez, et al., 2016). Material bulk densities defined as dry weight per unit volume of soil, considered both the solids and pore space for compaction analysis with dry weights and moisture content, these analyses were performed as protocolled by Lowery et al. (1996).

Physiochemical parameters (pH, EC and Eh) of all materials were analysed according to soil standard procedures described by Tedesco et al. (1995). A calibrated 3510 pH meter (Jenway; Staffordshire, UK), an AZ 86555 probe (AZ instrument corporation; Taichung City, Taiwan), and a 827 pH Lab probe (Metrohm; Herisau, Switzerland) were used for pH, EC and redox measurements, respectively. Measurements were conducted in triplicate.

3.4 Soil Fabrication

3.4.1 *Inoculum Preparation and Activation*

To ensure physiological adaptation and growth of the microbial community in bioaugmented Technosols, the incubation process was completed over seven days at 30°C (Schiraldi & De Rosa, 2014). Based on the methodology by Weiler et al. (2020), EM Pro-Soil was added in volumes of 250 mL to 2.5 g of dried MR per sterilised 250 mL Erlenmeyer flask, covered with cotton wool and aluminium foil. All flasks were placed on a platform shaker (Labcon; Mogale City, Gauteng, South Africa) at 120 rpm and 30 °C for seven days. Samples of 5 mL were taken daily to evaluate the effect of incubation on microbial diversity (through direct cell counting) and activity (through FDA analysis, refer to section 3.8.2).

Direct cell counting was performed with a Olympus CX40 Biological Upright Phase Contrast oil immersion microscope and haemocytometer (or counting chamber) (Olympus Corporation; Shinjuku City, Tokyo, Japan). A standard microscopy protocol was followed (ASTM D4455-85, 2002). The cell concentration per volume of suspension was determined from the following equation:

$$x_{cells} = \frac{x_{quadrant} \times 1000\ mL^3}{(4 \times Q_t) \cdot V_{quadrant}} \times DF \quad [2]$$

Where, x_{cells} is total cell count of the suspension, $x_{quadrant}$ refers to number of cells counted, Q_t is the number of squares per quadrant, $V_{quadrant} = 0.004\ mm^3$ is the volume of one quadrant, and DF is dilution factor.

3.4.2 *Technosol Fabrication and Incubation*

Technosols were prepared per pot; by mixing the raw materials (weight based on 500 mL pot volume, bulk densities, and correction factor) with a hand spade in a plastic tray until a homogeneous mixture had been achieved similarly to Weiler et al. (2020). Fabricated soils and agricultural soils without the addition of EM Pro-Soil were used as controls. To corroborate previous research by Weiler et al. (2020) on EM Pro-Soil in MR-amended coal-based soils, the inoculum was added at 2.5E7 cells per gram Technosol.

Soil incubation followed soil fabrication to evaluate the effects of inoculation on various Technosols and to kickstart microbe-mediated processes, especially nitrogen fixation (Chenu, et al., 2015). All Technosols and control soils were packaged in individual A4 plastic bags (Ziploc; San Diego, CA, USA) and placed in a 30 °C temperature controlled room for seven days similarly to Weiler et al. (2020). Each bag was mixed and aerated daily to reduce fungal growth and minimise clumping. After incubation, triplicate samples of all treatments were collected in Eppendorf tubes (2 mL) and stored at -20 °C. These samples were used during soil microbiome analysis.

Fabricated soils and controls were potted into 500 mL Polyvinyl Chloride (PVC) pots with drainage holes. All soils were transferred directly from the incubation bags (minimal moisture loss) into individual pots after mixing. It was assumed that negligible amounts of micro-plastics were transferred to soils and that the effects on plant growth were negligible compared to other abiotic variables.

3.5 Soil Preliminary Characterisation

Before plant growth experiments were conducted, Technosols and the controls were characterised for physiochemical and biological conditions. These properties provided information on the fabricated soil's ability to withstand abiotic stresses and support plant growth (Richter, 2007; Valarini, et al., 2003). In addition to, providing information on the influence of amendment dosage on soil quality (Herran Fernandez, et al., 2016). Substrate induced respiration experiments (refer to section 3.8.1) were performed to determine the initial microbial biomass present within Technosols, DNA extraction and qPCR amplification for initial microbiome profiling (refer to 3.8.4), field capacity tests for water holding capacity (Mills & Fey, 2004; Lowery, et al., 1996), and pH, EC and Eh measurements.

Field capacity were determined using 100 g of each fabricated soil, placed onto separate water-wetted filtration paper in a funnel on 250 mL Erlenmeyer flasks. The weight of the dry and wet filtration papers were recorded for each sample. Subsequently, 250 mL of tap water measured in a volumetric cylinder was poured over each soil filled funnel. After a 24h drainage period, the volume of water collected in each flask was measured with a volumetric cylinder. The difference between the volumes of water

initially added and drained was determined (water retention). From which, FC per pot of Technosol was determined using initial soil weights.

3.6 Plant Growth Experiments

Research on fabricated soils make use of plant growth experiments to evaluate soil health and quality (Firpo, et al., 2021; Prado, et al., 2020; Deeb, et al., 2017). In this investigation, two seasonal plant growth trials were performed. The experiments were aimed at evaluating physiochemical and biological potential of CW-based Technosols, and the changes thereof with seasonal variation. All experiments were monitored in a determined frequency.

3.6.1 *Growth Conditions and Setup*

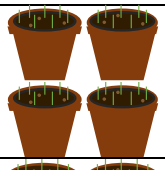
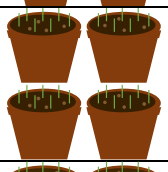
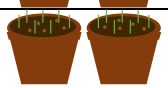
Following soil fabrication, pots were seeded and initially monitored in the laboratory (24-h-day) at constant abiotic conditions to ensure seedling emergence (Sayuti & Hitchmough, 2013). Whereafter, pots were moved to a greenhouse located on UCT Upper Campus. Plant growth experiments were conducted in a greenhouse at controlled conditions (16-h-day/8-h-night cycle) to simulate endemic site conditions. Relative humidity, temperature and dew point were automatically recorded every hour for the duration of each plant growth trial. The pots were randomly placed onto a single-tier stainless steel plant rack. This ensured that specific Technosols weren't negatively affected by spatial variation within the greenhouse, which aligns with the assumption made throughout the study. Pots were perpendicular to the greenhouse racks and weren't rotated during the trials to mimic stationary plant growth in agriculture.

3.6.2 *Plant Growth Trials*

3.6.2.1 Initial Plant Growth Trial

The Technosols and setup from the intial growth trial are summarized in Table 5. In this trial, compost was used as a control. The growth trial commenced on 10th March and was terminated on 9th May (total of 60 days).

Table 5: Initial teff growth trial in bioaugmented and biostimulated CW-based Technosols. Here, I represents inoculated and NI represents non-inoculated treatments.

	Components (wt. %)			Bioaugmentation	Greenhouse
Technosol ID	CW	MR	Compost	EM Pro-Soil	With teff
CW+MR2.5%; I	97.5	2.5	0	Yes	
CW+MR2.5%; NI				No	
CW+MR5%; I	95	5	0	Yes	
CW+MR5%; NI				No	
CW+MR7%; I	93	7	0	Yes	

CW+MR7%; NI				No	
Control	NA	NA	100	No	

Each treatment was performed in duplicates and teff were grown in each of the pots. This growth trial was performed during the summer months of Cape Town, South Africa. The temperature, dew point and relative humidity conditions within the greenhouse during the initial growth trial are summarized in Figure 12.

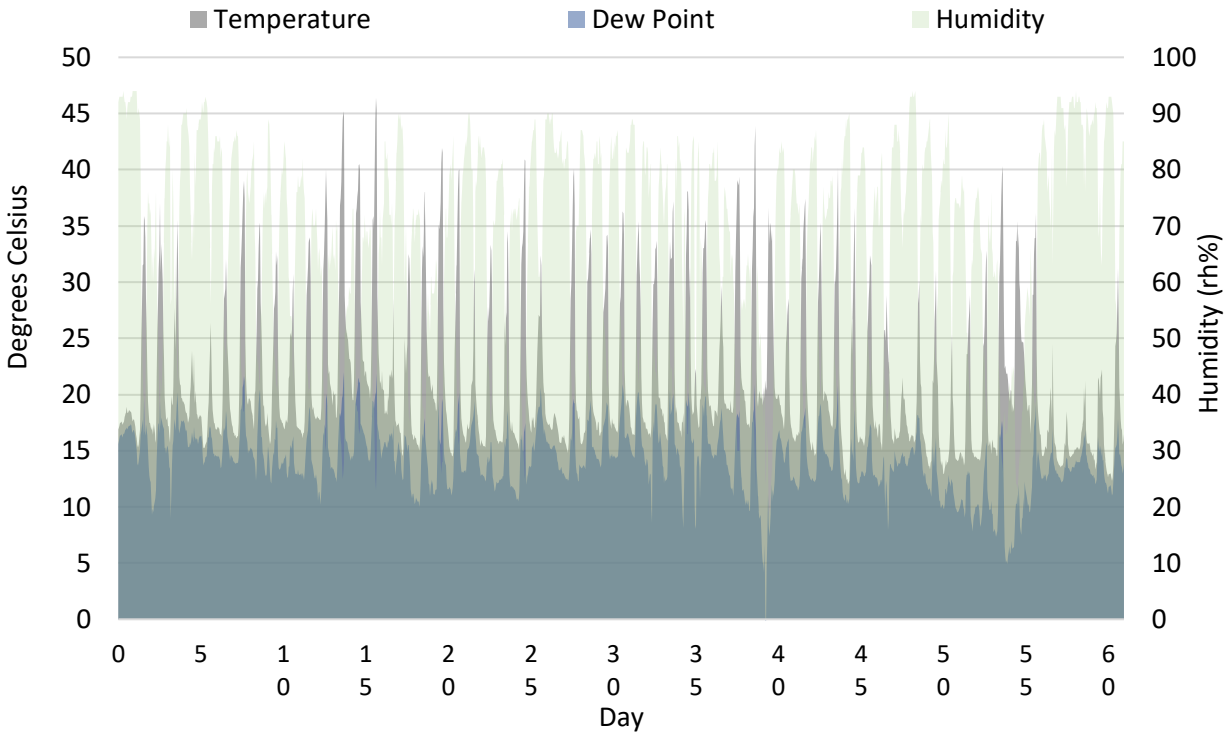


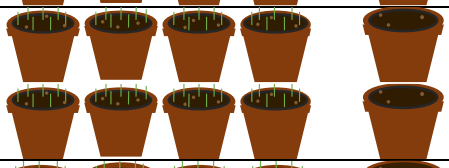
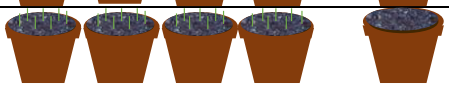
Figure 12: Daily greenhouse temperature, dew point and humidity conditions within the greenhouse for the duration of the initial plant growth trial (60 days) in summer.

Irrigation in the initial growth trial was based on the initial field capacity of the fabricated soils and not on the daily FC, thereby disregarding evapotranspiration. Furthermore, two pots per treatment type resulted in a too small sample (n) for error consideration. Non-planted controls were not considered in this trial, adding to the peculiarities of the initial growth experiments.

3.6.2.2 Final Plant Growth Trial

The structure of the final plant growth trial is summarized in Table 6. An optimised irrigation strategy proceeded from the initial trial to evaluate evapotranspiration, accompanied by non-planted controls for the effects of plant growth on soil properties, and a better suited control soil; potting soil, was employed as a precedent for Technosol performance. This growth trial commenced on 16th April till 7th July (82 days).

Table 6: Final teff growth trial in bioaugmented and biostimulated CW-based Technosols. Here, I represents inoculated and NI the non-inoculated treatments.

	Components (wt.%)			Bioaugmentation	Greenhouse	
Technosol ID	CW	MR	Potting Soil	EM Pro-Soil	With teff	No teff
CW100%; I	100	0	0	Yes		
CW100%; NI				No		
CW+MR2.5%; I	97.5	2.5	0	Yes		
CW+MR2.5%; NI				No		
CW+MR5%; I	95	5	0	Yes		
CW+MR5%; NI				No		
CW+MR7%; I	93	7	0	Yes		
CW+MR7%; NI				No		
Control	NA	NA	100	No		

Each treatment was performed in quadruplicates and were evaluated with and without Teff. Non planted treatments (no teff) were used as controls to evaluate the effect of plants on soil properties such as nutrients availability and uptake. All treatments except pure potting soil (control) were evaluated with and without the inoculum to investigate soil related benefits from inoculation (Weiler, et al., 2020). The final growth trial was performed during autumn and winter, where, the recorded temperature, dew point and relative humidity within the greenhouse are summarized in Figure 13.

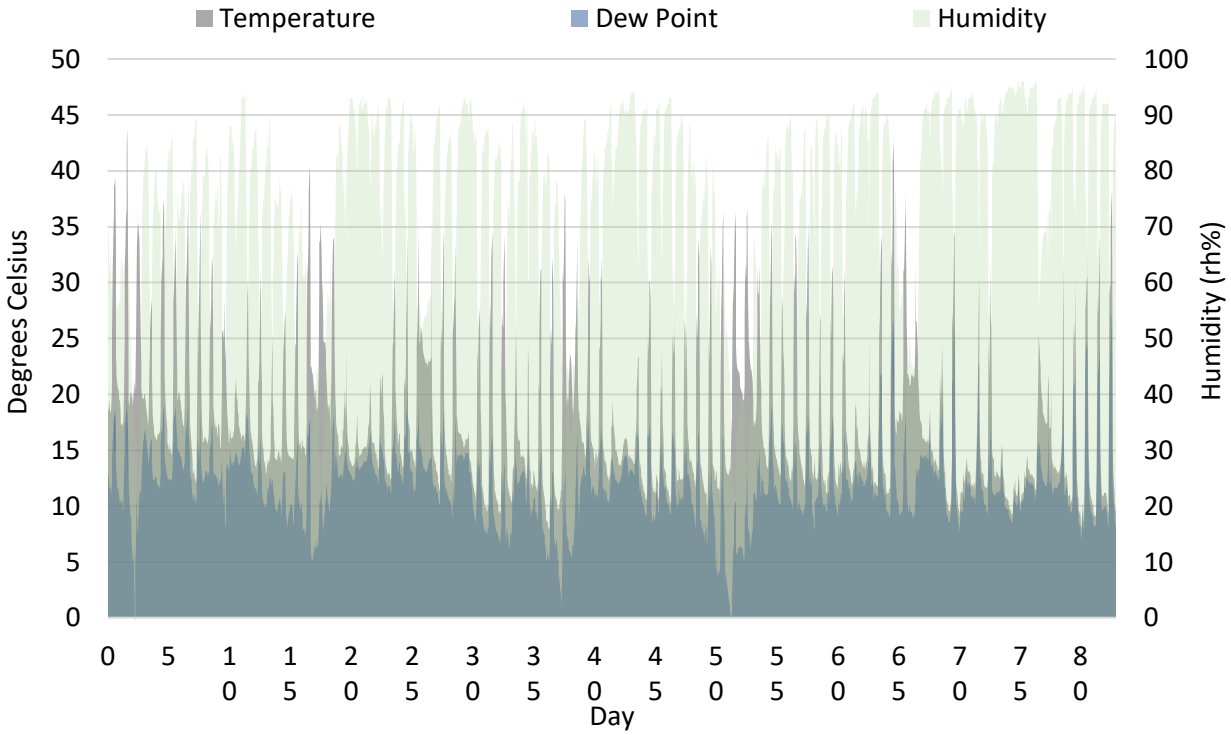


Figure 13: Daily greenhouse temperature, dew point and humidity conditions in the greenhouse for the duration of the final plant growth trial (82 days) in winter.

3.6.3 *Seed Sowing*

Eragrostis tef seeds, often used in seed mixtures for rehabilitation of mine sites (Amaral Filho, et al., 2020) and known for its durability and tolerance to saline soil conditions (Dame, 2018), were used in this study. Structural impedances from malt residue application was identified from delayed seedling germination in a small scale test on teff germination in various Technosols (refer to Appendix B: Feasibility Study on Plant Growth Trial Data). Hence, a single-seed sowing technique was developed and employed for the two growth trials (labelled; initial and final plant growth trials) to support seed germination. Sowing seeds on the soil surface and optimising the germination thereof would be beneficial when considering implementation processes of this rehabilitation scheme. The sowing process is illustrated in Figure 14.

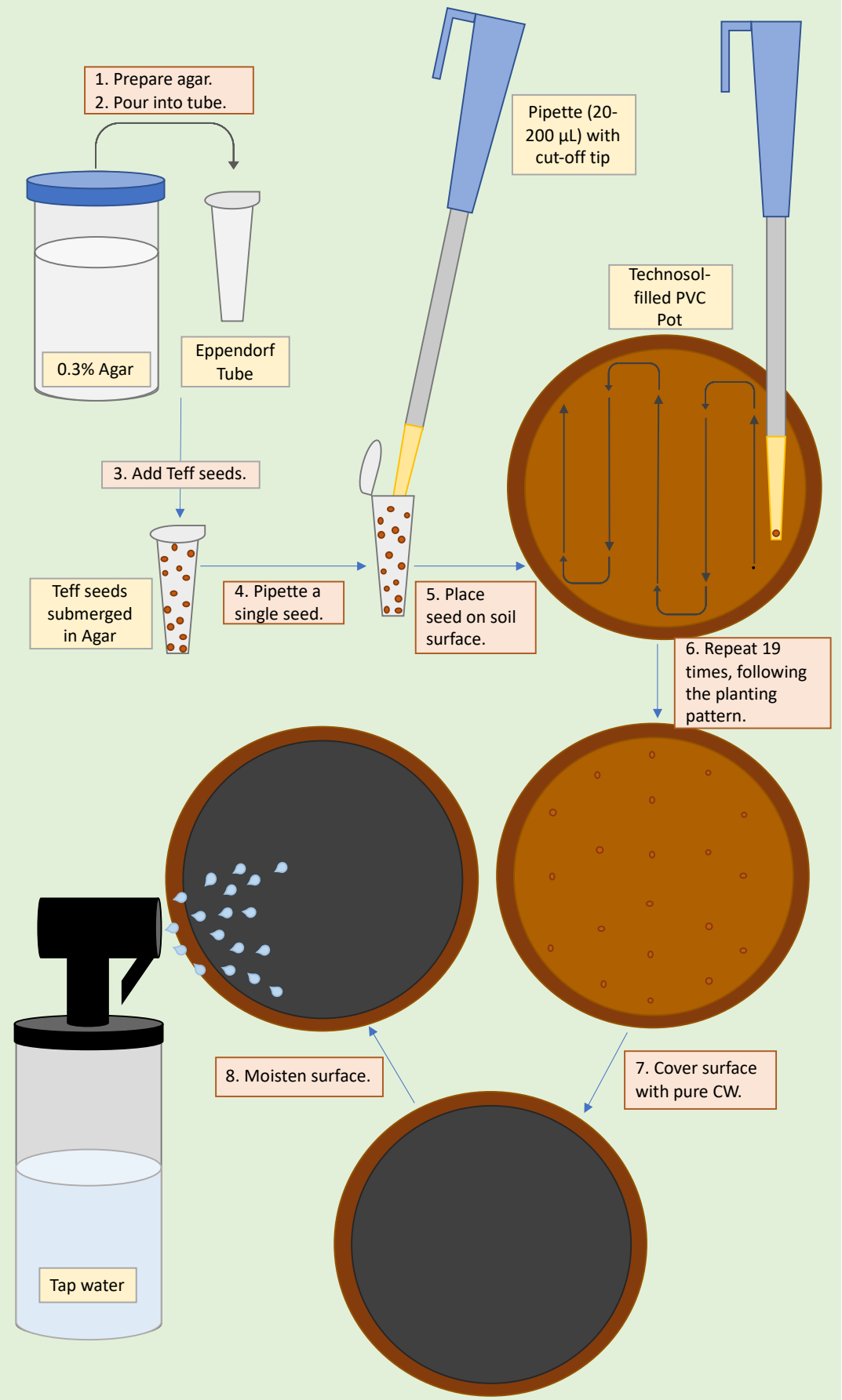


Figure 14: Single-seed sowing process.

In previous trials, the small size of the teff seeds resulted in multiple seeds sown where only one seed should have been. Therefore, teff seeds were suspended in 0.3% (w/v) agar for optimal sowing efficiency. From which, individual seeds were placed with a micropipette (20-200 µL) on the surface of each fabricated soil. Sown seeds were covered with a thin (4 mm) layer of pure CW and moistened with tap water. 20 Seeds were sown per pot (2 replicates in the initial growth trial, 4 replicates in the final growth trial) to ensure sufficient seedling emergence for each growth trial.

3.6.4 *Seedling Emergence and Plant Survival Rates*

Daily monitoring included seedling quantification for seedling emergence and plant survival rates. Seedling emergence assays were conducted in the first 20 days after planting analogously to Weiler et al. (2020), whereafter, the number of plants per pot were reduced to six per pot or maintained if already less than 6, to minimize competition between plants. Seedlings were removed with garden tweezers to prevent disruption of root growth in adjacent plants. Plant survival tests included quantification of the daily number of plants in all pots placed within the greenhouse until plant maturity. A plant growth cycle was monitored from seed planting until all shoot heights stabilized, inflorescence had been initiated and maturity had been reached in all plants similarly to Weiler et al. (2020). Literature suggests a maturity period for teff grass between 60 to 120 days (Ketema, 1991).

3.6.5 *Irrigation and Evapotranspiration Analysis*

Crop irrigation in greenhouse plant growth studies requires daily assessment (Nikolaou, et al., 2019). All pots were watered daily to sustain plant growth. During the seedling emergence period, sprouts are very fragile and susceptible to pests, fungi and dehydration. Therefore, the pots were kept in the laboratory for seven days until the seedling emergence rates had stabilised (Sayuti & Hitchmough, 2013). Here, pots were placed in tap water-filled trays (15 mm) and covered with plastic film. Small holes were made in this film to allow necessary oxygen transfer whilst minimizing evaporation. The layer of water created a humid environment ideal for seedling germination. Seedlings were quantified each day and moisture levels were adjusted with a water spray-bottle containing lab tap water (pH between 7.6 and 8.6).

Greenhouse irrigation was done on a field capacity basis. Each pot was weighed daily, and rain water (collected in a water tank outside of the greenhouse) was added to keep all soils in pots at the respective 50% field capacity level as suggested by Chessa et al. (2016). This ensured optimised irrigation (no dehydration or over-watering) of teff. Daily irrigation data for the final plant growth trial was used to determine evapotranspiration rates for Technosols and estimations on water requirements for implementation.

3.6.6 *Plant Heights and Growth Rates*

Throughout each plant growth trial, plant heights were recorded to determine growth rates in Technosols. Plant heights were measured from the soil surface to the longest apical meristem similarly to Weiler et al. (2020). Shoot heights of all teff per pot were recorded at the start and end of every working week. Heights were measured with a stainless steel 60 cm ruler. Representative plants for all Technosols were photographed once weekly for a visual diary of teff development within all Technosols.

3.7 Final Soil and Plant Biomass Characterisation

3.7.1 *Above and Below Ground Plant Biomass Yields*

Upon termination of a growth trial, all pots were transferred back to the laboratory for analysis. The pots were weighed and the final number of plants per pot were quantified. As per the standard operating procedure, visible plant biomass (shoots) were cut and dried in a constant flux oven at 60 °C for 48 hours to determine the above ground plant dry biomass per pot and teff yield per Technosol (SERAS, 1994).

Soil-like substrates were dried for seven days (room temperature; 22 °C) until all moisture had been lost (Amaral Filho, et al., 2020) before teff roots were removed by hand and rinsed with water similarly to Weiler et al. (2020). From the standard operating procedure, roots were dried in a 60 °C constant flux oven for 48 hours, after which it was weighed to quantify the yield of below ground biomass per Technosol (SERAS, 1994).

3.7.2 *Plant Biomass Characterisation*

Collected teff biomass were characterised according to major, minor and trace elements similarly to Amaral Filho et al. (2020). Results inferred on phytoremediation potential of teff, soil biogeochemical processes and the potential of using teff as cattle feed. Dried plant biomass (shoots and roots) were analysed for Na, Mg, Ca, K, P, Si, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg, and Pb using ICP-MS. CHNS analyses on the dried plant shoots were also performed with a Perkin Elmer 2400 CHNS Analyzer. Elemental analysis was outsourced to the centre for analytical facilities (CAF) at the University of Stellenbosch (SUN).

3.7.3 *Seed Production, Yield and Fertility*

Teff seeds were collected to evaluate grain yield and fertility thereof. The seed collection process consisted of placing the shoots in plastic bags, and rubbing it to release the seeds within all spikelets. Shoots and seeds were transferred to a sieve (mesh size 20; 840 microns) and rubbed through repeatedly, allowing all grains and some biomass to pass. Subsequently, seeds and remaining shoot biomass were placed onto a sheet of paper, using the electrostatic forces between paper and plastic, the remaining fine plant biomass were carefully separated from the heavier seeds. The seeds per plant per pot of Technosol were weighed and placed into clean, labelled Eppendorf tubes (1.5 mL) for additional fertility tests.

To evaluate the extended effects of mine waste based soils and commercial inoculums on grain fertility and quality of collected teff seeds from both trials, small scale germination tests were conducted for 14 days (OECD, 2006). Seeds from both trials were sowed (using the single-seed sowing method) according to their original labelled pot number, onto pure CW in open petri dishes. CW as medium eliminated variability that could be introduced from natural soil due to varying physiochemical characteristics and microbial communities. Petri dishes were placed in trays containing a 5 mm layer of tap water, and plastic film were placed on top to create an ideal environment for germination. The surface of the petri dishes were moistened and monitored daily. Seedling emergence rates were determined.

3.7.4 *Leachate Analysis*

The microbial-meditated cycling of Fe and S elements are interlinked; thus, ferrous and total iron and sulfate leaching analysis were performed. Leachates were collected from final soil field capacity tests.

Ferrous and total iron concentrations in the collected leachates were determined by spectrophotometry with 1-10 phenanthroline following standard laboratory procedures (Centre of Bioprocess Engineering Research, 2018). Roughly; for ferrous iron concentrations; leachate samples were filtered to remove suspended solids. Whereafter, 2 mL ammonium acetate buffer solution (stock solution), 2 mL 1-10 stock phenanthroline solution and 1 mL leachate sample were added to each empty, clean test tube and vortexed for 1 minute. Three blanks (2 mL acetate buffer, 2 mL 1-10 phenanthroline solution and 1 mL deionised water) were prepared for auto-zeroing of a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, Massachusetts, United States). Five minutes were allowed for the chelation reaction between ferrous iron and phenanthroline to form orange-red complexes. After which, test tubes were vortexed and 1 mL per sample was transferred to a clean cuvette to record absorbance measurements (510 nm). A standard curve ranging from 0-50 mg/L Fe^{2+} was prepared to ensure proportionality. For total iron concentrations in soil leachates, the reaction volumes (including blanks) were returned from the cuvettes to test tubes with one micro-scoop hydroxylamine, and vortexed (30 s) to ensure all hydroxylamine had dissolved. To allow for Fe^{3+} reduction by hydroxylamine, samples were left for 5 minutes. Finally, samples were vortexed (30 s) and poured into individual, clean cuvettes for absorbance measurements (510 nm). If absorbance measurements of blanks were above 0.04, samples were contaminated and fresh blanks had to be prepared.

Sulfate turbidimetric analysis was performed based on the standard protocol (American Public Health Association, American Water Works Association, and Water Environment Federation, 2017). Roughly; leachate samples were filtered to remove suspended solids of which 5 mL of appropriately diluted sample (1 to 4 dilution with deionised water) was added to a test tube with 0.25 mL of stock conditioning solution and one micro-scoop of finely ground (20 to 30 mesh) barium chloride crystals. Three blanks (5 mL deionised water, 0.25 mL conditioning solution and one micro-scoop of $BaCl_2$ crystals) were prepared. All samples and blanks were vortexed for 1 minute, before transferring 1 mL sample to a clean cuvette for absorbance measurements (at 420 nm) in a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, Massachusetts, United States). Absorbance measurements were correlated to sulfate concentrations from the prepared sulfate standard curve (0-50 mg/L SO_4^{2-}). Additional leachate TOC tests were performed using a Multi N/C 3100 TOC analyser (Analytik Jena; Reinhach, Switzerland).

3.7.5 *Soil Physiochemical Analysis*

Triplicate samples of soil in each pot were collected in Eppendorf tubes (2 mL) and stored at -20 °C. These samples were used in the soil microbiome analysis (refer to Chapter 3.8). The soil CEC, potential acidity (H + Al), T value, OM, and elemental content (C, H, TN, S, P Bray II, K, Ca, Mg, Na, Cu, Zn, Mn, B, Fe) were measured according to the procedure developed by Tedesco et al. (1995), performed by Bemlab Soil Analysis, Somerset West. Soil field capacity, pH, EC, and Eh were measured as previously detailed in section 3.5. The results addressed the agricultural potential of using Technosols as topsoils.

3.8 Soil Microbiome Analysis

The methods chosen to investigate the Technosol microbiology is supported by literature on soil microbiology (section 2.6.4). Biological soil properties were evaluated to support physical and chemical characteristics that discern the effects of biostimulation and bioaugmentation on plant-microbe interactions to perform soil processes.

3.8.1 *Microbial Biomass through Substrate Induced Respiration*

Microbial biomass through substrate induced respiration experiments based on the protocol by Jaggi (1976) were performed prior to and after plant growth as an indication of the rate and persistence of microbial proliferation in the various Technosols (Graca & Abelho, 2020).

All samples were performed in triplicate with four blank samples. Roughly; 60 mg glucose was added to 20 g Technosol in a 250 mL Erlenmeyer flask and incubated (22 °C) for two hours. Subsequently, an alkali trap was made by placing a 50 mL plastic beaker containing 10 mL of NaOH (0.125 M) in each of the soil and glucose filled flasks, sealing with parafilm for incubation (4 hours at 22 °C). Finally, NaOH was titrated with HCl (0.125 M). Using the titrate volume per sample, the carbon (mg) per gram of soil was determined with the following equation (Jaggi, 1976):

$$C_{mic} = 30(BL - SA) \times \frac{C_{HCl} \times k \times 1000}{\rho_{CO_2} \times SW \times 4} \quad [3]$$

Where, C_{mic} represents the carbon content in microbial biomass (mg C per kg soil), BL is the mean of the titrated HCl volume of the blanks (mL), SA is the titrated HCl volume of a sample (mL), C_{HCl} represents the HCl concentration, k corresponds to 22 mg carbon dioxide per 1 mL of 1 M HCl, 1000 converts g soil to kg, ρ_{CO_2} is carbon dioxide density (mg/mL) at 22 °C, SW is sample weight (g) and 4 is the conversion factor of 4h to 1h incubation.

3.8.2 *Microbial Hydrolytic Activity through Fluorescein Diacetate Assay*

Analysis on FDA (easily hydrolysed to fluorescein by various enzymes) was a kinetic and rapid method for measuring total microbial activity (Schumacher, et al., 2014). FDA analysis through spectrometry was optimised for Technosols in the background of CW (refer to Appendix A: FDA Optimisation). All samples were performed in triplicate to ensure statistically relevant results. Soil samples (10 mg soil with 9.90 mL 1xPBS in a 50 mL Centrifuge/falcon tube) were prepared. In an organics fume-hood, a 2 mg/mL FDA/Acetone solution was prepared by adding 10 mL pure acetone to 0.02 mg stock fluorescein diacetate in a 50 mL falcon tube. 1 mL of this solution was added to 49 mL of 1xPBS in a sterilised 50 mL Falcon tube to prepare a FDA/PBS (50 µg/mL) solution. Subsequently, 0.2 mL of the FDA/PBS solution was added to each of the soil samples that were placed on a rotary shaker for incubation (10 min at 37 °C). Sub-samples (500 µL) were taken into 2 mL Eppendorf tubes. After 10 mins, hydrolysis was terminated by pipetting 500 µL chloroform into each tube, closing the lids and vigorously shaking all tubes containing the sub-samples (working in an organics fume-hood). All samples were centrifuged at 13 krpm for 10 min in a Universal 320 centrifuge (Hettich; Tuttlingen, Germany). From which, 300 µL of each supernatant was syringe filtered into a qPCR tube. Fluorescence (RFU) was measured with a Quantus™ Fluorometer

(Promega; Madison, Wisconsin, USA), and correlated to the amount of soil per sample form dry weight measurements.

3.8.3 *DNA Extraction and Quantification*

Quantification of soil extractable genomic DNA (gDNA) was performed to evaluate changes in the Technosol microbiome structure. DNA extraction on soil samples (collected upon soil fabrication and post-plant growth trial) were performed by using the Machery-Nagel NucleoSpin® Soil kit (Düren, Germany) as per manufacturer's description. Initial nucleic acid quantification and quality was assessed with the NanoDrop® Spectrophotometer (Thermo Fisher Specific; Waltham, Massachusetts, USA). Preceding dilution of the DNA for qPCR analysis, the DNA was also quantified using the Quantus™ Fluorometer (Promega; Madison, Wisconsin, USA) system as described by the manufacturer for double stranded DNA. DNA aliquots containing 1 ng/µl gDNA were prepared and used for subsequent qPCR analyses.

3.8.4 *Profiling of Microbial Community through qPCR amplification*

The total bacteria, archaea and fungi represented within the gDNA was determined by 16S rRNA real time (RT)-qPCR analysis to profile the soil microbial community changes with plant growth as recommended by Sansupa et al. (2017). Each sample was analysed in triplicate through three separate DNA extraction samples. KAPA SYBR® FAST qPCR Master Mix (2X) Universal (KAPA Biosystems; Cape Town, South Africa) was used to carry out the analysis in Rotor-Gene Q equipment (Qiagen; Hilden, Germany). Total bacteria analysis was performed under the following optimised conditions: 10 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 3 s, annealing for 15 s at 63 °C, and fluorescence measurement at 80 °C. Universal archaea and total fungal analysis followed the same process with annealing at 60°C. Every extraction was conducted in a 15 µL volume containing 1 µL of gDNA template (1 ng/ µL), 200 nM of each primer, and 7.5 µL of the master mix. The specific primer pairs used for total bacteria, universal archaea and total fungal are referenced and summarized in the tables below.

Table 7: Universal primers for bacteria, archaea and fungi in RT-qPCR analysis.

		Bacteria	Archaea	Fungi
Forward Primer	Name	27F	Arch787F	5.8F
	Sequence	AGR GTT YGA TYM TGG CTC AG	ATT AGA TAC CCS BGT AGT CC	GAT GAA GAA CGC AGC GAA ATG
Reverse Primer	Name	1492R	Arch1043R	28R
	Sequence	TAC GGY TAC CTT GTT ACG ACT T	GCC ATG CAC CWC CTC T	ATT GAT ATG CTT AAG TTC AGC GGG
Positions (nt)		20	272	163
Amplicon size (bp)		8-27	787-806	74-94
Reference		(Frank, et al., 2008; Bomberg, et al., 2019)	(Fischer, et al., 2016; Yu, et al., 2005)	(White, et al., 1990; Bergman, et al., 2007)

Standard curves for total bacteria, archaea and fungal were produced from 16S rRNA gene standards (10 ng/ µL). Ten-fold serial dilutions of known copy numbers of the plasmid DNA (the range 10^2 to 10^7 copy numbers) were analysed in triplicate to produce the standard curves. Amplification efficiencies were

greater than 97% and the coefficient of determination (R^2) for standard curves were above 0.98. All results were processed with the QIAGEN Q-Rex software.

Primers targeting genes encoding the catalytic enzymes responsible for nitrogen-fixation (nifH), and denitrification (nirK, nirS, nosZ) similar to those applied by Gupta et al. (2012) were considered to understand shifts in microbial community functions (Hafeez, et al., 2012). The gene name and primer pairs are referenced and summarized in the table below. The oligonucleotides (primers) were purchased from Inqaba Biotec (Menlo Park, South Africa).

Table 8: Nitrogen cycling primers for RT qPCR analysis.

		nirS	nirK	nosZ	nifH
Protein/Function		Cytochrome cd1 nitrite reductase – denitrification	Nitrite reductase (Cu containing)-denitrification (nitrite to nitric oxide)	Nitrous oxide reduction - denitrification	Nitrogen fixation
Forward Primer	Name	nirS_916F	nirk876	nosZ2F	nifH112F
	Sequence	GTS AAC GTS AAG GAR ACS GG	ATY GGC GGV CAY GGC GA	CGC RAC GGC AAS AAG GTS MSS GT	GGI TGY GAY CCN AAV GCN GA
Reverse Primer	Name	nirS_1332R	nirk1040	nosZ2R	nifH482R
	Sequence	GAS TTC GGR TGS GTC TTG A	GCC TCG ATC AGR TTR TGG TT	CAK RTG CAK SGC RTG GCA GAA	GCR TAI ABN GCC ATC ATY TC
Positions (nt)			876-1040		112-482
Amplicon size (bp)		409	184	267	390
	Reference	(Pereg, et al., 2018; Throback, et al., 2004; Wang, et al., 2019)	(Pereg, et al., 2018; Henry, et al., 2004; Bru, et al., 2011)	(Henry, et al., 2006; Pereg, et al., 2018)	(Widmer, et al., 1999; Levy-Booth & Winder, 2010)

The operating protocol for the nirS primers set was optimised at: 10 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 3 s, annealing for 20 s at 62 °C, extension at 72 °C for 20 s, and fluorescence measurement at 80 °C. Analysis on nirK, nosZ and nifH genes followed the same protocol; however, annealing (for 20 s) occurred at 64 °C, 68 °C, and 58 °C, respectively. All the results were processed with the QIAGEN Q-Rex software. Each extraction was conducted in a 15 µL volume containing 1 µL of gDNA template (1 ng/ µL), 800 nM of each primer, and 7.5 µL of the master mix. Plasmids containing nirS, nirK, nosZ, or nifH gene fragments were used to generate the standard curves for each. Standard curves were produced similarly to that of the general primers. Amplification efficiencies were greater than 94% and the coefficient of determination for the standard curves were above 0.98.

CHAPTER 4

4 RESULTS & DISCUSSION

This chapter considered plant growth and the microbial ecology of biostimulated and bioaugmented coal-based Technosols. Two seasonal plant growth trials were conducted as outlined in Figure 10. From which, soil properties were evaluated according to the analysis detailed in Chapter 3. The results for both trials on plant growth and development, soil and plant biomass characterisation, and the soil microbiome are critically discussed in this chapter. This provided insight into the feasibility of a bioaugmented CW-Technosol when implemented as a topsoil (Chapter 5).

4.1 Material Characterisation

The physiochemical conditions of the raw materials used for Technosol fabrication and controls are tabulated in Table 9.

Table 9: Physiochemical analysis of raw materials; CW, MR, Compost and Potting Soil for Technosol fabrication.

		CW	MR	Compost	Potting Soil
Parameter	Unit	Value	Value	Value	Value
pH		7.20	5.71	8.09	6.05
EC	µS/cm	2030	690	797	893
Eh	mV	185	190	236	337
Moisture Content$_d$	%	2	7	4	6
Field Capacity	%	48	44	39	49
Bulk Density	g/mL	0.94	0.18	0.71	0.98
C	%	53.9	45.3	21.0	10.3
H	%	3.09	6.92	3.12	2.06
N	%	1.45	3.11	0.58	0.31
S	%	1.19	BDL	BDL	BDL
Al	ppm	33500	7.56	7700	14800
Fe	ppm	18200	97.0	5760	19000
Ca	ppm	29100	1960	13500	15500
K	ppm	2300	298	4580	10700
Mg	ppm	3000	1500	1810	32900
Na	ppm	400	121	1320	192
P	ppm	900	4860	1440	2800
Si	ppm	Nq	767	712	1650
B	ppm	Nq	3.15	14.3	6.24
V	ppm	40.7	0.0378	11.6	27.7
Cr	ppm	36.4	0.751	11.1	63.8
Mn	ppm	200	34.6	61.1	179
Co	ppm	3.57	0.357	1.61	19.6
Ni	ppm	12.6	0.167	3.55	43.7
Cu	ppm	10.9	10.1	16.8	40.3
Zn	ppm	9.96	82.0	99.0	43.7
As	ppm	BDL	0.185	6.60	3.66
Se	ppm	BDL	0.0675	0.349	0.415
Sr	ppm	384	7.61	83.2	78.6
Mo	ppm	1.07	0.931	0.324	1.02

Cd	ppm	BDL	0.0319	0.122	0.147
Sn	ppm	BDL	0.0267	1.166	0.830
Sb	ppm	BDL	0.0107	0.331	0.147
Ba	ppm	377	7.84	34.2	158
Hg	ppm	BDL	0.0116	0.0315	0.0462
Pb	ppm	8.32	0.0677	10.6	10.6

From Table 9, CW is carbonaceous (total carbon content of 53.9%) and slightly alkaline (pH of 7.20). Microorganisms from bioaugmentation are required to produce substrates through soil carbon mineralisation necessary for microbial respiration and SOM as illustrated in Figure 6 and as shown in the Fungcoal technique (Sekhohola-Dlamini, et al., 2022). The low salinity of MR is favourable as saline conditions are detrimental to plant and microbial growth (Boyrahmadi & Raiesi, 2018). Furthermore, MR had a moisture content (M_d) 3.5-fold of CW. Thereby, suggesting improved water permeability and retention, and ion mobilisation in fabricated soils with MR amendments as shown by Amaral-Filho et al. (2020). The high nitrogen content of MR (10-fold more than potting soil) was expected to result in improved plant growth and N cycling microbe proliferation introduced into Technosols by EM Pro-Soil. The same accounts for phosphorus; a very valuable root growth nutrient abundant in MR but not in CW (5-fold less). Minor and trace elements in CW and MR are all within standard levels that correspond to the commercially available compost and potting soil, except for Mn, Mo, Sr, V and Ba. However, these trace elements are commonly extracted by hyperaccumulating plants (Hrynkiewicz, et al., 2018) and microbes such as *Bacillus* (Wood, et al., 2016), which is present within the applied inoculum (refer to 3.4.1). Therefore, mobilisation of these elements into the teff plant organs were expected in Technosols with higher dosages of MR. In MR, the zinc content is almost 2-fold compared to potting soil. Implying, potential beneficial effects for teff germination and chlorophyll development in Technosols with higher concentrations of MR. Conversely, it could result in zinc toxicity in soils amended with excess of organic matter. In addition, high Fe and Al concentrations within CW is of concern for soil microbial activity and metal leaching. Microbial biomass and activity was investigated in section 4.3.3 and total iron leaching tests were performed. In support, research by Sekhohola et al. (2017) and Amaral Filho et al. (2020) on revegetation in mine tailings, reported no deleterious socio-environmental effects when organic amendments are applied at 2% to coal mine waste soil substrates.

From the physiochemical analysis of the raw materials, the potential for a soil-like substrate of CW+MR is evident. Teff growth in all Technosols with various ratios of MR was evaluated in favourable (initial growth trial in Chapter 4.2) and unfavourable (final growth trial in Chapter 4.3) conditions to determine the effects of including bioaugmentation with biostimulation on coal-based Technosols.

4.2 Initial Plant Growth Trial

The initial plant growth trial was structured to answer the key questions regarding amendment application as biostimulation. The aim was to determine if the inclusion of amendments to CW benefited soil fertility in conjunction with bioaugmentation, and if an increased ratio of organic material as amendment enhanced microbial biomass, improved soil structure and supported plant growth in a bioaugmented-Technosol.

There are some issues intrinsic to the use of pots in soil experiments which should be considered for the initial and final plant growth trials results. Soil in pots tends to compact, water is lost between the soil and pot walls, and preferential paths affect irrigation. Thus, plant growth, root structures and yields differ from plants grown in fields.

4.2.1 *Plant Development & Performance*

Seedling emergence and survival rates are primary indicators of plant growth performance in a soil, and were recorded throughout this study. Seedling emergence refers to the rate at which seeds germinate and develop from the moment photosynthetic autotropism is initiated from interaction with ecological variables, whereas survival rates correlate the plant growth to the initial number of seeds sown (Campbell, 1985). High seedling emergence and plant survival rates, especially under various environmental conditions, are indicative of good plant development and seed quality (De Ron, et al., 2016).

Average rates of seedling emergence per Technosol type in the initial plant growth trial are summarized in the Figure 15 and Table 22 in Appendix C: Initial Plant Growth Trial Data. Seedling emergence occurred at a 66% faster rate due to the single-seed sowing method where soil structure did not impede sprouting. An average of 75% seeds had germinated by day 3 in bioaugmented CW+MR7%, compared to 5% after day 7 that was reported in the preliminary run.

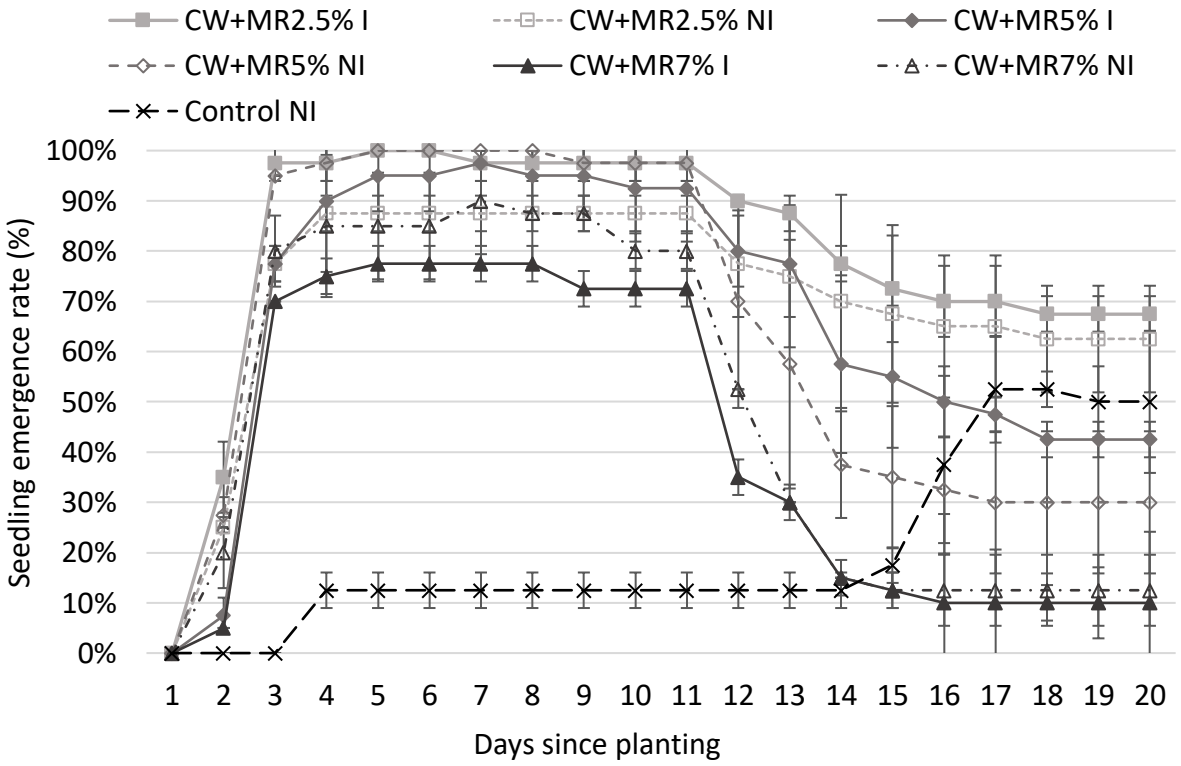


Figure 15: Average rate of teff seedling emergence (expressed as percentage with standard deviation) relative to the number of seeds planted (n=20) in MR amended coal-based Technosols and 100% compost as the control for 20 days after planting before the number of seedlings per pot were reduced to six, in

the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

On average, germination occurred on the first day after planting, excluding compost. CW+MR2.5% had the highest initial germination rates; 98% of the planted seeds had emerged in the inoculated treatment two days after planting with a peak at 100% after three days. The germination rate in compost were evidently slow; 13% on day 3, with a peak of 53% on day 16. The physiochemical properties of compost tabulated in Table 9 showed an abundance of nutrients Na, Ca, H; however, valuable P and K were inadequate for teff germination when compared to potting soil. By the 6th day after planting, the rate at which plants emerged had peaked in all Technosols except compost. Subsequently, the pots were moved from the lab into the greenhouse after day 7.

In the greenhouse, temperatures were high and fluctuating, as seen in Figure 12**Error! Reference source not found.**. This, along with the change from a 24h-day period in the laboratory to a 16h-day/8h-night in the greenhouse, shortened the photosynthesis period and resulted in seedling death. Hence, an overall drop in seedling emergence rates seen after day 7, except for compost (already at a low 13%). Temperatures dropped on the 9th and 10th day since planting which resulted in a spike in humidity conditions on day 10 in the enclosed greenhouse. These conditions are adverse for teff growth, ensuing seedling death in all pots. The lingering effects of these abiotic variables were noticeable when looking at the attenuated number of plants in the days following. Fungal growth and nematodes persisted during this period, as high humidity in the greenhouse in summer months invoked susceptance to pestilence. Visible fungi were abundant in CW+MR7% and utilised the MR as OM feedstock. Initial soil pockets from the heterogeneous 7 wt.% MR and CW mixture, were quickly inhibited by flies and larvae that deposited their eggs and fed on teff endosperms, inhibiting plant growth. At the end of the seedling emergence period, teff in compost did not perform well as seen in the figure above, yet, inoculated Technosols with MR dosages of 2.5% and 5% (w/w) outperformed the non-inoculated controls.

The number of plants were reduced to 6 per pot on day 21 to mitigate plant deaths, and minimise competition between plants for nutrients, space and water. Following, the number of plants remained constant in both non- and inoculated CW+MR2.5% and CW+MR5%. Teff plants in all Technosols with 7% (w/w) MR were visibly more fragile, thin and smaller in size compared to the other soil substrates for both inoculated and non-inoculated treatments. Nematodes remained present on days 21 and 22 in the identified poor-performing soils. The inability of compost and CW+MR7% to support plant growth is apparent in Figure 16. The graph depicts the final number of plants that weren't able to grow in the Technosols by the end of the initial growth cycle.

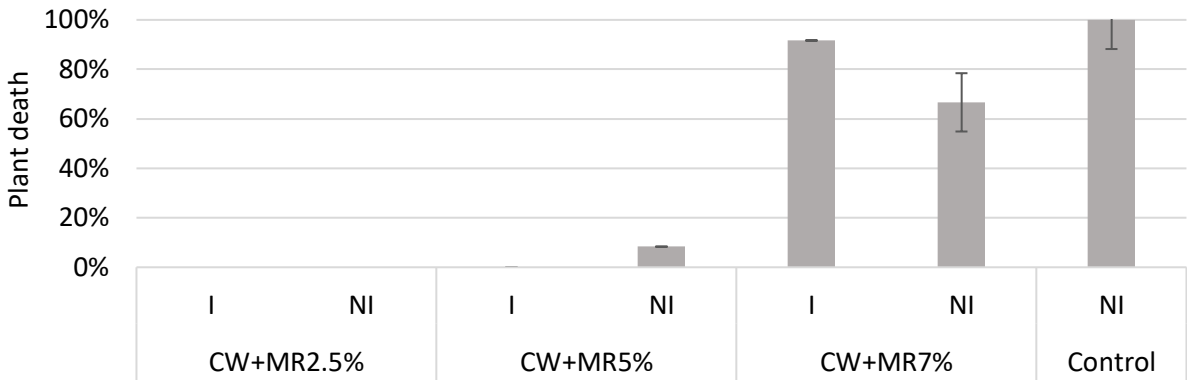


Figure 16: Average percentage and standard deviation of plant death in MR amended coal-based Technosols and pure compost as the control, 60 days after planting for the initial plant growth trial in summer as an indication of the soil's ability to maintain teff development. Where, I refers to inoculated and NI to non-inoculated treatments.

The effect of amendment dosage on plant survival is observed in Figure 16. A fine balance exists between the ratio of amendment to parental material, and the effect thereof on the relationship between plants and associated microbes. Research on soil amendments reported that shifts in the soil microbial community after inoculation is dependent on type of soil; thus, emphasizing the significance of bio-stimulation and the effect thereof on the success of bioaugmentation (Bakhoum, et al., 2012). In all Technosols amended with 2.5% and 5% (w/w) MR, the number of plants remained constant until the end of the growth trial (60 days). Suggesting that these fabricated soils have the ability to support a teff growth cycle. Another growth trial was necessary to determine the seasonal effect on teff growth in these Technosols.

Shoot heights of all plants were recorded to evaluate teff development and biomass yields. The cumulative growth rates of teff (cm) per day are presented for all Technosols in Figure 17.

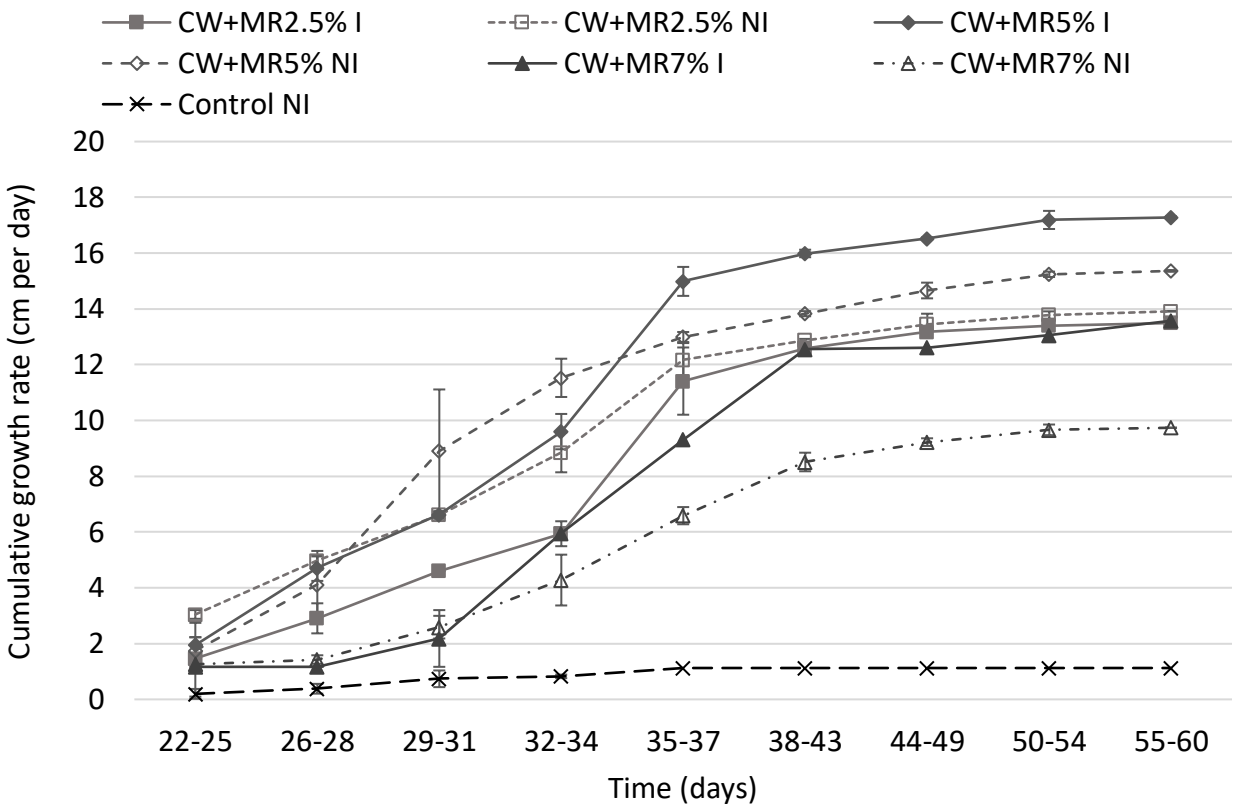


Figure 17: Cumulative above ground teff growth rate (cm per day) and standard deviation in Technosols amended with MR during the initial plant growth trial in summer, with compost as the control. Where, I represents inoculated and NI is non-inoculated treatments.

Rapid plant growth occurred mid-way through the greenhouse study, between days 26-37 since planting, as seen in Figure 17. Here, daily average greenhouse temperatures never dropped below 20 °C (refer to Figure 12), promoting teff growth. In the succeeding days, plant mechanisms and energy-use shifted from plant growth to inflorescence. Stationary teff growth rates during this period (days 55-60) are clear in Figure 17.

The teff growth rates in Figure 17 showed that soils amended with 5% (w/w) unmineralized organic material as MR were best able to support above ground plant growth. Teff in bioaugmented CW+MR5% were the tallest and reached shoot heights up to 75 cm. Microbial biomass experiments were performed (section 4.2.3) to determine if this MR as biostimulation was advantageous to both plants and microorganisms. The presented results coincide with the seedling emergence results in Figure 15. Representative pots of each Technosol type after the growth cycle, are shown in Figure 18. From Figure 17 and Figure 18, a greater dosage of MR did not enhance plant growth and teff was not able to grow in compost as control.

CW+MR2.5%; I	CW+MR2.5%; NI	CW+MR5%; I	CW+MR5%; NI	CW+MR7%; I	CW+MR7%; NI	Control

Figure 18: Representative pots with teff grown for 60 days in malt residue amended coal-based Technosols with and without bioaugmentation. Where, I refers to inoculated treatments, and NI is non-inoculated, with pure compost as the control.

Teff is a short-day plant (Van Delden, et al., 2011) and signal to flowering in teff is influenced by photoperiodism. Subsequently, when the day-night period shortened as summer transitioned into autumn, bolting was initiated and the spikelets produced seeds. Figure 19 graphically summarizes the average weight of seeds produced in teff per Technosol type and the days-till-bolting. There is no data for the control, compost, as no plants survived after the 60-day growth period. The earliest signs of inflorescence were in bioaugmented CW+MR2.5% and CW+MR5% 40 days after planting as shown in Figure 19, implying that these engineered soils were best able to provide the necessary nutrients, structural support for plant development. Soil microbial activity analysis (refer to chapter 4.2.3) were performed in support. There is a positive correlation (R^2 = 0.9) between grain yield and above ground plant dry biomass production for each Technosol as shown in Figure 53 (Appendix C: Initial Plant Growth Trial Data). As anticipated, a low seed productivity was obtained in compost and CW+MR7% soils.

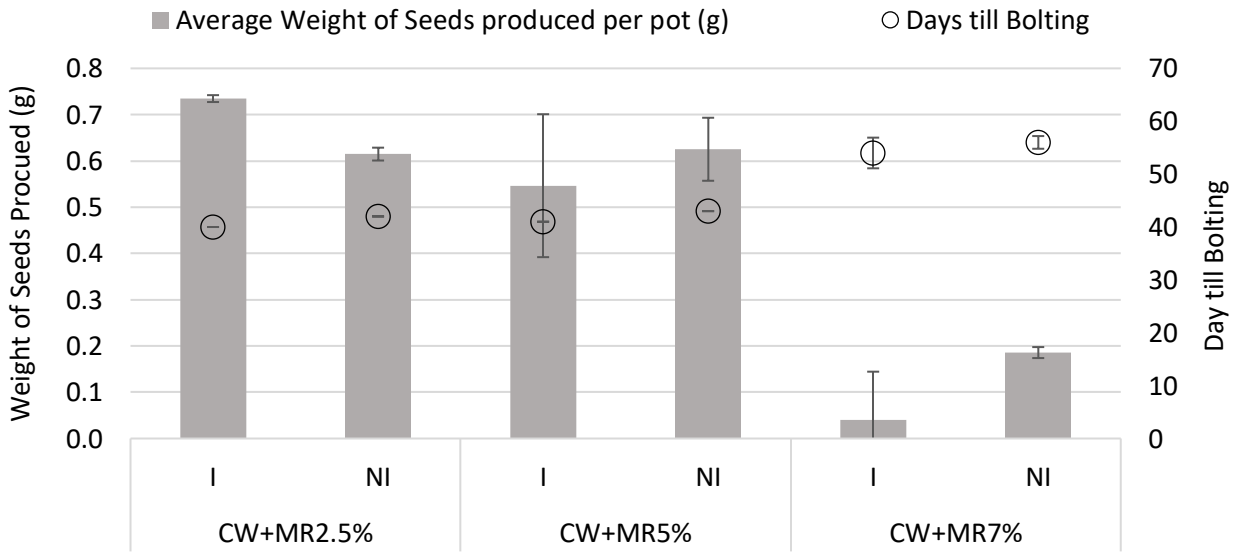


Figure 19: Average and standard deviation results of seed production achieved in teff per MR amended coal-based Technosol as influenced by bolting time during the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

Teff is a rapid growing annual grass capable of producing 4.1 tonnes biomass per hectare on average (Ketema, 1991). Various socio-economic benefits from revegetation with teff would therefore arise. The initial plant growth cycle lasted 60 days; this corresponds to the desired reported turn-around times for teff cultivation (Ketema, 1991). The greenhouse conditions during this trial suited teff growth as plant maturity were reached quickly whilst producing sufficient biomass. The above and below ground plant dry biomass were quantified as summarized in Figure 20.

Expectedly, biomass yields in compost and CW+MR7% (both NI and I) were negligible. This is supported by literature on standard SOM levels that recommend between 3-6% in arable land for productivity (Fenton, et al., 2008). Results for CW+MR2.5% were consistent with minimal variation in shoot biomass between inoculated and non-inoculated treatments. The most biomass was produced in CW+MR5%, corroborating results by Weiler et al. (2020). As an anomaly, 1.59 g of above ground dry biomass per 100 g of soil was produced in one of the non-inoculated CW+MR5% pots; it provided valuable information on the soil's ability to support plant growth. The below ground dry biomass production in CW+MR5% far exceeded that of other treatment types, suggesting that complex, functioning root structures developed. Root structures helped stabilise soil aggregates and improve water retention (refer to section 4.2.2). Consequently, the results for high seedling persistence, plant height and growth, and biomass production motivate for a 5% (w/w) MR dosage when the inoculum is applied. Thus, it is evident that biostimulation supported plant growth in coal-based bioaugmented-Technosols.

When considering the greenhouse plant yield data, it must be noted that field production yields of teff are generally measured in an exponentially greater ratio of teff plants per field to the ratio of teff plants in the pots for this study. This can negatively influence the comparisons made between greenhouse trial data and potential field results.

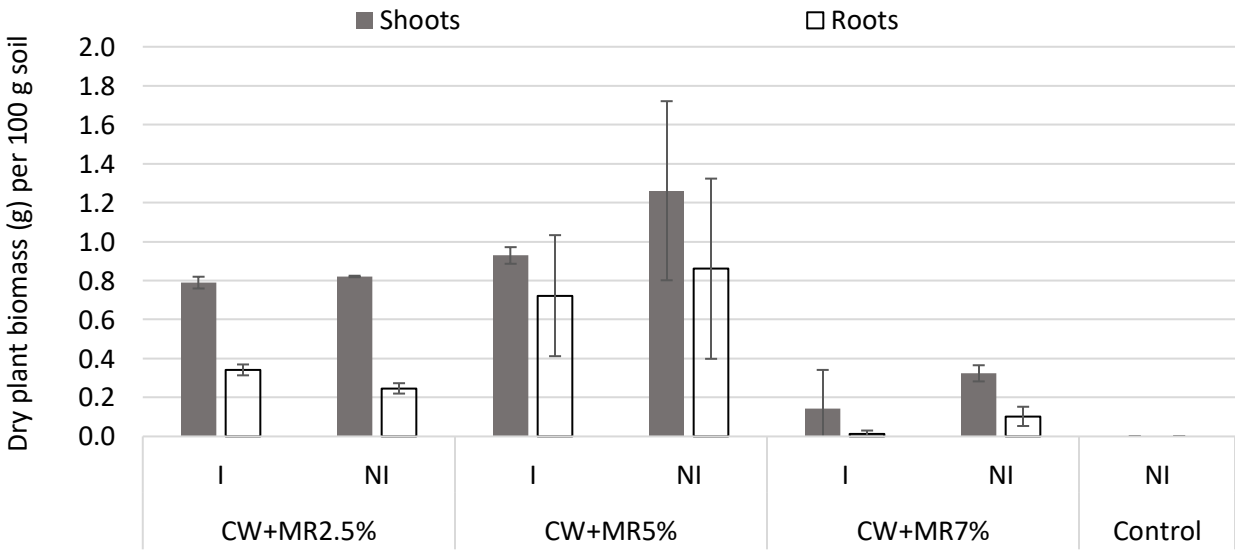


Figure 20: Average above (shoots) and below (below) ground teff dry biomass produced per 100 g of coal-based Technosol with standard deviation after the initial plant growth trial in summer, with compost as the control. Where, I represents inoculated and NI is non-inoculated treatments.

Although the results for plant growth and development in best-performing bioaugmented CW+MR5% provided an indication of the Technosol's ability, soil characterisation and soil microbiome analysis were needed to evaluate soil fertility.

4.2.2 *Characterisation of Technosols*

Technosols in this study were characterised according to pH, Eh, EC, water holding capacity and elemental content by following the methodology that was detailed in Chapter 3. Soil pH, redox, and electrical conductivity evolves with vegetation (Cervantes, et al., 2011). The changes in pH, Eh, and EC in the coal-based Technosols before and after teff growth are graphically presented in Figure 21, Figure 22, and Figure 23, respectively.

From Figure 21, pH conditions of the investigated Technosols before plant growth all ranged between 7-8. Amaral Filho et al. (2017) conducted research on alkaline coal-based Technosols and concluded that acidic soil pH inhibits plant growth and increases the potential for aluminium toxicity in coal waste-based soils (Amaral Filho, et al., 2017). This coincides with the good plant growth achieved in CW+MR5% with stabilised pH values above 7.

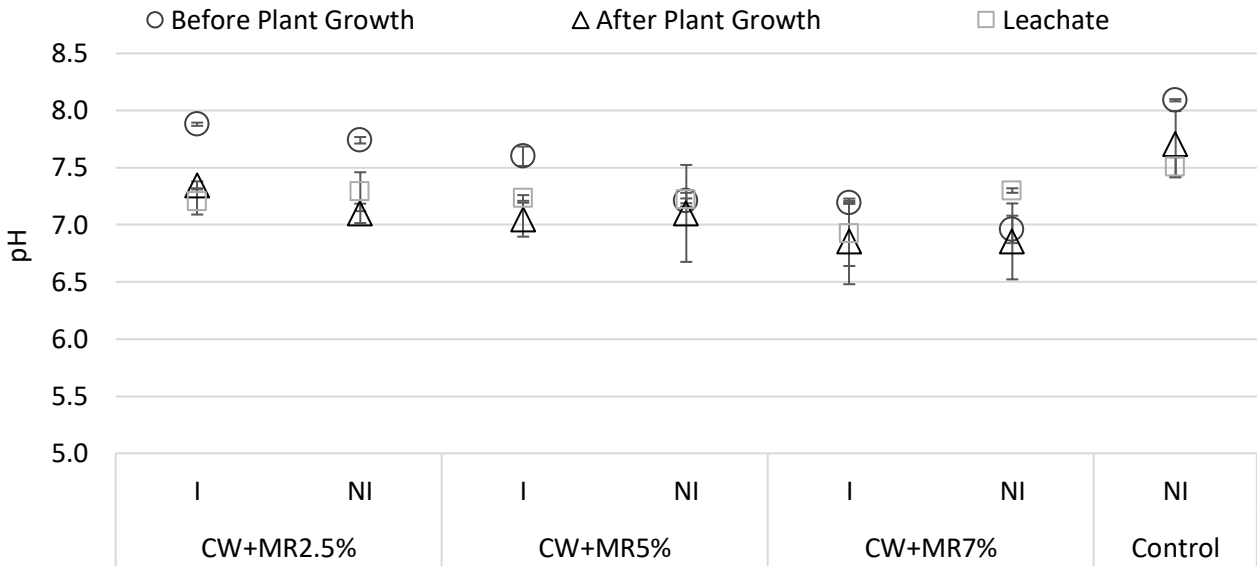


Figure 21: Average pH and standard deviation of vegetated, MR amended coal-based Technosols and associated leachates before and after the initial plant growth trial in summer with compost as the control. Where, I represents inoculated and NI is non-inoculated treatments.

From Figure 21, initial pH levels increased with inoculation. Although the applied inoculant was characterised as acidic, it was evenly dispersed throughout the soil-like substrates and added in very small volumes (based on the desired number of microbial cells per gram Technosol) that evidently did not reduce soil pH. Rather, microbial metabolism increased extracellular pH upon incubation (enhanced microbial growth as shown by SIR analysis). Furthermore, Sanchez-Clemente et al. (2018) reported that pH change during microbial growth is dependent on the carbon source. Thus, different trends might occur when different amendments to MR are utilised.

Initial pH levels of Technosols decreased when MR dosage increased, as MR was initially characterised by an acidic pH (refer to Table 9). This trend persisted with plant growth. However, as previously detailed, teff is commonly cultivated in soils with pH values between 5.5 and 7.5 (Prado, et al., 2020; AGT Foods Africa Pty Ltd., 2017). Therefore, higher amendment dosages negatively influenced teff growth through soil pH reduction. Furthermore, the alkalinity of compost decreased the availability of many soil nutrients that are important for plant development (Prado, et al., 2020).

pH levels of all Technosols were reduced after a plant growth cycle, especially in bioaugmented-Technosols amended with 2.5% and 5% (w/w) MR with final values between 7.0 and 7.5. Vegetation in non-inoculated CW+MR5% did not cause significant pH modification. Perhaps due to the lack of native microorganisms (examined by microbial biomass experiments) that facilitate ion mobilisation to regulate soil pH, as illustrated in Figure 5. Cervantes et al. (2011) reported that Technosols with vegetation have a lower pH (between 6.0 - 7.0) than those without (pH between 6.5 – 7.5) due to nutrient ion uptake and SOM generation. To fully evaluate the influence of amendment dosage through soil pH on teff growth, the final plant growth trial investigated the change in pH in vegetated and non-vegetated soils.

Leaching refers to the loss of soluble soil and plant nutrients following irrigation. Primary factors effecting leaching are soil structure and composition, and plant species. The highest volumes of leachates after the plant growth trial were collected from compost due to poor plant growth as previously discussed and poor soil structure (deduced from poor water retention in Figure 24), and from non-inoculated CW+MR2.5%. 84.4% of the water poured into compost were collected as leachate, and 75.4% in non-inoculated CW+MR2.5%. Conversely, only 46.5% leached out of inoculated CW+MR5%. This is an indication of sufficient WHC in CW+MR5% to prevent nutrient loss. Thus, a higher MR dosage decreased leaching by acting as a structural ameliorant.

Literature suggests that a higher CW content would increase the concentrations of metal and mineral leached (Komonweeraket, et al., 2015). However, from Figure 21, none of the soil substrates were strongly acidic or basic. Implying a lower probability of metal(loid) leaching that reportedly follow amphoteric leaching patterns (Cui & Li, 2016; Mahedi, et al., 2019). Total iron and sulfate, and TOC, TIC and TN were analysed to support. The pH of Technosol leachates were slightly higher than the final soil pH values, except for inoculated CW+MR2.5% and the control. This was expected as these soils were characterised by the highest pH values of the investigated treatments before teff growth.

The change in redox conditions with teff growth in Technosols is graphically illustrated in Figure 22. Before plant growth, the redox potential of all Technosols ranged between 200-300 mV, except for CW+MR2.5%. Expectedly yet contrary to pH conditions, inoculation with EM Pro-Soil slightly decreased redox conditions in Technosols before and after teff growth as the redox potential of a medium decreases during bacterial growth (Reichart, et al., 2007). Bioaugmentation with the activated, growing inoculum before plant growth should augment microbial-meditated redox processes and continue with vegetation. However, this was only seen in bioaugmented CW+MR5% (4% decrease after plant growth), of which, consumption of readily available, labile OM from MR and oxygen by microorganisms for respiration, and

potential reducing root exudates contributed to the Eh reduction (Husson, 2013). Unchangingly, suggesting that this fabricated soil is better suited for microbial assisted geochemical processes.

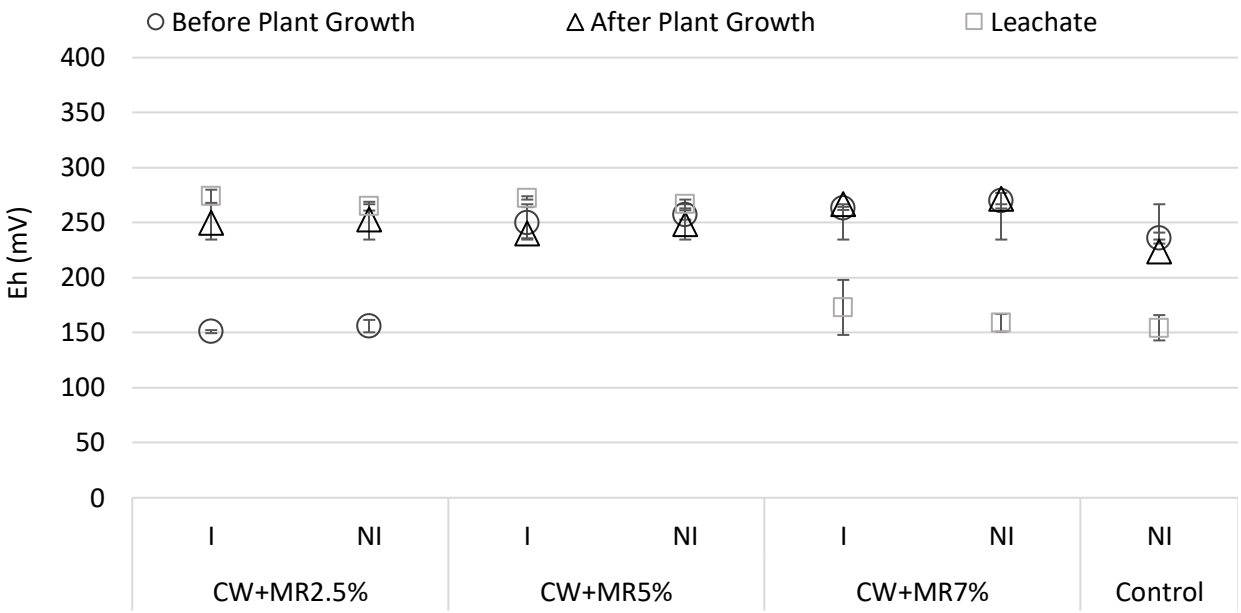


Figure 22: Average redox potential (Eh) and standard deviation of vegetated, MR amended coal-based Technosols and associated leachates, with compost as the control, before and after the initial plant growth trial in summer.

Literature describes a decrease in Eh when OM increases as a result of increased carbon dioxide emissions (Husson, 2013; Gardiner & James, 2012). However, this was not the case in this growth trial where Eh values increased with every dosage of MR. From Figure 22, potentials of CW+MR2.5% increased with vegetation, potentially due to higher oxygen diffusion rates relative to oxygen consumption rates between the engineered soils and microbes (Husson, 2013). Leachate redox potentials correspond to the final soil Eh after plant growth, except for CW+MR7% and the control that were incapable of supporting plant growth. Soil agglomeration and clumping caused saturation and poor water permeability in CW+MR7%. This limited mass transfer, specifically oxygen diffusion, and resulted in reducing conditions (Sondergaard, 2009).

Electrical conductivity measurements provide information on salinity conditions within soils as summarized in Chapter 2. Results for EC change in coal-based Technosols with and without bioaugmentation after one cycle of teff growth are presented in Figure 23.

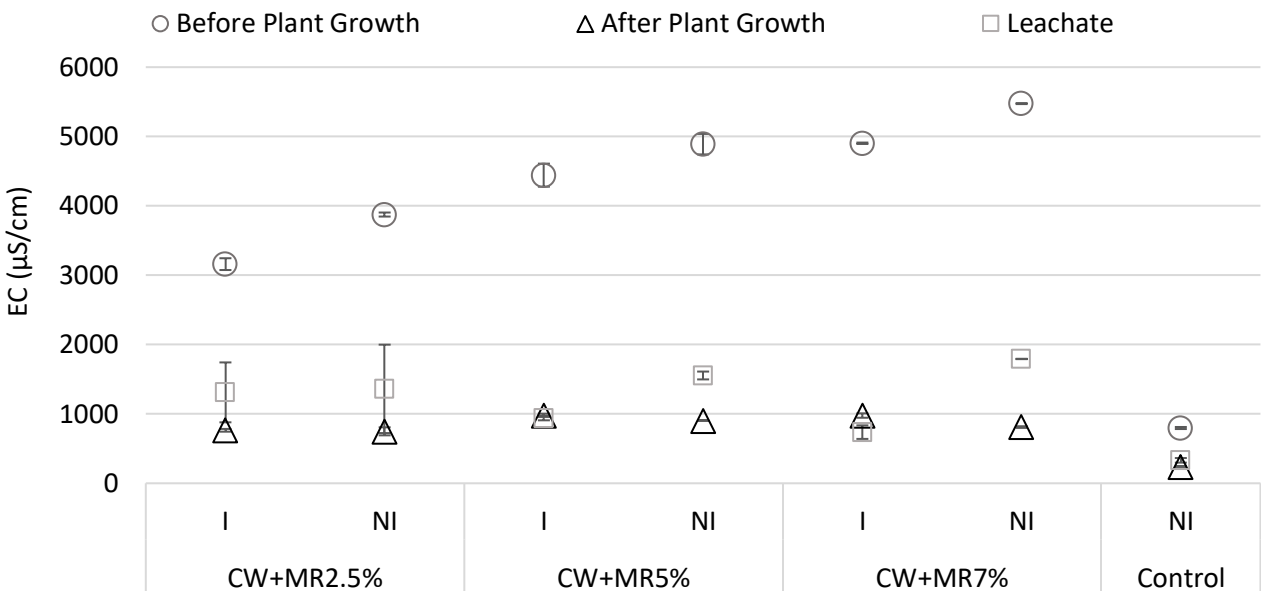


Figure 23: Average electrical conductivity (EC) and standard deviation of vegetated, MR amended coal-based Technosols with compost as the control and associated leachates before and after the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

In Figure 23, it is apparent that initial soil salinity is directly proportional to the amendment dosage, and inversely proportional to bioaugmentation; mimicking the observed trends in soil initial and leachate redox conditions. Here, an increase in EC is an indication of augmented salt concentration (primarily sodium, nitrates, sulfates, potassium and ammonia (NRCS, n.d.)) from organic material (MR) and associated, available minerals and nutrients (as seen in Table 9). As with agricultural practices, EC levels increase with fertilization dosage and poor soil structure (Conover, et al., 1992).

The initial EC values above 4000 µS/cm of CW+MR7% and non-inoculated CW+MR5%, indicated that the Technosols were saline, especially CW+MR7% (Boyrahmadi & Raiesi, 2018). Salinity is detrimental to plant growth (Boyrahmadi & Raiesi, 2018) and microbial growth (Yan, et al., 2015). Structurally, the coal-based fabricated soils amended with 7% MR were heterogeneous in texture with mineral and organic soil colloids (chemically active, finer soil fractions from the high ratio of organic material). The large surface area from the colloid pores reduced the availability of organic substrates through adsorption, which delayed microbial-associated pollutant degradation (Nannipieri, et al., 2003).

Post plant growth, conductivities of non-inoculated Technosols (and associated leachates) were marginally lower than that of the inoculated treatment type. Suggesting improved nutrient salt cycling within the bioaugmented-Technosols from applied exogenous microorganisms. EC of all Technosols were were decreased by 500% and stabilised to below 1000 µS/cm (non-saline conditions) by the end of the trial from plant-microbial interactions that enhanced cation uptake. Irrigation throughout the plant growth trial had also contributed to lowered ion/salt concentrations by mass transfer within the Technosols (as seen in Table 10) (NRCS, n.d.).

Water holding capacity (WHC) is an universal parameter widely used to identify declining soil quality (Mills & Fey, 2004; Lowery, et al., 1996). The WHC results are shown in Figure 24. Before teff growth, all

Technosols were capable of maintaining a desired 50% FC. This ensures good mass transfer within the soil to support nutrient cycling, oxygen diffusion, microbial respiratory functions, and root and shoot growth (Lowery, et al., 1996; Amaral Filho, et al., 2017).

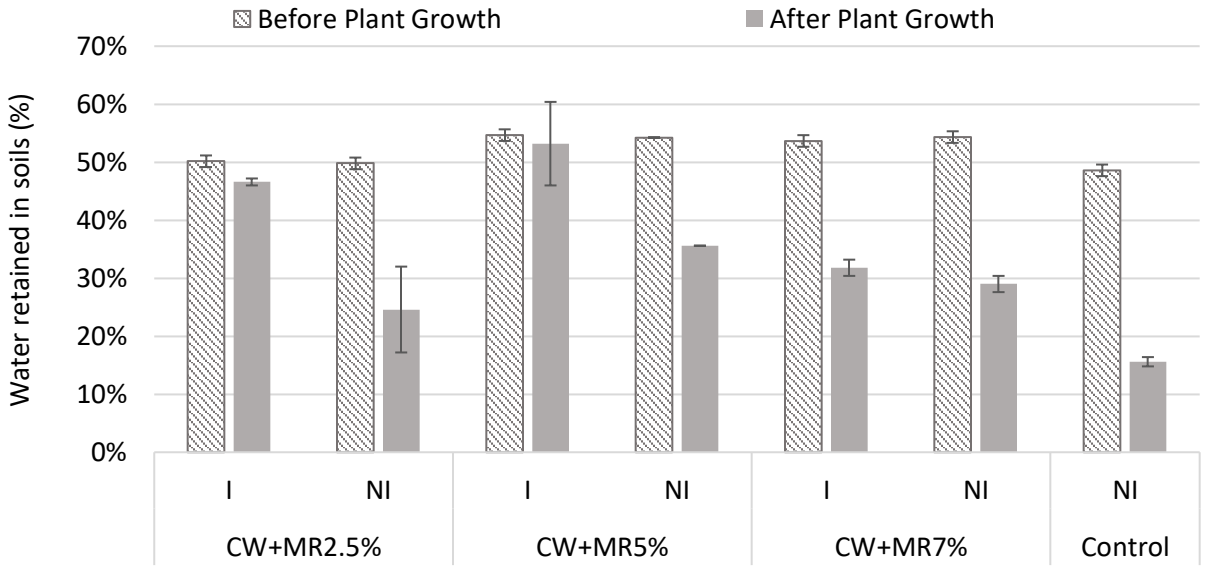


Figure 24: Average percentage water retained in MR amended coal-based Technosols and compost as the control with standard devition before and after teff growth in the initial plant growth trial in summer. Where, I represents inoculated and NI represents non-inoculated treatments.

WHC is dependent on soil structure (porosity) and texture (Lowery, et al., 1996). Coal tailings are sandy and fine in texture, with 2% moisture content (from Chapter 4.1); it does not retain water well. Amending CW with MR ameliorated the Technosol's water retaining capabilities and altered the soil texture to loam. Thus, it was expected that the fabricated soils with higher percentages MR would demonstrate higher water retaining capabilities compared to those with less MR. This was true for the initial WHC as shown in Figure 24 but not for post plant growth WHC. Here, WHC in inoculated and non-inoculated CW+MR7% significantly decreased. With time, the initial high water retention and underdeveloped root systems in CW+MR7% resulted in soil compaction, altering the soil texture and eventually preventing water percolation (Prado, et al., 2020). Bioaugmented CW+MR5% performed the best in terms of WHC after vegetational growth, with only 2% lower than before plant growth.

In anthropogenic soils, the soil microbiome is essential to soil agglomeration (Margerison, et al., 2020; Rivas-Perez, et al., 2016). This is evident from the higher WHC in the bioaugmented-Technosols compared to vegetated non-inoculated controls (refer to Figure 24). Thereby, supporting the use of bioaugmentation in coal-based Technosols for plant growth. Further research was needed to compare results against the performance of agricultural soils; this was investigated in the final plant growth trial.

The macro- and micronutrient contents of the Technosols were investigated as part of soil quality analysis (Botta, 2015). Technosols were analysed for C, N, S, P Bray II, K and micronutrient (Ca, Mg, Na, Cu, Mn, Zn, Fe, B) content after the greenhouse pot study finished. The results are summarized in Table 10.

Table 10: Chemical properties (average values and standard deviation) of MR amended coal-based Technosols with and without bioaugmentation and compost as the control after the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

Chemical Property	Unit	CW-MR2.5%				CW-MR5%				CW-MR7%				Control
		I		NI		I		NI		I		NI		NI
		Value	STD	Value	STD	Value	STD	Value	STD	Value	STD	Value	STD	Value
C	%	49.4	0.13	47.1	3.71	49.3	2.46	48.0	5.37	51.0	0.27	49.4	1.58	10.2
N	mg/kg	0.68	0.0	0.68	0.0	0.7	0.0	0.71	0.01	0.73	0.0	0.74	0.01	0.33
P (Bray II)	mg/kg	68.3	6.65	65.8	2.83	157	9.19	134	17.0	259	0.0	238	14.1	549
K	mg/kg	399	27.6	176	73.5	438	85.6	235	146	375	20.5	252	112	3780
S	mg/kg	766	9.19	656	91.9	1033	137	636	37.5	889	84.2	768	71.4	89.0
Cations (cmol/kg)	Ca	31.4	2.83	27.8	7.71	14.0	0.28	15.0	0.42	15.2	0.78	14.5	0.64	17.8
	Mg	1.85	0.07	1.70	0.14	2.15	0.50	1.85	0.21	2.20	0.0	2.35	0.07	6.60
	K	1.04	0.09	0.45	0.18	1.13	0.24	0.60	0.37	0.96	0.06	0.65	0.29	9.70
	Na	0.92	0.04	0.58	0.07	1.08	0.32	0.72	0.16	0.64	0.04	0.48	0.22	5.60
Ca	%	89.2	0.91	90.7	2.69	76.4	4.75	82.6	3.76	78.0	0.74	80.6	3.30	44.8
Mg	%	5.28	0.62	5.70	0.96	11.7	2.21	10.2	1.00	11.6	0.49	13.1	0.43	16.62
K	%	2.94	0.03	1.60	1.00	6.14	1.05	3.29	1.97	5.07	0.09	3.60	1.63	24.4
Na	%	2.61	0.31	2.00	0.73	5.83	1.49	3.96	0.79	3.36	0.33	2.65	1.23	14.1
Cu	mg/kg	3.95	0.21	3.95	0.21	4.00	0.42	4.55	0.35	4.30	0.28	4.60	0.42	4.60
Zn	mg/kg	3.40	0.28	3.50	0.0	5.05	0.35	5.40	0.28	6.70	0.42	7.50	0.28	51.5
Mn	mg/kg	23.8	0.42	24.5	0.0	22.1	0.42	22.1	0.28	22.1	0.64	22.1	0.14	23.0
B	mg/kg	0.61	0.08	0.85	0.04	0.81	0.18	0.80	0.09	0.91	0.13	0.82	0.13	3.30
Fe	mg/kg	52.4	0.21	61.6	1.13	64.6	2.62	74.1	3.11	87.1	0.14	93.4	2.83	124
T Value	cmol/kg	35.2	2.81	30.5	7.59	18.4	0.77	18.2	0.31	19.0	0.80	17.9	0.06	39.7
S Am.acet	mg/kg	3200	368	2655	757	1595	35.4	1665	35.4	1700	99.0	1605	35.4	87.8
CEC	mg/kg	14.2	0.20	14.2	2.45	14.1	0.06	13.8	0.98	11.7	0.43	12.9	0.66	16.0

The total carbon content (%) varied between 47% and 51% for all coal-based fabricated soils, which correlates to the mean TOC commonly found in South African soils (between 30-75%) (du Preez, et al., 2011). Expectedly, soil organic carbon content was directly proportional to the weight percentage of MR in each Technosol with the highest organic carbon content in inoculated CW+MR7%. Additionally, bioaugmented Technosols had higher levels of macro-nutrients (organic C, N, P and K) compared to the non-inoculated controls after teff growth, thereby, supporting the first objective.

The visibly lower macro-nutrient content of non-inoculated CW+MR2.5% was likely as a result of nutrient leaching. High volumes of increased salinity leachates were collected from these soils, indicating mineral salt accumulation (NRCS, n.d.). Whereas, high macronutrient contents and WHC results for inoculated CW+MR5% suggests structural support for teff growth and nutrient cycling. Compost contained high levels of potassium. This contributed to the poor teff growth and biomass production previously discussed, as excessive K inhibits the uptake of other cations into plant organs (Rawat, et al., 2016).

The results for metal(loid) content of fabricated soils after an initial growth cycle are graphically summarized in Figure 25.

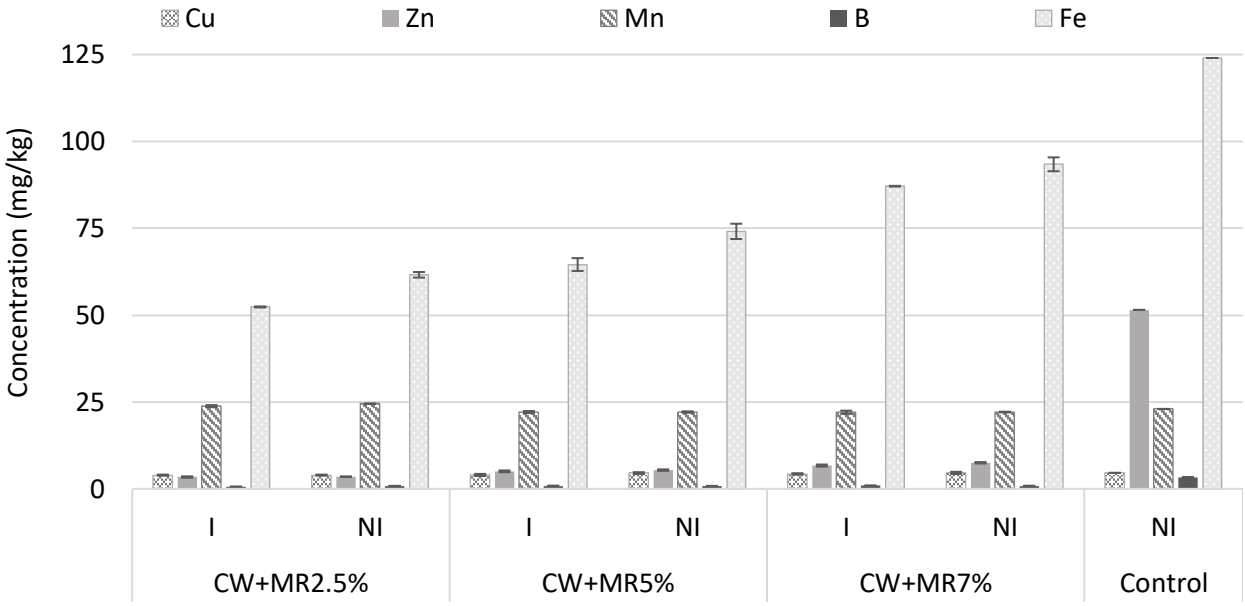


Figure 25: Average metal(loid) concentrations and standard deviations in vegetated, MR amended coal-based Technosols with and without bioaugmentation and compost as the control, after the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

The figure shows that iron concentrations within the engineered soils were eminent, whereas Cu, Zn and B were inferior. This was expected, as coal tailings have an iron content of 18 200 ppm (in Table 9). Thus, iron leaching was of concern, and motivated total and ferrous iron assays that were implemented in the final plant growth trial (discussed in Chapter 4.3.2). Iron levels increased with amendment dosage as per material characterisation (Table 9). Fe, Cu, Zn, B, and Mn levels were less in bioaugmented treatments compared to the non-inoculated controls, indicating that added exogenous microorganisms were capable of translocating these metals as illustrated in Figure 7. It is recommended to characterise the Technosols before plant growth for metal(loid)s to better evaluate the effect of inoculation on soil metal(loid) solubility.

Technosol metal(loid) concentrations were compared to South African national soil standards for metals and metalloids (as depicted in Table 4) to determine if below hazardous levels. It is evident that Cu, Mn and Zn concentrations of all coal-based Technosols after teff growth were below the screening values, except Mn concentrations were higher in non-inoculated CW+MR2.5% and compost, the more alkaline soils (refer to Figure 25). Mn could be leached from these soils that showed poor water retention. This is contrary to research by Komonweerakat et al. (2015) who found increased concentrations of Mn in leachates from fly ash mixed soils with decreased pH values. However, Zn, Fe and Cu are amphoteric in leaching (Cui, et al., 2019) and are expected to leach at very basic and acidic conditions which were not characterised in any of the Technosols.

Considerable levels of soluble iron were present in CW+MR7%, which reduces soil microbial diversity (Firpo, et al., 2014). Thus, along with high salinity (Boyrahmadi & Raiesi, 2018), low water retention capabilities and cation exchange capabilities (1.2 fold lower than CW+MR5%) after plant growth (Prado, et al., 2020; Lowery, et al., 1996), and structural impedance to seedling emergence and root development

(Macia, et al., 2014; Herran Fernandez, et al., 2016), the conditions for sustaining an active and functioning soil microbiome were inadequate in CW+MR7%.

Characterisation of above and below ground dry biomass presented information on the nutrient solubility, metal uptake in teff. The results are summarized in Table 11 and Figure 54 illustrated in Appendix C: Initial Plant Growth Trial Data.

Table 11: Elemental analysis of above (shoots) and below (roots) ground teff dry biomass cultivated in MR amended coal-based Technosols with and without bioaugmentation in the initial plant growth trial in summer. Where, I represents inoculated and NI is non-inoculated treatments.

		CW+MR2.5%				CW+MR5%				CW+MR7%			
		Shoots		Roots		Shoots		Roots		Shoots		Roots	
Symbol	Unit	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
Na	g/kg	2.33	0.985	2.03	1.33	2.04	0.712	0.962	0.77	2.21	0.664	2.07	0.71
Mg	g/kg	3.17	2.73	2.85	2.48	2.43	2.62	2.57	2.20	3.42	2.34	3.40	2.30
Ca	g/kg	4.33	4.35	19.3	16.4	2.69	3.68	23.6	17.7	4.13	4.91	21.1	19.6
K	g/kg	20.6	14.8	5.03	3.16	19.1	16.3	2.06	1.86	20.4	16.6	3.46	1.70
P	g/kg	4.21	3.61	1.34	1.19	3.63	4.23	1.35	1.11	3.73	4.01	2.96	1.70
Si	g/kg	2.70	2.72	0.184	0.176	2.22	2.48	0.126	0.287	3.19	3.21	3.91	0.333
B	mg/kg	5.05	6.35	24.2	24.8	5.47	6.69	23.8	26.3	7.91	6.94	81.3	22.4
Al	g/kg	0.0430	0.098	20.8	21.2	0.0403	0.0400	24.0	26.0	0.0868	0.0279	10.9	17.3
V	mg/kg	0.0948	0.139	24.5	20.9	0.0610	0.0680	29.4	26.8	0.105	0.0579	24.8	20.8
Cr	mg/kg	1.52	1.66	55.9	44.7	1.07	1.83	42.1	35.6	0.796	0.770	28.3	26.6
Mn	mg/kg	113	109	176	174	106	107	170	147	75.5	62.7	174	127
Fe	g/kg	0.0738	0.0922	14.7	11.2	0.0572	0.0638	17.0	14.1	0.105	0.0641	14.7	11.0
Co	mg/kg	0.232	0.404	12.6	68.5	0.289	0.220	9.06	8.23	0.300	0.187	6.80	7.49
Ni	mg/kg	0.976	1.09	32.7	26.0	0.641	0.953	24.8	23.8	0.719	0.987	14.8	19.7
Cu	mg/kg	5.56	8.71	34.9	36.2	6.32	6.20	28.4	29.0	6.85	6.83	26.8	31.4
Zn	mg/kg	54.2	61.7	211	246	32.6	30.5	48.1	54.0	46.8	32.9	38.3	37.9
As	mg/kg	0.578	0.785	8.02	5.68	0.466	0.405	5.93	5.60	0.728	0.440	7.60	4.49
Se	mg/kg	0.0498	0.154	1.05	0.878	0.113	0.0617	1.12	1.10	0.0392	0.0508	0.844	0.943
Sr	mg/kg	31.6	39.8	251	214	22.3	30.1	294	259	23.4	32.6	280	240
Mo	mg/kg	0.727	0.713	2.14	1.16	0.639	0.668	1.15	1.64	0.833	0.609	3.22	1.80
Cd	mg/kg	0.0428	0.0193	0.131	0.156	0.0536	0.0331	0.0716	0.0751	0.0570	0.0358	0.0957	0.110
Sn	mg/kg	0.0682	0.0822	1.46	1.20	0.0535	0.0713	1.23	1.56	0.0864	0.0768	1.47	1.21
Sb	mg/kg	0.0306	0.0343	0.366	0.0187	0.0283	0.0260	0.0207	0.472	0.0496	0.0227	0.574	0.429
Ba	mg/kg	14.2	17.2	278	237	9.81	14.9	351	306	5.84	6.75	301	239
Hg	mg/kg	0.0119	0.0135	0.181	0.467	0.0083	0.0092	0.190	0.186	0.0111	0.0145	0.215	0.153
Pb	mg/kg	0.155	0.249	12.9	11.8	0.132	0.143	10.6	10.1	0.139	0.108	9.89	7.32

From the table above, the macronutrients Na, Mg, Ca, K, and P were identified, which were more abundant within teff shoots than the roots, whereas, Ca, Al and Fe concentrations were significantly higher in the below ground biomass, especially of inoculated CW+MR5%. Initial material characterisation showed high Al and Fe content of CW and MR (refer to Chapter 4.1). Thus, the results for high Fe and Al metal concentrations in the fabricated soils were expected.

The macro-content of Na, Mg, K, and P in the best performing bioaugmented CW+MR5% shoots and roots were lower than inoculated CW+MR2.5% and CW+MR7%. Potentially due to augmented microbial metabolism, enzymatic activities and chlorophyll production to which K, Mg, Na, Ca and P are vital (Rawat, et al., 2016). This is supported by the higher soil levels of Na, Mg and K in bioaugmented CW+MR5% compared to the other inoculated treatments (refer to Table 10). Phosphorus was higher in CW+MR7% due to a greater MR dosage. From CHNS results (Figure 54 in Appendix C) for the above ground teff dry biomass, it is clear that there was little variation between the CHNS content within the shoots of the Technosols. Carbon concentrations in all were above 40% with negligible amounts of sulfur.

The difference in macro, micro-, and trace elements levels in above and below ground plant biomass between the various fabricated soils suggest that nutrient cycling and metal(loid) translocation have occurred at different rates. This was expected, as these processes are dependent on the soil microbial communities (Tiwari, et al., 2021). Therefore, it was necessary to evaluate the Technosols' biological properties.

4.2.3 *Soil Microbiome Analysis*

The microbial biomass of the fabricated soils were determined with and without inoculation, before and after incubation through substrate induced respiration (SIR) tests. The same technique was performed for post plant-growth analysis, as suggested by Weiler et al. (2020). The results for the initial growth trial, presented as available carbon (mg) per kilogram soil, are summarized in Figure 26.

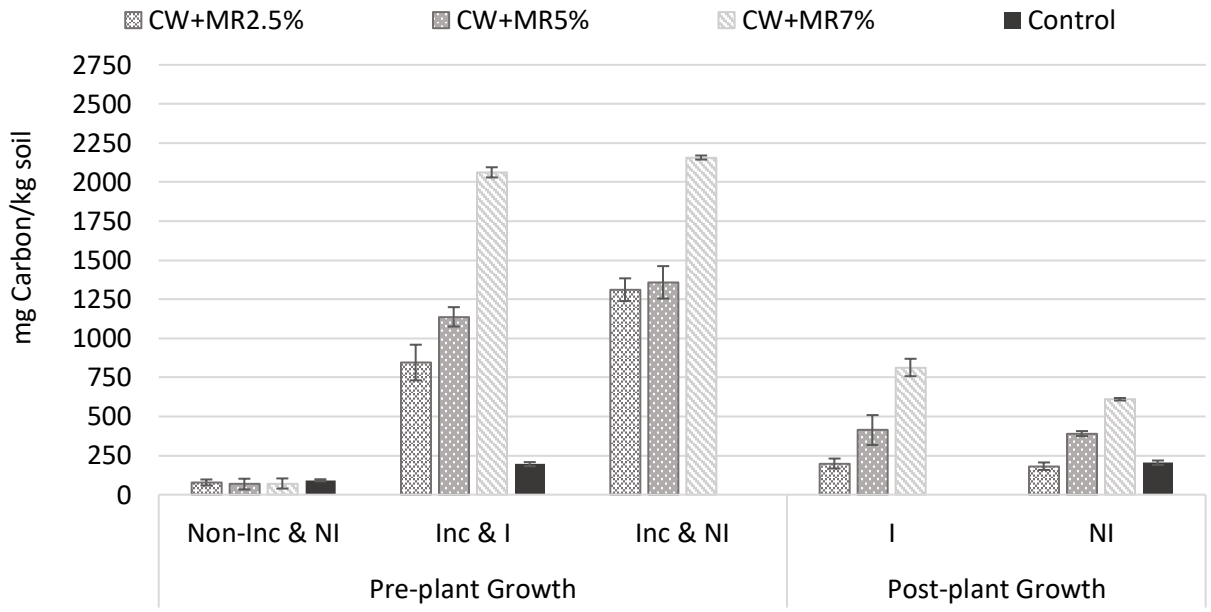


Figure 26: Average microbial biomass and standard deviation in MR amended coal-based Technosols with and without bioaugmentation, and compost as the control, before and after plant growth in the initial growth trial in summer. Where, Non-Inc is non-incubated, NI is non-inoculated, Inc represents incubated and I refers to inoculated treatment types.

Microbial proliferation was promoted by incubation. This is seen by the notably higher microbial biomass achieved after incubation compared to before (17-fold for inoculated CW+MR5%) in Figure 26. Strikingly, non-inoculated fabricated soils had a greater abundance of microbial biomass than the inoculated treatments before plant growth commenced. Ezeokoli et al. (2020) reported on the sensitivity of mine waste-based soil microbial communities to abiotic and physicochemical soil characteristics such as temperature, pH, sodium and calcium soil levels. As corroborated by Vrabl et al. (2019), incubation rapidly activated fungal growth by quickly adjusting metabolism rates to the increase in temperature, that further prolonged the lag phase of bacterial growth and resulted in the formation of hot-spots and uneven spread of microbial biomass within the soils. Therefore, not providing an accurate representation in the SIR analysis. Additionally, Rousk & Baath (2007) suggested a negative correlation between nitrogen

addition by amendments and bacterial growth, whilst nitrogen stimulated fungal growth in soils. Thereby, corroborating the results as MR was characterised by 3.11% nitrogen, and higher N concentrations were reported in the non-inoculated fabricated soils compared to the inoculated treatments after plant growth. From Figure 26, the microbial biomass was proportional to ratio of added OM as MR before and after vegetation, with the greatest microbial biomass in vegetated, inoculated CW+MR7%. This was anticipated from the carbonaceous nature (45.3% carbon) of MR. However, microbial biomass relates to combined respiration of soil microorganisms (Rousk & Baath, 2007). To further investigate the discretion between microbial biomass in Technosols, the microbial communities were profiled in the final growth trial to identify the bacterial and fungal abundance within all soils after incubation.

Expectantly, bioaugmented CW+MR5% had 6% more microbial biomass than its non-inoculated control after a growth cycle. Suggesting that the added exogenous microorganisms were able to proliferate and adapt with plant growth whilst increasing soil fertility as recommended by the manufacturers of EM Pro-Soil (Efficient Microbes, 2006). Furthermore, achieving the project's primary objective.

Moreover, the standard error in microbial biomass results was due to the sensitivity of soil microbiomes to biotic and abiotic factors. Several environmental factors, such as plant species, soil water content, accessible carbon, soil mineral matrix, temperature, location, pH, salinity, inoculum type, and the interaction between microbes, play a role in the growth, abundance, and persistence of soil microorganisms (Nannipieri, et al., 2003; Margerison, et al., 2020; Tiwari, et al., 2021). Error between samples per treatment type was inevitable as it is realistically impossible to collect a representative sample of each Technosol. To determine if the inclusion of amendments as biostimulation to coal mine waste benefited soil fertility when applied with bioaugmentation, pure CW as a control was included in the subsequent growth trial.

4.3 Final Plant Growth Trial

The final plant growth trial aimed at answering the key questions regarding the effects of bioaugmentation and biostimulation to increase soil fertility, enhance plant growth and reduce metal(loid) solubility in coal-based Technosols with seasonal changes. The experiments were performed according to the methodology detailed in Chapter 3. The agricultural potting soil (control soil) used in this growth trial provided information on teff growth and the performance thereof in naturally occurring soils with optimised fertility and quality as a benchmark for the anthropogenic soils. Plant growth was evaluated in 100% CW (both I and NI), including the previously investigated Technosols. This informed on the value of biostimulation with MR as an OM amendment and structural ameliorant to CW. In addition, the difference between physical, chemical and biological properties of vegetated and unvegetated fabricated soils were determined in this trial.

4.3.1 *Plant Development & Performance*

The average rate of seedling emergence per treatment type over a 20-day period is presented in Figure 27 below and **Error! Reference source not found.** in Appendix D. Plant growth and development measurements included teff shoot heights, water retention, number of plants per pot, and days till bolting, all recorded until the teff plants had matured. Seasonal changes were identified from collected

daily greenhouse temperature, dew point and relative humidity data that were compared to the initial trial.

A natural disaster occurred in Cape Town near UCT during the first five days of this trial. During this time, access to campus was limited and no data could be collected for seedling emergence, hence the missing data in Figure 27. From the germination results in the initial trial (discussed in 4.2.1) it was expected that germination in this trial commenced on the first day after planting.

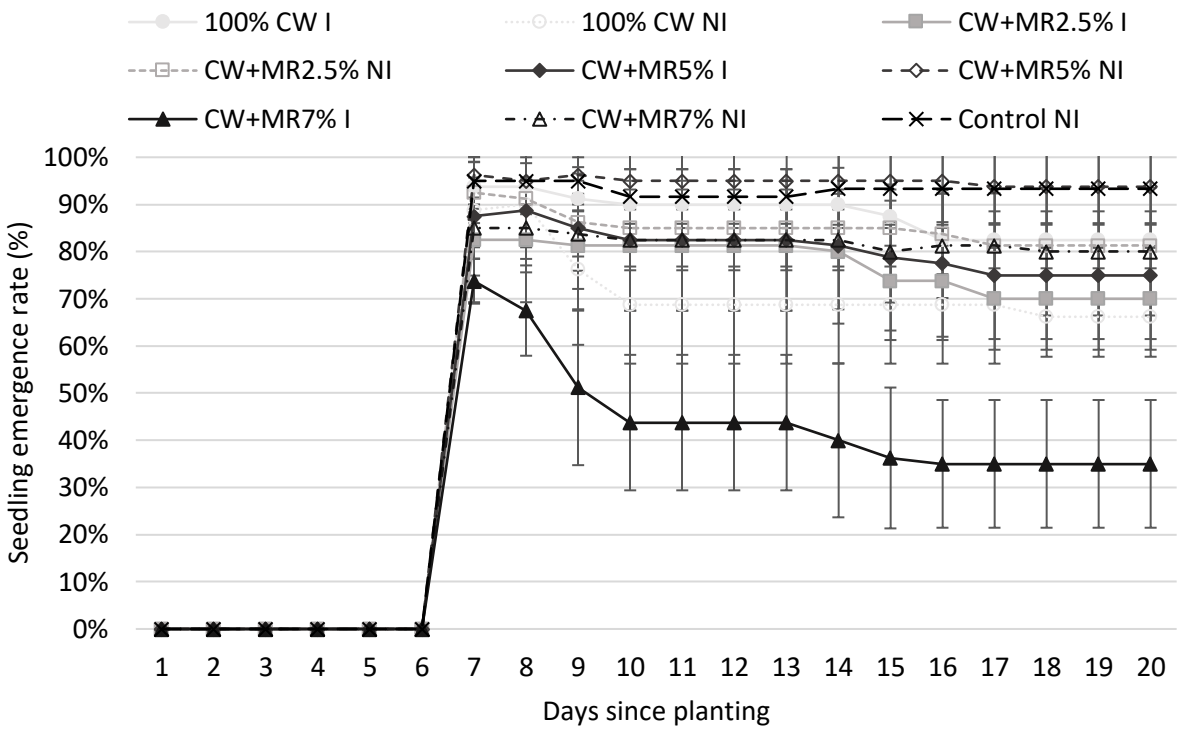


Figure 27: Average rate (expressed as percentage) and standard deviation of teff seedling emergence per MR amended coal-based Technosol with and without bioaugmentation, and potting soil as control, relative to the number of seeds sown (n=20), during the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

Similarly to the initial growth trial, teff germination rates within bioaugmented and biostimulated fabricated soils were marginally slower compared to that in only biostimulated soils. Perhaps due to the delayed bacterial growth. Novo et al. (2013), Firpo et al. (2014), Hernan Fernandez et al. (2016) and Amaral Filho et al. (2020) underlined that pedogenesis in anthropogenic soils differ from natural soils, as there is at times an inherent exotic distinction between parental materials and amendments to the natural environment. Material degradation often must occur first, consequently delaying other soil functions (e.g., supporting seed germination) and the process of reaching equilibrium between soil health, quality and fertility (Rivas-Perez, et al., 2016).

Inoculated CW+MR7% demonstrated poor performance in supporting seed germination as seen in Figure 27**Error! Reference source not found.**. After 7 days, the average emergence rates for inoculated and non-inoculated CW+MR7% were 1.2- and 1.1-fold lower (respectively) than that of inoculated and non-

inoculated CW+MR5%. It continued to decrease with time due to persistent fungal growth issues. Fungal growth occurred predominantly on the surface of the incubated fabricated soils with 7 wt.% MR as discussed in Chapter 4.2.3. The humid conditions (from covered, moistened soils), carbon and nitrogen-rich MR, and nutrient-dense teff endosperms provided feedstock and favourable conditions for fungi development, whilst bacterial communities lagged (Rousk & Baath, 2007). The relocation of the pots from the laboratory to the greenhouse ceased excessive fungal growths. By the end of the seedling emergence period, the average number of seedlings that emerged in CW+MR5%-NI were similar to that in the control soil. CW+MR5%-I showed the highest percentage of seedling emergence of all inoculated soils, confirming the performance of CW+MR5% achieved in the initial trial during favourable conditions. Inoculated 100% CW treatments showed potential in supporting teff growth, with 83% by the end of the seedling emergence period compared to 66% in its non-inoculated control.

In the greenhouse (conditions presented in Figure 13), the number of plants per pot declined in all treatments. The change in photosynthesis period (from a 24h-day to 16h-day/8h-night) and abiotic conditions (lower temperatures and undulating humidity) resulted in plant deaths. A significant decline in temperature was recorded from day 7 (20.3 °C average) till day 20 (14.9 °C average), delaying seedling growth. Similarly to the first trial, nematodes and flies preceded in this growth cycle. Fly larvae growth ensued from the fluctuations in humidity caused by sporadic rain storms during the trial. Fly strips were put up in attempt to mitigate flies/larvae from feeding off teff endosperms. Nonetheless, teff seedlings in Technosols with high dosages of MR were adversely affected, inferring seedling deaths.

The number of plants that have established or died by the end of the growth cycle relative to the number of seeds planted per Technosol type, indicates the ability of the fabricated soil to function as a healthy topsoil. This is summarized in Figure 28 below and in Figure 55 (Appendix D). The potential of CW with biostimulation of 2.5% and 5% (w/w) MR as a topsoil is clear; inoculated CW+MR5% and non-inoculated CW+MR2.5% consistently supported all teff plants for a growth cycle in unfavourable conditions.

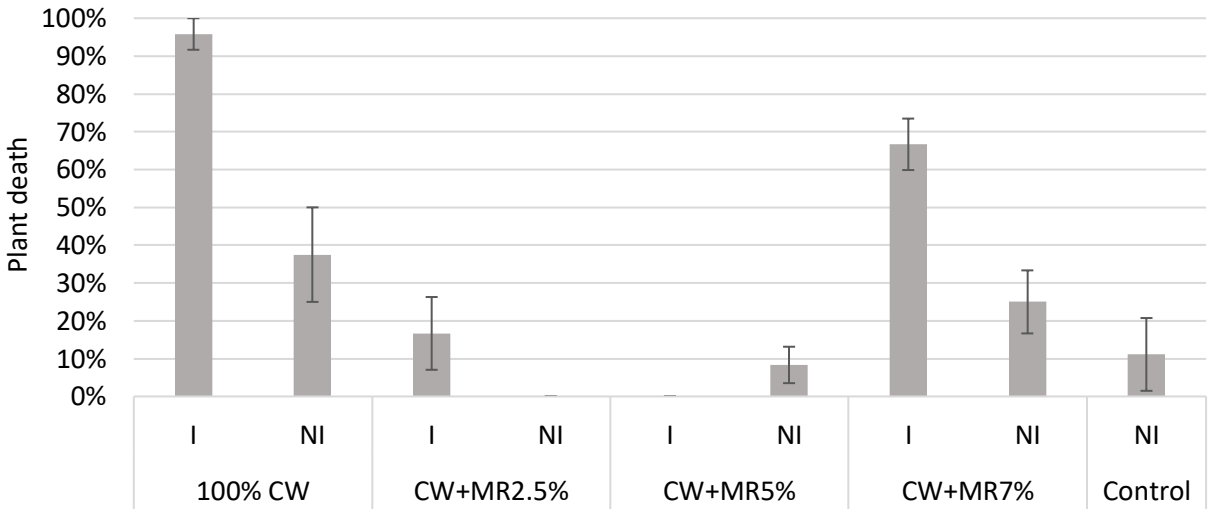


Figure 28: Average percentage and standard deviation of plant deaths in MR amended, coal-based Technosols with and without bioaugmentation, and potting soil as the control, after the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

Plant growth and development in 100% CW were limited, with only 4% healthy teff by the end of the trial in inoculated pure CW. The inadequate P and K levels, and low moisture content (2%) of CW (outlined in Table 9) cannot in its entirety sustain plant growth.

The average above ground teff growth rate (cm/day) per pot of Technosol were determined as per the initial growth trial, and illustrated as cumulative growth in Figure 29.

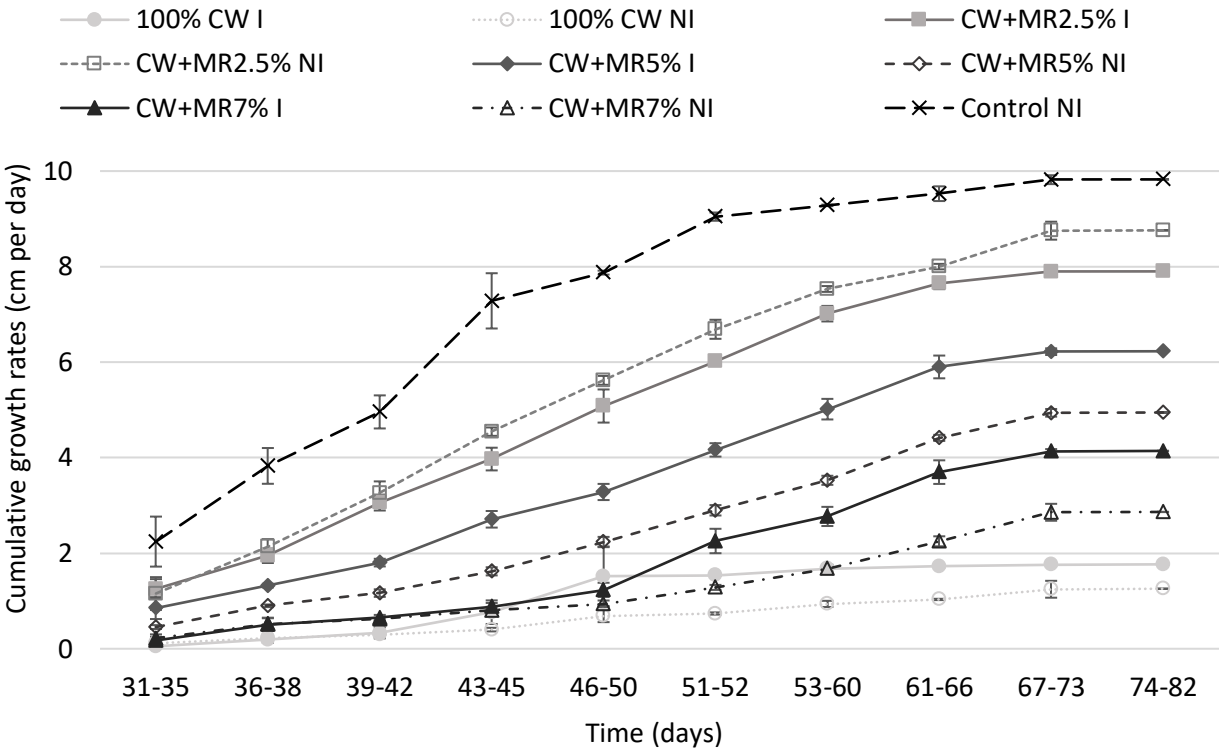


Figure 29: Cumulative above ground teff growth rates (cm per day) and standard deviations in MR amended coal-based Technosols, with and without bioaugmentation, and potting soil as the control, during the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

Significant growth occurred between days 36-42 and 51-60 as seen in Figure 29, as seedlings established and extended their root networks. During these periods, greenhouse conditions were stable and there were no competition for resources. Average daily greenhouse temperatures ranged between 20.6-18.1 °C between days 36-40, and 18.2-18.4 °C between days 51-58 (refer to Figure 13). After these periods, temperatures continuously dropped to 14.6 °C on day 45, and 12.2 °C on day 70. From Figure 29, it is clear that teff cultivated in the control performed the best and teff growth in 100% CW and CW+MR7% were very poor. Of the inoculated fabricated soils, the highest above ground growth rates were in CW+MR2.5% followed by inoculated CW+MR5%. Plant development obtained within the best performing Technosols (CW+MR2.5%; NI and CW+MR5%; I) were resemblant to that achieved within the control soil. Representative pots of each Technosol type after the growth cycle, are shown in Figure 30 (bioaugmented) and Figure 31 (without bioaugmentation).

Control	CW+MR2.5%; I	CW+MR5%; I	CW+MR7%; I	100% CW; I

Figure 30: Representative pots illustrating the above ground teff growth achieved in the biostimulated and bioaugmented coal-based Technosols relative to the control (potting soil) in the final plant growth trial after 82 days.

Control	CW+MR2.5%; NI	CW+MR5%; NI	CW+MR7%; NI	100% CW; NI

Figure 31: Representative pots illustrating the above ground teff growth achieved in the biostimulated and non-inoculated coal-based Technosols relative to the control (potting soil) in the final plant growth trial after 82 days.

High biomass generation and healthy, developed root systems are an indication of the soil's ability to accommodate phytoextraction (Hrynkiewicz, et al., 2018). A healthy soil microbiome stimulates both root proliferation and shoot development through nutrient cycling, carbon and nitrogen sequestration, and the degradation of organic material (Margerison, et al., 2020; Novo, et al., 2013). Therefore, yields for above and below ground plant dry biomass after the final growth trial were determined and illustrated in Figure 32.

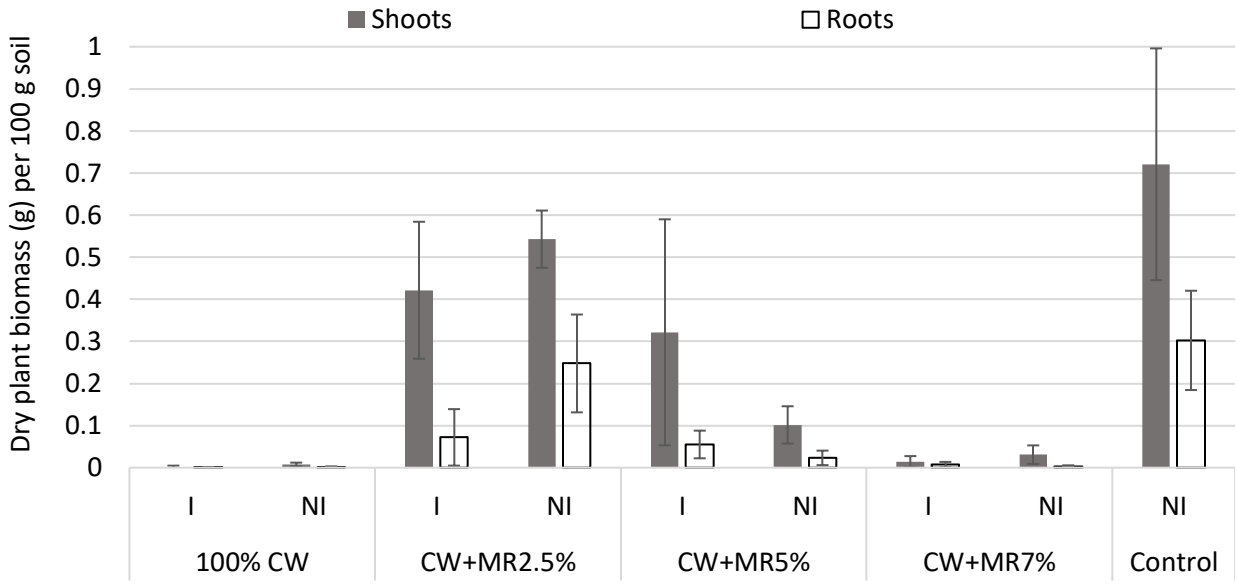


Figure 32: Average and standard deviation above (shoots) and below (roots) ground teff dry biomass produced per 100 gram soil (MR amended coal-based Technosols with and without bioaugmentation, and potting soil as the control) after 82-days in the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

In Figure 32, teff in potting soil (control) had the highest above ground dry biomass production. Root structures in the control were long and fibrous; creating a large surface area for mass transfer between plant organs, microbial cells and soil matrix. Hence, correlating to the good teff growth and performance seen in the control. Variation in biomass yields between pots were significant in teff shoots cultivated in CW+MR2.5%-I, CW+MR5%-I and in the control, due to the interdependence between biotic and abiotic factors during soil processes (Sauer, 2015). Non-inoculated CW+MR2.5% generated the highest above ground dry biomass in all Technosols; corroborating the previously presented seedling survival and teff height results for this engineered soil. As previously found, a higher ratio of OM added as amendment did not enhance plant growth in a bioaugmented coal-based Technosol. The results are comparable with those presented by Amaral Filho et al. (2020), Firpo et al. (2015), and Weiler et al. (2018), where the addition of 2 to 5 wt.% OM enhanced shoot and root productivity, and acted as a physical ameliorant in the coal waste soils.

As anticipated, there is a positive correlation (R^2 = 0.91) between above and below ground teff dry biomass production since leaf growth and seed production ensues root evolution (Tiwari, et al., 2021). Non-inoculated CW+MR2.5% performed the best of all Technosol types. As determined from the initial

growth cycle, 100% CW and CW+MR7% were not able to sustain teff growth and produced negligible yields, depicted in Figure 33 illustrating the root structures in all bioaugmented Technosols after the final growth trial.

Species diversity is maintained by root exudates (Thavamani, et al., 2017; Tiwari, et al., 2021; Margerison, et al., 2020). Thus, results suggest that bioaugmentation and biostimulation with 2.5% and 5% MR (wt.%) supported soil microbes to establish, to develop root structures that enhanced soil water retention.

100% CW	CW+MR2.5%; I	CW+MR5%; I	CW+MR7%; I

Figure 33: Visual representation of teff root development in bioaugmented coal-based Technosols after 82 days in the final plant growth trial in winter.

Overall, higher biomass (above and below) yields were achieved in the initial trial; implying that conditions were more favourable for teff growth compared to those in the final trial. During summer, inoculated CW+MR5% outperformed the other fabricated soil types in terms of teff germination, soil quality, shoot heights and biomass production. However, in winter, non-inoculated CW+MR2.5% showed the most potential in supporting germination, seedling growth, root development, biomass production and shoot heights. Probably due to seasonal changes and modified irrigation schemes and the effects thereof on the soil inoculum. Analysis on soil biological properties were performed to further investigate effects of bioaugmentation on Technosol health.

In the final stage of plant growth, small florets form in dense spikelets to produce grains (seeds). The daylight hours progressively shortened during the autumn to winter growth trial. The attenuated exposure to light encouraged flowering as teff is a short-day plant and its developmental response is affected by photoperiodism. The continuous drop in temperatures and rise in humidity during the final plant growth phase (seen in Figure 13) were unfavourable and significantly delayed inflorescence compared to the initial trial. The days till bolting were correlated to the average weight of seeds produced per pot of all Technosols, and graphically summarized in Figure 34.

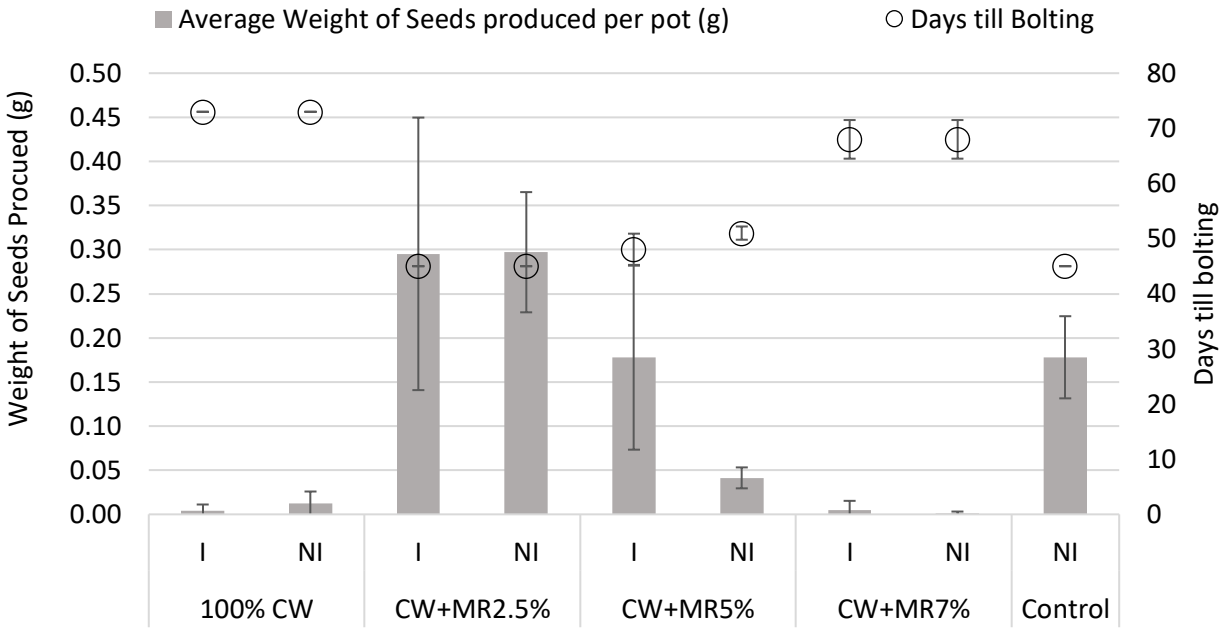


Figure 34: Average teff grain yield (g) and days till bolting with standard deviations in all coal-based Technosols, with and without bioaugmentation, and potting soil as the control, in the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

Of the bioaugmented Technosols, CW+MR2.5% was best able to support inflorescence and seed production, and bolting was initiated on day 45 (5 days later than in the initial, summer growth trial) along with teff in potting soil. Three days later, flowering occurred in teff in CW+MR5%, followed by CW+MR7% and then 100% CW. This trend of inflorescence and seed production in Technosols were congruent to the initial growth trial results.

The correlation between plant dry biomass and seed production for teff cultivated in all Technosols after the final growth trial is presented in Figure 56 in Appendix D. From the coefficient of determination (R^2 = 0.9), it is evident that higher above ground plant biomass resulted in higher seed production. The biomass and seed production in coal-based Technosols amended with 2.5% (w/w) MR were comparable to that obtained in potting soil (grain yield outweighs that of the control). Additional fertility tests (as detailed in Chapter 3) were conducted to investigate the effect of soil health on grain fertility. The results are graphically presented in Figure 57 in Appendix D. Of the seeds collected from bioaugmented Technosols, CW+MR2.5% seemed the most fertile with seedling germination at 91% followed by CW+MR5% at 81%. Interestingly, seeds collected from teff cultivated in non-inoculated Technosols germinated faster than those from bioaugmented fabricated soils. The germination rates equalised by the 6th day after planting, whereafter those from the inoculated treatments outperformed the controls. This trend in NI vs I germination mimicked the seedling emergence results reported at the start of the final growth trial. By the end of the fertility test period, germination rates of seeds harvested from shoots in the inoculated treatments for CW+MR2.5% and CW+MR5%, exceeded those originally sown in these engineered soils. An indication of cycle-to-cycle growth stability from including 2.5-5 wt.% MR as biostimulation in bioaugmented Technosols.

The daily water requirements from soil FC data infer water usage demands of plants and soil microorganisms, and evapotranspiration rates. The figure below cumulatively summarizes the water requirements per kilogram of fabricated soil over a 30-day period.

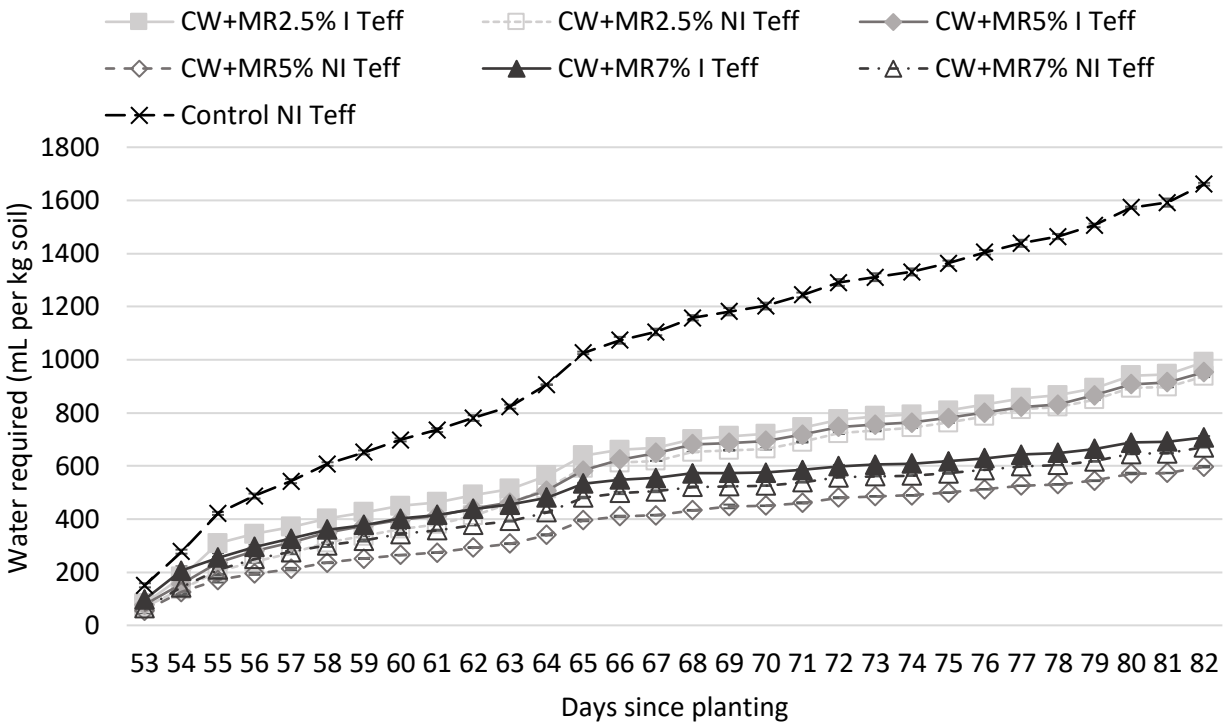


Figure 35: Cumulative water requirements and standard deviation per kilogram of vegetated MR amended coal-based Technosol, with and without bioaugmentation, compared to the control (potting soil) over a 30-day period. Where, I refers to inoculated and NI refers to non-inoculated treatments.

Teff growth within coal-based Technosols should restore moisture and minimise the risk of soil erosion when implemented as a rehabilitation strategy at mines (Karaca, et al., 2018). As MR was also used as a structure ameliorant to minimise water percolation (Lamb, et al., 2014), it was anticipated that Technosols with the highest ratio of MR (7 wt%) would have the lowest average daily water consumption, provided that plant growth is maintained. This trend remains, especially within bioaugmented fabricated soils. However, the overall evapotranspiration rates observed in CW+MR5%; NI are the lowest and most favourable when considering implementation. The figure suggests that large volumes of water could be saved when implementing Technosols as topsoils for revegetation with teff compared to agricultural soil. This is further evaluated in Chapter 5.

The results for the best performing fabricated soils; CW+MR2.5% (Figure 36) and CW+MR5% (Figure 37) against the control are further discussed. The average daily volume of water required per pot of soil are presented from the first day of maintaining 50% FC per pot till the end of the growth period, against the corresponding greenhouse temperatures.

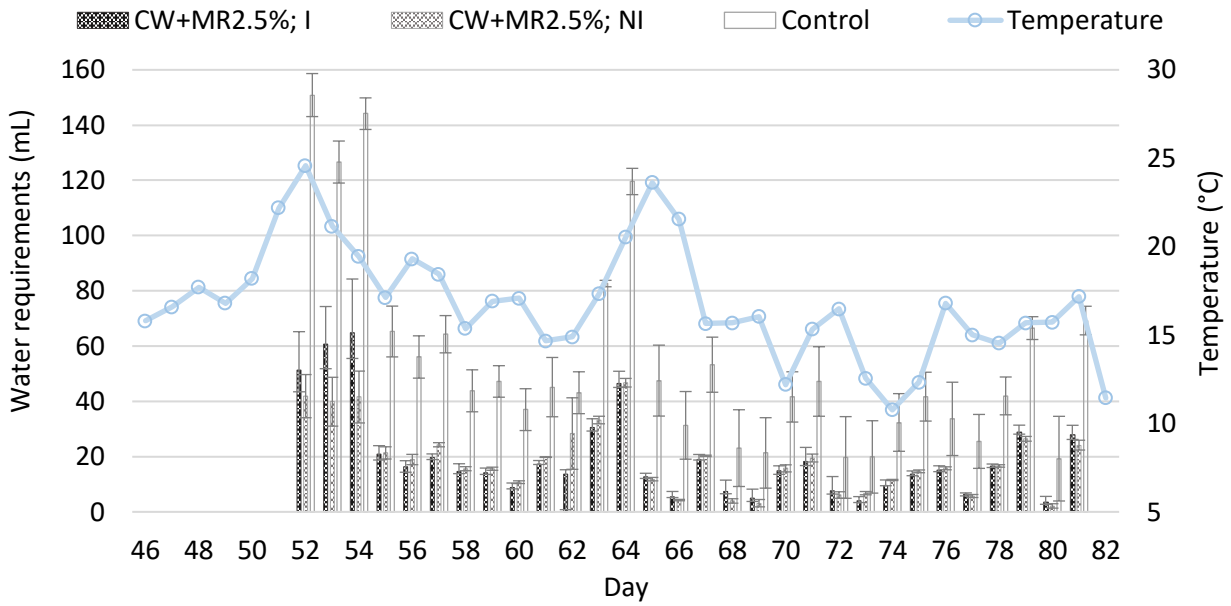


Figure 36: Average daily water requirements with change in greenhouse temperature for vegetated CW+MR2.5% and potting soil as the control during the final growth trial winter. Where, I represents inoculated and NI is non-inoculated treatments.

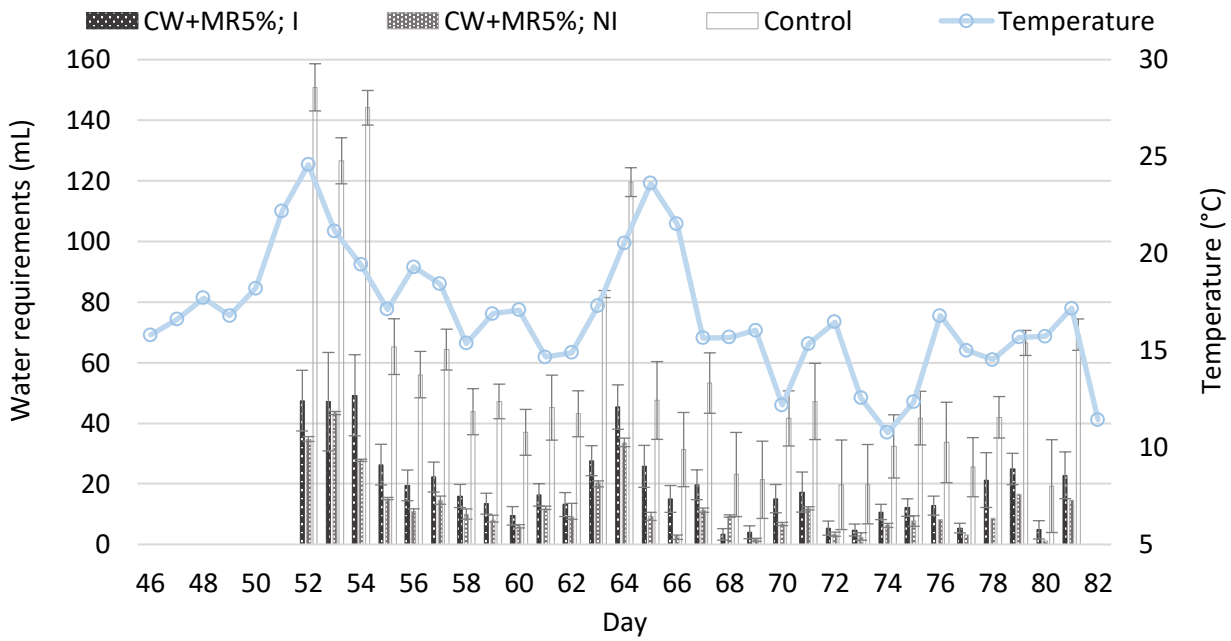


Figure 37: Average daily water requirements with change in greenhouse temperature for vegetated CW+MR5% and potting soil as the control soil during the final growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

In the first three days of measurements (day 52-54 in all figures), water requirements in all treatment types reached a maximum. If augmented temperatures and reduced humidity, the water evaporation from topsoil surface and transpiration from stems, leaves and flowers are amplified. Upon analysis of the greenhouse data (refer to Figure 13), the temperature rapidly increased on day 50 (18.2 °C) and peaked at 24.6 °C on day 52. This rise in temperature accelerated teff growth (as seen between days 50-52 and

52-60) and resulted in higher water requirements per pot of soil relative to the other days where root growth had stabilised and the frequency and magnitude of climatic fluctuations were less. By day 66, CW+MR2.5% and CW+MR5% shoot growth decelerated as shown by the limited growth rates after this day in both figures. Stationary growth and colder conditions (illustrated in Figure 13), lead to reduced water requirements for the days succeeding.

When critically comparing the water requirements for the inoculated against non-inoculated CW+MR5% soils (Figure 37); it is evident that the water requirements in soils without bioaugmentation were less. A similar trend, but to a lesser extent, is seen for inoculated and non-inoculated CW+MR2.5% Technosols (Figure 36). Microbial activities and the associated rates thereof are controlled by soil water content (Paul, et al., 2003). Thereby, suggesting that the bioaugmented treatments which predominantly required more water to maintain a daily soil 50% FC, had more active microbial communities compared to non-inoculated soils. The shoot dry biomass in non-inoculated CW+MR2.5% were 1.3-fold of the inoculated treatment (refer to Figure 32). More aerial stomata biomass implies larger surface area available for photosynthesis and water transpiration, thus, higher water demands. However, the results allude that soil microbial functions required more water for mass transfer than the rates of evapotranspiration from thin, hairy grasses.

From assessment, it can be concluded that water requirements were reduced tremendously in CW-based Technosols relative to the control soil. To sustain 6 teff plants in bioaugmented CW+MR2.5% and CW+MR5%, 19.8 mL and 31.8 mL water per 500 mL of fabricated soil were respectively required every day, compared to 33.2 mL in the control.

4.3.2 *Characterisation of Technosols*

The primary key question; whether soil fertility and structure increased, metal(loid) solubility reduced, and plant growth enhanced in a bioaugmented-Technosol compared to the non-inoculated control, has been addressed in part by the previously discussed plant growth results. The results on plant performance demonstrated that an increased ratio of MR to coal based bioaugmented-fabricated soils, did not lead to successful seedling emergence, prolonged plant growth, nor to superior plant biomass yields. It was determined that a CW:MR ratio of 95:2.5-5 (w/w) provided the best performing soil matrix for teff growth. To further investigate the soil quality in these bioaugmented fabricated soils, physiochemical conditions were analysed as detailed in Chapter 3.7.

The results for average pH values of the examined Technosols before and after the final plant growth trial are graphically presented in Figure 38 (below) for vegetated soils and in Figure 58 (Appendix D) for non-vegetated. Leaching tests were performed after teff growth. The pH, Eh and EC of all soil leachates are summarized according to the soil conditions.

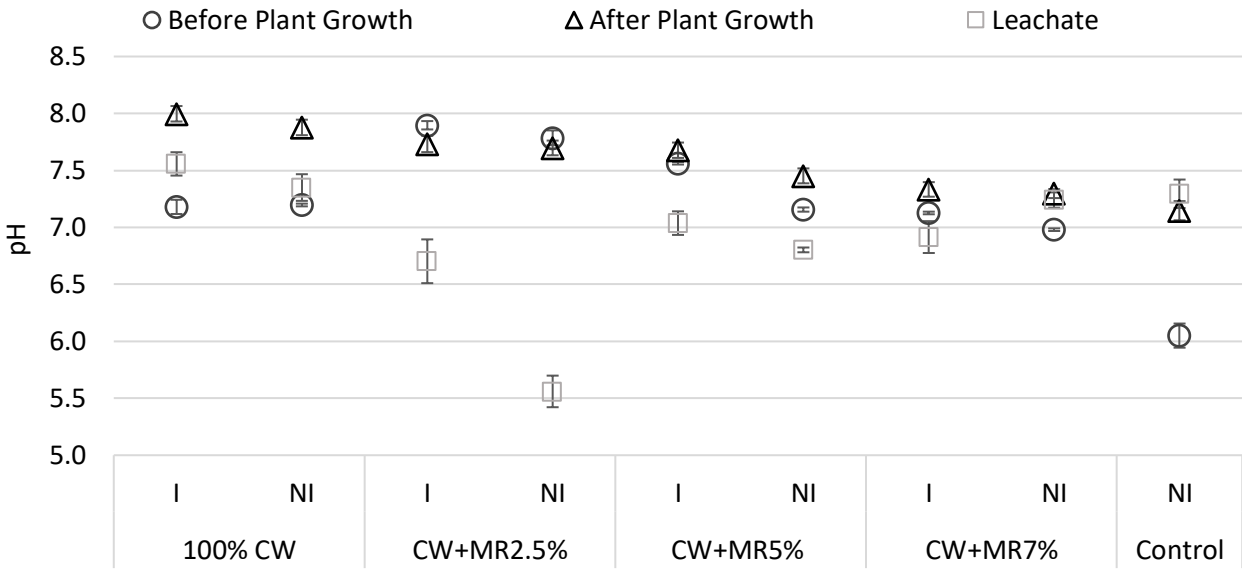


Figure 38: Average pH values and standard deviations of vegetated coal-based Technosols with and without bioaugmentation, and of associated leachates, with potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatments.

As previously shown, unprecedented teff growth was exhibited in the control which had the most acidic initial soil pH of the studied treatments. This is in line with the preferred soil pH conditions for teff. A declining pH with increasing OM levels were expected as described in literature (Husson, 2013). The initial Technosol pH values are all moderately alkaline (between 7 and 7.90). The addition of 2.5% MR as amendment (pH of 5.71) increased the initial acidity. As dosage of MR increased, initial pH decreased. This is corroborated by the results for the initial growth trial (refer to Figure 21) and the reduction in pH observed by Amaral Filho (2020) when MR was used in coal-based Technosols for plant growth in coal waste soils. This trend, along with the increase in pH in the presence of the inoculant compared to the non-inoculated treatments, correlates to the biogenic regulation of soil pH through MR application as biostimulant for microbial respiration (Sanchez-Clemente, et al., 2018; Neina, 2019). Interestingly, upon initial amendment application of 2.5% MR, soil pH increased by 1.1-fold. The corresponding decrease in Eh for this alkalinization illustrates the interdependence between pH-Eh in pedogenesis, and is perhaps indicative of microbial respiration from bioaugmentation or initial soil compaction upon MR application (Husson, 2013).

Biological functions in the rhizosphere induce soil pH changes to enhance proton motive forces across microbial cell membranes, by ensuring membranal diffusion of formic acid, acetic acid, and other short-chain fatty acids, and to promote microbial growth (Neina, 2019). Thus, it was expected that the microbial populations introduced through inoculation would be able to establish and proliferate in these initially alkaline Technosols (enhanced proton motive force).

From Figure 38, soil pH increased with teff growth in all treatments except CW+MR2.5%. According to Prado et al. (2020), the alkalising interaction of teff growth in MR amended CW-based Technosols are specific to teff as a pioneer species, suggesting rehabilitation of soil conditions through ion uptake into

plant organs. During anaerobic respiration, consumption of protons in reduction reactions result in a pH increase (Prado, et al., 2020) that lead to alkalinization. Additionally, the storage of rainwater (used for irrigation) could have contributed to pH alkalinity adjustments. During nitrogen sequestration when nitrate uptake is dominant, hydroxyl or bicarbonate ions are released by plants to regulate ion potential (Atlas & Bartha, 1998). This increases the soil pH. This phenomenon could explain to the slight increase in soil pH after teff growth in Technosols with 5% MR and in the control. Profiling the soil microbiomes for nutrient cycling genes (refer to Chapter 4.3.3) was performed in support.

The trends in change in soil pH with time in vegetated and non-vegetated pots are similar, revealing that Technosol pH was not independently regulated by teff growth. Thereby, eluding to the significance of abiotic variables (temperature, irrigation, exposure to light) and of microbiota on pH regulation as suggested by Atlas & Bartha (1998). Yet, the randomised pot placement in the greenhouse ensured equal exposure to sunlight and all pots were maintained at 50% FC, eliminating independent variation in Technosol pH from abiotic variables. The soil pH, especially that of metal-rich mine based soils, is also regulated by the release of cations from Ca, Mg, K and Na and Al during weathering with plant growth (Prado, et al., 2020). This is evident in the different concentrations (cmol/kg) of these cations between vegetated and non-vegetated Technosols (refer to Table 12 and Table 24, respectively).

From Figure 32, maximum biomass production in coal-based engineered soils occurred when the pH was closer to 7.5 (all CW+MR2.5% and inoculated CW+MR5%). This was expected as higher pH values lead to a higher bioavailability of macronutrients and reduced aluminium toxicity (Amaral Filho, et al., 2020). Conversely, lower pH values in the potting soil increased the bioavailability of micronutrients and phosphorus.

The neutralising effect on pH seen in inoculated Technosols with 2.5% MR with good teff growth were expected as most bacterial cells are neutrophiles and acidophiles (Atlas & Bartha, 1998). Minimal microbial abundance is expected in the evidently poor performing soils (100% CW and CW+MR7%). Husson (2013) and Mills & Fey (2004) suggest that pH is the primary parameter influencing soil microbial diversity and richness. Thus, it is expected that the soil microbiome structures will vary between the fabricated soils with varying pH values.

The pH of Technosol leachates were slightly above final soil pH values, except for inoculated CW+MR2.5% and the control. This was expected as these soils were the most alkaline (of the investigated treatments) before teff growth. Leaching results of the final growth trial showed that only 41% and 26% for inoculated and non-inoculated CW+MR2.5%, respectively, of the water entering, leached out. This coincides with the extensive root structures displayed in Figure 33. From literature, it was expected that a higher CW content would increase the concentrations of metal and mineral leached (Komonweeraket, et al., 2015). Following Figure 38, none of the substrates were strongly acidic or basic, implying a lower probability of metal(loid) leaching that follow amphoteric leaching patterns (Cui & Li, 2016; Mahedi, et al., 2019). Li et al. (2013) described that the release of heavy metals (e.g., Cu and Zn) from soils are influenced by climatic changes (temperature fluctuations), soil pH and irrigation. Thus, it would be valuable to investigate metal

leaching with time in the coal-based bioaugmented Technosols containing different ratios of amendments.

The pH values of collected leachates from inoculated and non-inoculated CW+MR2.5% and CW+MR5% were not in agreement with the initial growth round results, pH values after teff growth were noticeably lower. Yet, the pH of all 100% CW and CW+MR7% and associated leachates were similar in both trials. Implying that the better performing Technosols (based on teff performance), CW+MR2.5%-NI and CW+MR5%-I, contained more water-soluble nutrient ions from microbial-meditated material decomposition; hence, the reduction in leachate pH. The optimised irrigation in this growth trial could have contributed to this as in the initial trial optimised irrigation for 50% FC was not yet regulated.

The overall change in redox conditions within coal-based Technosols and associated leachates with Figure 39 (below) and without Figure 59 (Appendix D) vegetation are graphically presented.

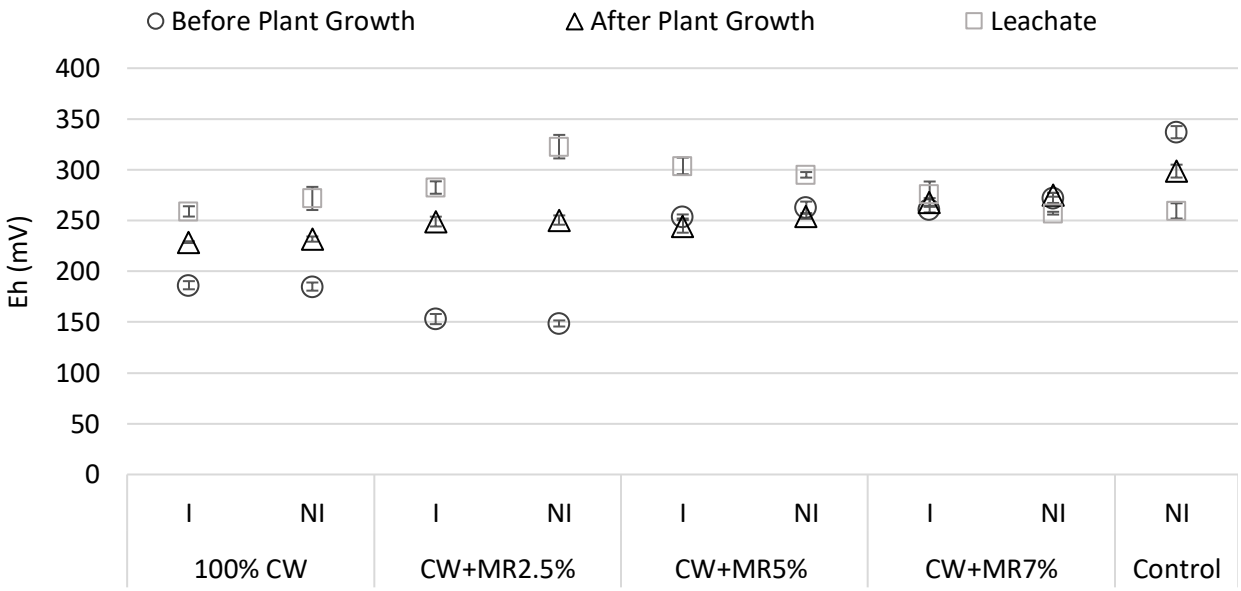


Figure 39: Average redox potential (Eh) values and standard deviations for vegetated coal-based Technosols with and without bioaugmentation, and of associated leachates, with potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatments.

The initial redox conditions of the fabricated soils correspond to those in Figure 22 of the initial plant growth trial, where, redox values increased with a higher dosage of MR and with teff transpiration (Prado, et al., 2020). The initial redox potentials correspond to the material characterisation of MR, characterised by a redox potential higher than that of CW. Conversely to pH, inoculation resulted in a decline in redox potential compared to non-inoculated treatments (also seen in the first growth trial) as bacterial growth (CO_2 emissions during respiration) is associated with a decrease in redox potential (Reichart, et al., 2007). This trend remains throughout the growth trial. Except, initial redox potential of inoculated CW+MR2.5% were 153 mV compared to 149 mV of the non-inoculated treatment. Yet, here the standard error is more significant than the non-inoculated soil-like substrate.

If the Technosols were aerobic, pyrite would oxidize and generate sulfuric acid, reducing the soil pH to 4.0 or less in the absence of neutralizers such as limestone (Arkesteyn, 1980). Under these acidic conditions, aluminium cations are liberated that are toxic to plant growth. Thus, the slightly anaerobic conditions (lowered Eh) were an indication of slow biological pyrite oxidation. The results were supported by Dong et al. (2020) who found undetectable pyrite oxidation at 650 mV even with associated acidophilic bacteria. Consequently, adding to the feasibility of coal-based Technosols as a topsoil for degraded coal mine land.

From Figure 39, the increase in redox potential in CW+MR2.5% and the decrease in CW+MR5% after teff cultivation directly resemble the results after the initial growth trial. However, as with pH values of Technosol leachates, the leachate redox conditions of this growth round differed from the initial trial. Leachates of the best performing Technosols had higher Eh values than the corresponding soil conditions in the final growth trial. The trend in Eh increase is supported by the leachate pH values that decreased, as previously discussed.

As seen with pH, soil reduction processes (photosynthesis, nitrogen fixation, etc.) modify the soil electro-neutrality (Husson, 2013). Yet, microbial diversity, especially bacteria, is controlled by changes in pH and Eh (Neina, 2019; Husson, 2013). Therefore, as previously mentioned, differences in soil microbial abundances were expected between the different types of fabricated soils with varying pH and Eh conditions.

Similarly to the initial growth trial, Technosols were also characterised according to electrical conductivity for it affects soil-water balance and microbial performance (Yan, et al., 2015). The results for vegetated soils are presented in Figure 40, below, and for non-vegetated soils in Figure 60 (Appendix D).

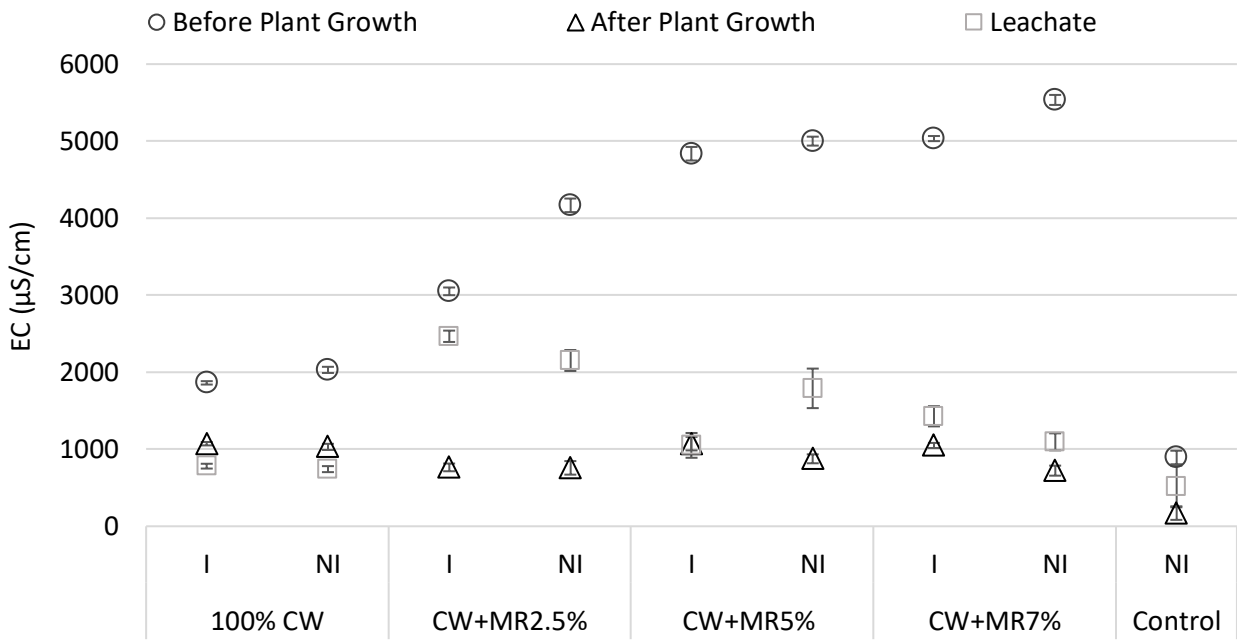


Figure 40: Average electrical conductivity (EC) values and standard deviations for vegetated coal-based Technosols with and without bioaugmentation, and associated leachates, with potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatments.

Initially, the salinity between the treatment types varied significantly. Before plant growth commenced, Technosol EC was positively influenced by the amendment dosage and negatively influenced by bioaugmentation when comparing fabricated soils of the same type. This supports the results obtained in the initial trial (refer to Figure 23 in section 4.2.2) and addresses the amalgamated contribution of parental materials and associated amendments to the overall soil characteristics described by Jordan et al. (2017), Novo et al. (2013), and Herran Fernandez et al. (2016). The trend of increasing Technosol EC with higher dosages of MR correspond to soil-water content (initially higher in Technosols with higher percentages of MR as seen in Figure 41) and OM content (higher EC when more OM) (Othaman, et al., 2020).

In terms of soil structure, clay-like soils commonly have higher salinity due to enhanced surface area between soil particles (Cervantes, et al., 2011). This was observed in both greenhouse trials in Technosols with 7% (w/w) MR, where, soil particles clumped together making it impervious to water. The sand-like 100% CW had the lowest initial EC as cation concentrations and moisture content were low. Non-inoculated CW+MR5% and all CW+MR7% treatments were initially characterised as very saline. High salinity (EC values above 5000 µS/cm) is deleterious to microbial growth and activity (Boyrahmadi & Raiesi, 2018). This corroborates the poor teff growth achieved in all CW+MR7%.

Soil EC values between 1000 – 2500 µS/cm are acceptable by standard (Allison, et al., 1954). When values increase, soil sodicity (high accumulation of sodium salt relative to other cation salts) increases (Amaral Filho, et al., 2020). Based on the agricultural standards, the initial EC of fabricated soils before plant growth were unsuitable (except for 100% CW). Even so, conditions in all Technosols were improved with teff growth. With vegetation, EC had stabilised in all treatments to levels below 1100 µS/cm, indicating that nutrient and metal(loid) uptake into plant organs, photosynthesis, root exudation and pollutant degradation processes occurred to control soil salinity (Cervantes, et al., 2011). As expected, by the end of the trial the control had the best teff development and performance with the lowest EC, compared to the worst performing treatment, inoculated 100% CW, with the highest EC.

From Figure 40, there are no trends in the salinity of Technosol leachates. Leachates from all CW+MR7% remained above 1000 µS/cm subject to the high initial soil salinity. The relatively high EC in CW+MR2.5% leachates can be explained by a high concentration of nutrient ions and salts due to active soil microbes and maximised irrigation.

The water uptake and cycling within soils are pivotal to biogeochemical transformations, plant and microbial growth, and soil-plant mass transfer (Lowery, et al., 1996). WHC of every treatment type was evaluated before and after the final growth trial. The results for vegetated and non-vegetated pots are graphically presented in Figure 41.

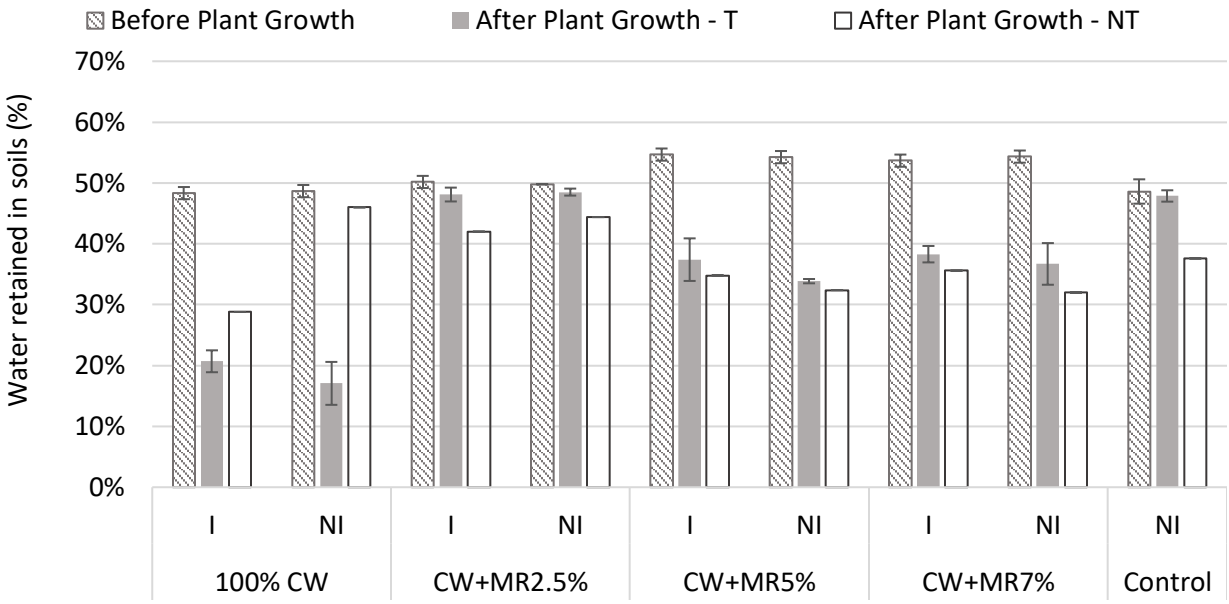


Figure 41: Average percentage and standard deviation water retained in vegetated and non-vegetated coal-based Technosols with and without bioaugmentation, and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types, T is for teff growth and NT is no teff growth.

There is no standard deviation for the pots with no teff as only one pot per treatment type were used as the control (refer to section 3.6.2.2). Initial WHC of all treatments averaged near 50%. However, WHC in 100% CW was poor (inoculated: -2.4 fold change; non-inoculated: -2.9 fold change), yet foreseeable as ultrafine coal tailings were homogeneous in structure, sand-like in texture with very small particle size (<2 mm), and incapable of supporting vegetational growth. Noticeable water channels were observed in 100% CW Technosols during the greenhouse trial which formed as a result of reduced soil porosity when aggregates are continuously exposed (Mills & Fey, 2004). Implementing 100% CW as a topsoil would result in extensive water run-off, crusting or gully erosion. Thereby, emphasizing the significance of amendments to ameliorate soil structure.

From Figure 41, WHC increased with an increasing ratio of MR. The results were consistent with the initial growth trial (refer to Figure 24) and auspicious to research by Amaral Filho et al. (2020), Firpo et al. (2015), and Weiler et al. (2018), where, the addition of OM benefitted biomass production and soil structure performance in coal-based soils.

By the end of the growth trial, WHC of all treatments decreased as seen in Figure 41, due to pedogenesis altering soil WHC (Amaral Filho, et al., 2020). After 82-days, the average percentage of water retained in CW+MR2.5% were the highest amongst all Technosols, suggesting good mass transfer for oxygen diffusion, cation translocation, carbon sequestration and nitrogen fixation (Lowery, et al., 1996). It also correlates to good shoot and root development achieved (refer to Figure 32). These results are consistent with those by Amaral Filho et al. (2017) who reported that a mixture of CW and native soil (in w/w ratio 3:1) amended with a lower ratio of 2% (w/w) OM resulted in better water percolation than the Technosol constructed from the same ratio of CW and native soil without OM by Sekhohola et al. (2017).

Similarly to the initial trial (refer to Figure 24), WHC of biostimulated and bioaugmented Technosols were superior to only biostimulated Technosols, due to inoculum microbes performing OM decomposition and soil aggregation to improve structural stability (Atlas & Bartha, 1998). Interestingly, WHC of non-vegetated pure coal-based Technosols were higher than those with vegetation, potentially as a result of water channels that formed around teff plants in the ultrafine coal tailings.

All CW amended with 7% MR had a slightly higher WHC than those with 5%. As previously mentioned, Technosols with 7 wt.% MR were more prone to compaction with decreased water drainage and high salinity that inhibited root development. The exposed OM in CW+MR7% was oxidised and attenuated microbial activities and nitrogen levels. Leaching results on 100% CW and CW+MR7% support the previously presented results; 79% and 75% of the water entering inoculated and non-inoculated 100% CW, respectively, leached out. Whereas, 62% and 63% of the water entering inoculated and non-inoculated CW+MR7%, respectively, were leached. The initial growth trial and literature on the effect of OM (when absent and in very high concentrations) on water drainage and infiltration are in agreement (Lowery, et al., 1996; Atlas & Bartha, 1998).

Macronutrients (C, N, S, P Bray II, K) are pertinent to soil development (Botta, 2015) and soil metal(loid) profiles influence plant growth and soil toxicity (Da Silva Rego, et al., 2016). Soil fertility measured after plant growth for vegetated and non-vegetated Technosols are shown in Table 12 (below) and Table 24 (Appendix D), respectively.

Table 12: Physiochemical characteristics of vegetated coal-based Technosols with and without bioaugmentation, and potting soil as the control, after 82 days in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

		100% CW		CW+MR2.5%		CW+MR5%		CW+MR7%		Control
Parameter	Unit	I	NI	I	NI	I	NI	I	NI	NI
C	%	50	46	50	50	52	50	51	50	15
TN	%	1	1	1	1	1	1	1	1	0
P (Bray II)	mg/kg	14	8	32	30	58	54	106	104	35
K	mg/kg	209	32	140	25	141	45	196	47	319
S	mg/kg	760	1076	709	732	648	709	1093	1037	136
Ca^{2+}	cmol/kg	33.1	35.4	31.7	32.8	29.8	28.9	24.8	25.3	23.8
Mg^{2+}	cmol/kg	1.9	1.7	1.7	1.7	1.9	1.8	2.3	1.9	10.4
K^{+}	cmol/kg	0.5	0.1	0.4	0.1	0.4	0.1	0.5	0.1	0.8
Na^{+}	cmol/kg	0.6	0.2	0.5	0.2	0.5	0.2	0.6	0.2	0.6
Ca	%	91.6	94.8	92.7	94.2	91.5	93.1	88.2	91.8	65.9
Mg	%	5.3	4.4	4.9	4.9	5.9	5.8	8.0	6.9	28.8
K	%	1.5	0.2	1.1	0.2	1.1	0.4	1.8	0.5	2.3
Na	%	1.6	0.5	1.4	0.7	1.5	0.7	2.0	0.8	1.6
Cu	mg/kg	2.0	2.4	3.6	3.3	3.8	3.5	3.1	3.4	4.0
Zn	mg/kg	1.3	1.2	2.5	2.3	3.9	3.1	4.5	4.5	14.0
Mn	mg/kg	23.9	22.5	23.2	23.6	23.5	23.1	23.1	24.1	67.0
B	mg/kg	0.4	0.3	0.5	0.5	0.4	0.6	0.7	0.6	0.6
Fe	mg/kg	34.2	33.8	41.2	33.9	62.1	54.2	56.8	60.5	273.7
S Am.acet	mg/kg	3185	3335	3145	3183	2883	2683	2653	2475	121
Resist.	(ohm)	278	413	350	403	280	315	240	313	623

T Value	cmol/kg	36	37	34	35	33	31	28	28	36
CEC	mg/kg	13	11	13	13	12	11	13	15	19
Stone	Vol %	1	1	1	1	1	1	1	1	2

Macronutrients are necessary for plant tissue development and enzyme activation (Ca and K), and chlorophyl production (Mg and Na) (Muindi, 2019; Rawat, et al., 2016). Thus, it was expected that higher concentrations of these ions would be cycled in bioaugmented-Technosols that showed good teff growth. The results support this, as after teff growth Ca levels were slightly less, and Mg, K, and N contents slightly more in bioaugmented-treatments than those with only biostimulation.

The soil cation concentrations correspond to the higher electrical conductivity in Figure 40 in bioaugmented Technosols compared to non-inoculated controls and potting soil. The control had the highest concentrations of K, Mg, Cu, Zn and B, which supports the high cation exchange capacity and T-value, implying that there were more nutrients in the soil matrix with a lowered probability of nutrient loss.

Upon analysis of the Technosol characterisation in Table 12, it is apparent that C, P, K, and Ca nutrient concentrations were in greater concentrations when MR was applied. MR was a source of key nutrients (summarized in Table 9), including N, Ca, K, Zn and P and imparted structure to the Technosols as previously discussed. It was evident that carbon sequestration occurred in vegetated MR-amended fabricated soils, as overall percentages total organic carbon were higher than in non-vegetated Technosols. Magnesium content was similar in all bioaugmented Technosols but 1.6-fold higher in CW+MR7%.

The P (Bray II) contents are graphically presented in Figure 62, where, P concentrations increase proportionally to MR dosage. The P content in bioaugmented CW+MR2.5% was most similar to the control, potting soil. Inoculated 100% CW contained only 0.4-fold that of the control, whereas inoculated CW+MR5% and CW+MR7% had 1.7-fold and 3-fold that of the control, respectively. When considering all results, it suggests that EM Pro-Soil and MR as biostimulant generated soil-like conditions with high phosphorus availability and soil charge capable of capturing and releasing the necessary nutrients during plant growth, whilst minimizing nutrient loss and metal leaching (Muindi, 2019).

Similar to the initial trial, treatments with bioaugmentation outperformed those without EM Pro-Soil in terms of K cycling, where inoculated CW+MR2.5% had accumulated 5.6-fold that of the non-inoculated treatment (refer to Figure 63 in Appendix D). Furthermore, K content increased as MR dosage increased. In the initial trial, the excess K from compost inhibited the uptake of other cations (Ca, Mg and Na) that were necessary for plant growth (Campbell, 1985), but in the final trial, insufficient K delayed plant maturity and increased susceptibility to pestilence (Rawat, et al., 2016) discerned from teff cultivated in non-inoculated 100% CW (K: 31.8 mg/kg) and CW+MR7% (K: 47.0 mg/kg).

Total sulfur after plant growth is graphically presented in Figure 64 (Appendix D). The results indicated that bioaugmented and biostimulated (with 2.5% and 5% MR) Technosols were best able to remediate soil S to suitable levels (709 mg/kg and 648 mg/kg, respectively) with teff growth. The S concentrations

were below the maximum national screening values for soils listed in Table 4. Whereas, S in 100% CW and CW+MR7% were eminent (1.7-fold the reference value). These results support bioaugmentation and biostimulation of coal-based Technosols but further research is required to evaluate soil S levels and pyrite oxidation with successive plant growth cycles.

The sulfur speciation on coal tailings indicated primarily pyritic content of 48.0%. It was expected that bioaugmentation and amendment application would reduce the pyritic sulfur content. Weiler et al. (2020) reported that CW amended with 5% OM showed a 50% decrease in pyritic sulfur levels with *Medicago sativa* (alfalfa) growth compared to unamended CW. Furthermore, the low soluble iron and sulfur results were in agreement with the low redox potentials and with the low iron and sulfate levels in Technosol leachates, suggesting that pyritic reduction occurred in all Technosols. A slow rate of pyrite oxidation in soil is desirable since sulfur species are transformed to compounds that are accessible and beneficial for plant and microbial growth and microbial proliferation (Weiler, et al., 2020; Dong, et al., 2020). Analysis on the Technosol elemental content prior to plant growth is necessary for validation.

To evaluate environmental effects of implementing coal-based Technosols as topsoils, sulfate and ferrous iron leaching analysis were performed. The results (refer to Appendix D) are summarized in Figure 68 for ferric iron, Figure 69 for ferrous iron, and sulfate in Figure 70. Ferric and ferrous iron concentrations in all leachates were negligible (all ferrous below 0.6 mg/mL, and all ferric below 0.35 mg/mL). Mahedi et al. (2019) described iron leaching patterns as cationic with a decrease proportional to an increase in soil pH conditions. All Technosols had pH levels between 6.98 and 7.90, thus, iron leaching is not a concern.

Sulfate is generated in Technosols during sulfur sequestration with added S from MR (0.67% S), irrigation with rainwater, leaf litter decomposition, and atmospheric deposition (Tabatabai, 1987). The results for sulfate leaching (refer to Figure 70) suggested a corresponding increase in the sulfate form, auspicious to results by Weiler et al. (2018, 2020). Sulfate production followed exposure of pyrite to aerobic conditions with microbes, and plant absorption of sulfur. Leaching of SO_4^{2-} was induced by the alkaline Technosol conditions with Ca, K and Mg cation dominance. The acidic profile of potting soil resulted in improved SO_4^{2-} absorption.

Technosol metal(loid) concentrations after teff growth are illustrated in Figure 42. From this graph, Fe and Mn levels were superior compared to other metals. Nonetheless, all metal(loid)s were below the national screening values (listed in Table 4). While Cu concentrations were similar across all engineered soils in the presence of MR, it was 1.5-fold lower in the absence thereof. Mn concentrations were similar across all treatment types but 3-fold more in the potting soil. All metal(loid)s were more abundant in the control, with 4.5-fold more Fe than the highest bioaugmented Technosol, CW+MR7%.

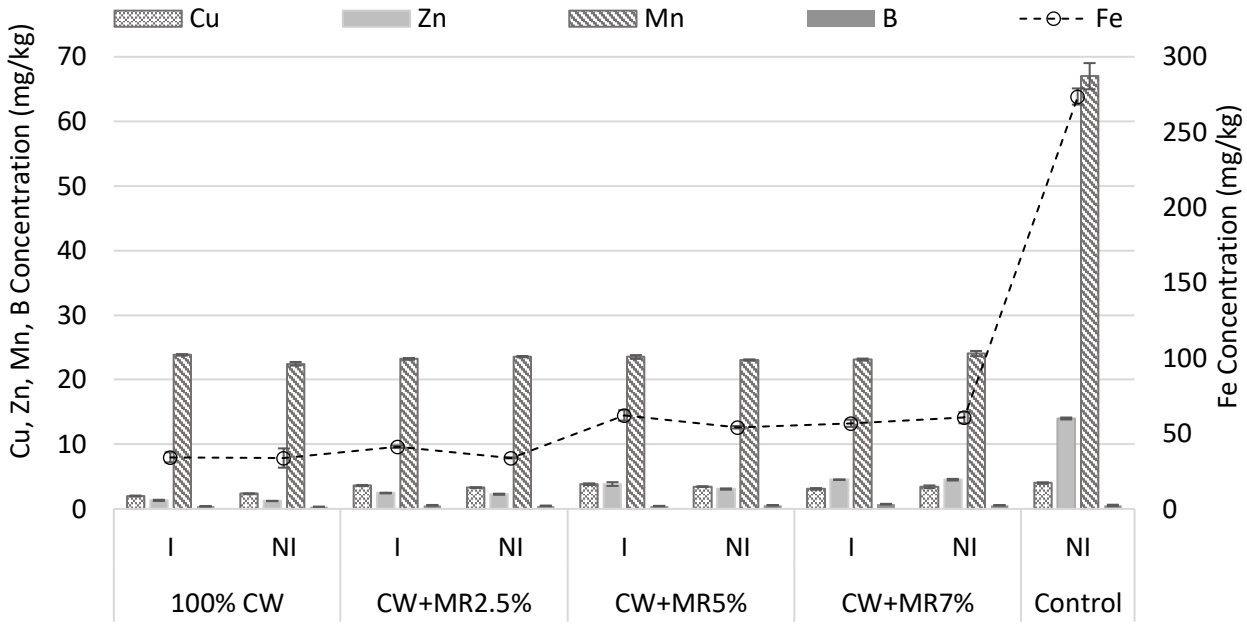


Figure 42: Metal(loid) (Cu, Zn, Mn, B, Fe) concentrations and standard deviations in vegetated coal-based Technosols, with and without bioaugmentation and potting soil as the control, after 82 days in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Teff roots and shoots grown in the best performing CW+MR2.5% and CW+MR5% Technosols were analysed for major, minor and trace elements, and compared to those cultivated in the control, potting soil. The results are summarized in Table 13.

Table 13: Elemental characterisation of above (shoots) and below (roots) ground teff dry biomass cultivated for 82 days in coal-based Technosols, with and without bioaugmentation, amended with 2.5% and 5% (w/w) MR against potting soil as the control in the final growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

		CW+MR2.5%				CW+MR5%				Control	
		Shoots		Roots		Shoots		Roots		Shoots	Roots
Symbol	Unit	I	NI	I	NI	I	NI	I	NI	NI	NI
Na	g/kg	0.546	0.492	1.47	0.321	0.608	0.675	2.23	1.64	0.274	0.340
Mg	g/kg	3.65	4.04	3.29	2.22	3.43	4.28	4.69	7.93	3.40	19.8
Ca	g/kg	5.32	6.21	14.1	19.8	6.50	9.71	14.8	17.4	3.59	14.6
K	g/kg	14.9	7.69	1.85	0.988	15.5	7.79	2.55	1.20	18.1	6.55
P	g/kg	3.27	1.39	0.848	0.736	2.64	1.71	1.12	1.07	2.74	0.969
Si	g/kg	1.62	1.76	0.273	0.187	2.41	2.87	0.229	0.566	1.21	0.372
B	mg/kg	9.46	5.00	21.7	24.4	7.87	9.30	23.2	33.5	7.31	12.9
Al	g/kg	0.144	0.151	18.7	27.4	0.159	0.318	16.8	13.6	0.0868	11.9
V	mg/kg	0.169	0.162	18.0	25.6	0.175	0.325	16.3	14.4	0.177	26.5
Cr	mg/kg	1.92	6.48	33.4	39.1	1.55	1.07	27.9	20.6	0.630	184
Mn	mg/kg	202	104	134	143	117	98.5	126	109	22.2	210
Fe	g/kg	0.0951	0.0845	7.71	11.8	0.117	0.172	7.00	5.20	0.0683	11.1
Co	mg/kg	1.098	0.477	7.95	8.67	0.564	0.416	6.70	5.87	0.0395	33.0
Ni	mg/kg	1.393	1.68	22.3	26.6	1.05	1.62	19.1	17.0	0.803	96.6
Cu	mg/kg	14.4	11.9	33.9	33.4	14.2	13.4	31.8	31.5	8.10	45.5
Zn	mg/kg	86.2	39.3	41.1	26.3	76.0	46.8	45.1	31.2	81.4	53.9
As	mg/kg	0.127	0.130	2.53	3.66	0.156	0.322	2.39	2.70	0.260	1.51
Se	mg/kg	0.135	0.0798	0.808	0.966	0.0790	0.0810	0.735	0.678	BDL	0.551

Sr	mg/kg	70.2	76.9	208	270	63.8	85.5	205	250	25.9	62.4
Mo	mg/kg	0.736	0.538	1.32	0.693	0.804	0.695	1.76	2.70	1.49	2.12
Cd	mg/kg	0.364	0.0955	0.381	0.135	0.172	0.0900	0.300	0.290	0.785	0.663
Sn	mg/kg	0.0299	0.0282	0.954	1.335	0.0330	0.0440	0.861	0.741	0.0341	0.912
Sb	mg/kg	0.0584	0.0346	0.0280	0.0166	0.0190	0.0250	0.0453	0.0715	0.0164	0.0632
Ba	mg/kg	13.0	5.25	211	292	12.1	9.77	191	157	34.3	101
Hg	mg/kg	0.0137	0.0165	0.136	0.178	0.0210	0.0280	0.113	0.0970	0.0166	0.0492
Pb	mg/kg	0.143	0.149	5.75	8.42	0.152	0.248	5.12	4.56	0.170	12.4

Similar to the initial growth round, macro elements (Na, Mg, K,P) were more concentrated in the shoots compared to the roots, except Ca. The higher concentrations of calcium in plant roots are indicative of microbial respiration in the rhizosphere as high concentrations of carbon dioxide increased Ca solubility and availability (Atlas & Bartha, 1998). Interestingly, shoots and roots of teff in inoculated CW+MR2.5% and CW+MR5% had lower concentrations of Ca than the non-inoculated controls. Yet, CW+MR5%-I had a higher concentration of calcium cations (29.8 cmol/kg) in the soil matrix than its control treatments (28.9 cmol/kg). In the initial growth trial, all teff below ground biomass from inoculated treatments contained more Ca than the non-inoculated controls, indicating the effects of seasonal variation on microbial functions for metal uptake. The abundance of macronutrients in inoculated CW+MR2.5% and CW+MR5% compared to the control validated the value of cultivating teff in Technosols for otherwise nutrient-deficient coal mining waste (Rawat, et al., 2016; Truter, 2007).

The elemental analyses showed favourable results for teff grown in bioaugmented CW+MR5%, with K concentrations in teff shoots similar to those in the control and 1.4-fold more than shoots in CW+MR2.5%-I. Phosphorus levels were 1.3-fold higher in the roots of CW+MR5%-I compared to CW+MR2.5%-I, suggesting enhanced P solubility in the soil-like substrate. Campbell (1985) described greater rates of phosphate uptake associated with plants in soils with rhizosphere microbes compared to sterile soils. The microbes produce acids that dissolve the mineral group, apatite, which releases soluble forms of phosphorus (Atlas & Bartha, 1998). Thus, linking to the augmented P (Bray II) levels in the soil matrix of inoculated CW+MR5%, compared to the non-inoculated control in Table 12. These results support that of the initial greenhouse growth trial.

Once again, Al and Fe concentrations were relatively high in the root structures as a result of the initial concentration of these metals within the soil parental material and increased Fe solubility from microbial mechanisms (Hrynkiewicz, et al., 2018) detailed in Chapter 2. Teff shoots in CW+MR5%-I had the highest Al (16 800 ppm) and Fe (97 000 ppm) content compared to bioaugmented CW+MR2.5%. This corresponds to the Fe content in the soil-like mixture matrix that was 1.5-fold that of CW+MR2.5%-I. Nonetheless, iron leaching was prevented as previously discussed. Furthermore, minor and trace metal(loids) such as Ni, As, Sr, and Hg, were present in lower concentrations in CW+MR5%-I compared to the inoculated Technosol with 2.5% MR, implying that 5% MR amendment with bioaugmentation sufficiently reduced metal(loid) solubility.

The above ground teff dry biomass were analysed for CHNS (refer to Figure 65, Appendix D) to further investigate these effects. The carbon content of teff shoots in the final growth trial were similar (above 40.7%) between all treatment types, and the sulfur contents were negligible relative to reference values

(Table 4). This compared well with the results for the first cycle of teff growth. From Figure 65, it was clear that bioaugmentation in soils amended with 2.5% and 5% (wt.%) MR increased nitrogen and phosphorus uptake into plant above ground organs. Smith & Daft (1978) reported on beneficial associations between teff and myrcorrhizal fungi. Thus, when investigating plant-microbe interactions to determine nutrient absorption from soil and plant tolerance to abiotic variables, it is recommended to microscopically look at a cross section of the teff rootlet to determine which mycorrhizal associations (ectotrophic or endotrophic) have established after a growth cycle in coal-based Technosols (Atlas & Bartha, 1998).

Collected leachates from all Technosols were analysed for TOC, TIC and TN (refer to Figure 43, below and Figure 71, Appendix D).

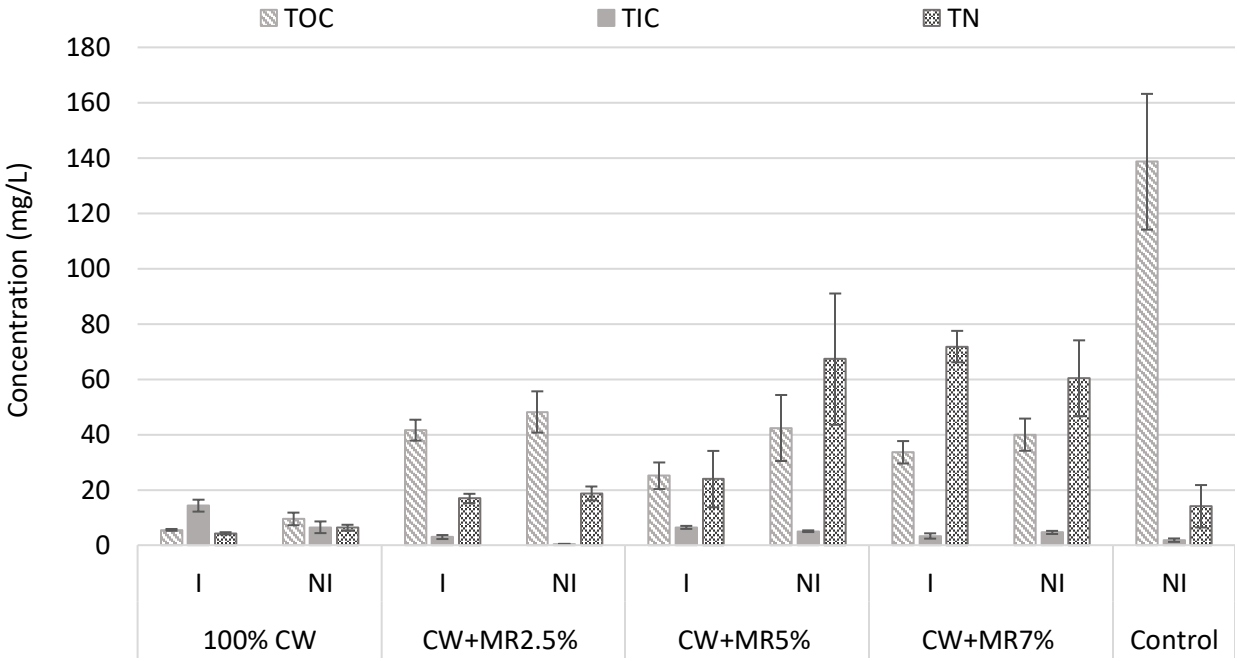


Figure 43: Total organic & inorganic carbon and total nitrogen concentrations (mg/L) in leachates from vegetated coal-based Technosols and potting soil as the control, collected from the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

TOC in potting soil leachates was 3.3-fold that of inoculated CW+MR2.5%, potentially as a result of enhanced C sequestration from enhanced microbial activities in the control. TN in potting soil leachates was similar to that of CW+MR2.5%-I; corroborating the plant growth results highlighting this fabricated soil as a best performing Technosol. Low TOC and TN leachates results in CW+MR2.5%-I and CW+MR5%-I compared to their non-inoculated controls and compared to CW+MR7% treatments, indicated that bioaugmentation with biostimulation in coal-based Technosols enhanced C and N mineralisation and the uptake of nutrients into plant organs.

TN in Technosol leachates followed the same trend as in the soil matrix, where, nitrogen contents increased with MR dosage. Adding MR to CW accelerated the breakdown of organic material (Zhang, et al., 2010), resulting in more TOC and TN in leachates of MR-amended Technosols. TN in bioaugmented

CW+MR7% was 12-fold that of 100% CW. The effects of biostimulation and bioaugmentation were evident from the TOC in 100% CW with no amendments that was 5-fold less than inoculated CW+MR2.5%, an indication of low microbial activities and inadequate OM mineralisation in pure CW (Cervantes, et al., 2011).

4.3.3 *Soil Microbiome Analysis*

In fulfilment of this investigation's third hypothesis, the fabricated soil microbiomes were analysed according to the methodology in Chapter 3.8, and the results are discussed in this section.

Cell counts were performed in parallel with FDA analysis to evaluate the effect of inoculum activation (prior to soil fabrication) on microbial diversity and proliferation. Cell counts after the seven-day incubation period showed a 1.45-fold increase in the microbial population during the activation process. As a result, a lower volume of activated inoculum was required (1.5-fold less than non-activated EM Pro-Soil) for bioaugmentation per gram of Technosol, thereby lowering associated costs.

The activation process was further evaluated with FDA and SIR, of which the FDA results are graphically summarized in Figure 72 till Figure 76 in Appendix D. A direct correlation between concentration of microbial cells (cells/mL) and activation period was found through fluorescence (RFU) measurements. Microbial activity increased by 1.1-fold with the incubation period. These results were consistent with the substrate-induced respiration (SIR) results as shown in Figure 44.

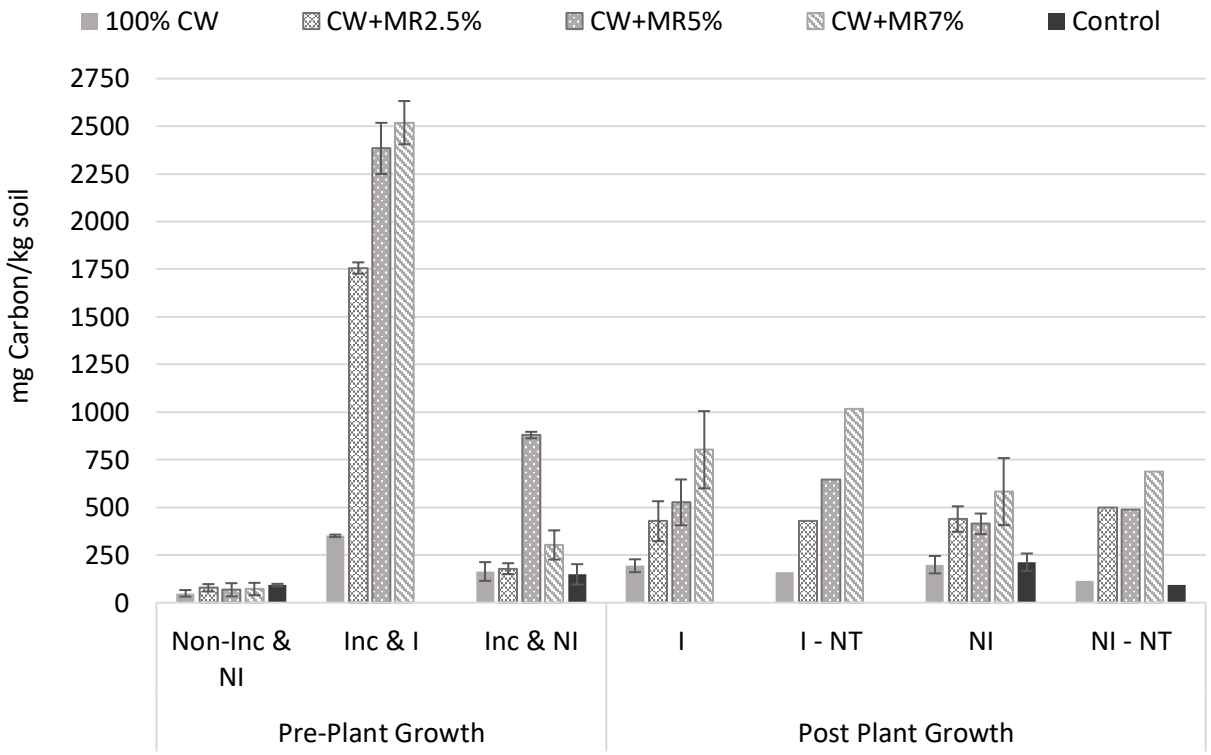


Figure 44: Average microbial biomass and standard deviation in vegetated and non-vegetated (NT) coal-based Techosols, with and without bioaugmentation and potting soil as the control, before and after the final plant growth trial in winter. Where, Non-Inc is non-incubated, NI is non-inoculated, Inc represents incubated, and I refers to inoculated treatment types.

Inoculation and incubation resulted in a 2.7-fold increase in microbial biomass in CW+MR5%, and a 10-fold increase in CW+MR2.5%. This is consistent with Weiler et al. (2020), and in agreement with the results of the initial growth trial (presented in Figure 26). As expected, inoculation significantly increased microbial biomass compared to non-inoculated soils before plant growth. In CW+MR5%, bioaugmentation increased the biomass by 2.7-fold, and 9.8-fold in CW+MR2.5%. Figure 44 also visually shows greater microbial biomass in inoculated treatments after teff growth, especially CW+MR5%-I. The carbon content per weight of Technosol in bioaugmented CW+MR5% was 21.5% more than non-inoculated CW+MR5%, 16.6% more than non-inoculated CW+MR2.5%, and 59.5% more than the control by the end of the greenhouse study.

The link between MR dosage and microbial biomass suggests great potential for CW+MR7%-I in supporting microbial functions; however, SIR results also included fungal biomass and as previously discussed, mould growths in CW+MR7% treatments were detrimental to teff germination and development. Furthermore, results suggest that bacterial growth was delayed by osmotic stress from large relative conductivities between the soil-like mixture and microbial community (Thornton, 1912), affecting microbial abundances. Nevertheless, in terms of microbial biomass, the SIR results clearly indicate that the inclusion of amendments to coal mine waste benefit soil fertility in conjunction with bioaugmentation.

It was expected that the microbial biomass results would support the conditions in which the studies were conducted and the plant biomass yields achieved. However, overall microbial biomass in treatments from the final trial was significantly more than after the initial growth trial: 2.1-fold more in inoculated CW+MR5% and 1.3-fold more in CW+MR2.5% in the final trial. Perhaps an indication of the influence of irrigation regimes (maintaining 50% of the maximum water retention) on soil pH and consequently on soil microbial abundances (Atlas & Bartha, 1998). Li et al. (2021) showed that soil bacterial communities' structure and function were more strongly affected by irrigation than nitrogen fertilization in *Triticum aestivum* (winter wheat) grown in a semi-arid environment (also prevalent to eMalahleni from this study).

FDA analysis after plant growth was performed to investigate microbial activities within coal-based Technosols with and without bioaugmentation. FDA was optimised for coal-based Technosols (refer to Appendix A). The effectiveness of using chloroform to terminate hydrolysis was corroborated by Schumacher et al. (2014), and it was assumed that all living organisms were removed from MR through sterilisation. It was determined that microbially-emitted fluorescence in the background of CW was directly proportional to microbial cells per mL soil for a 10-minute incubation period. FDA results for soils before and after the final growth cycle were presented accordingly in Figure 45. The microbial activity and function were expected to be superior in Technosols with improved teff growth compared to the poor performing fabricated soils (100% CW and CW+MR7%) as soil enzyme levels fluctuate with soil OM and microbial composition (Weerasekara, et al., 2017).

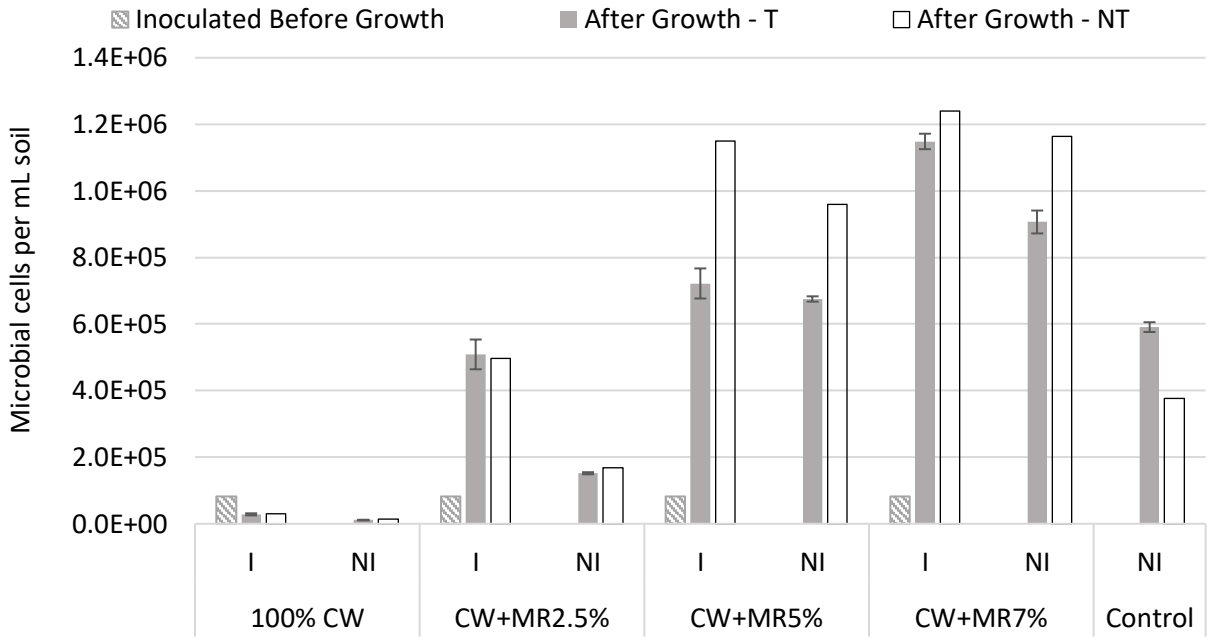


Figure 45: Average estimated concentration and standard deviation microbial cells per coal-based Technosol, with and without bioaugmentation and potting soil as the control, with teff (T) and without teff (NT) after 82 days in the final plant growth trial in winter. Where, I is inoculated and NI is non-inoculated treatments; T represents soils with teff growth and NT is the no teff control of each treatment type.

From Figure 45, microbial concentrations per volume of soil increased with bioaugmentation and biostimulation. Adding amendments that are easily metabolizable to CW enhanced microbial growth in treatments without vegetation compared to the Technosols with vegetation and no amendments (100% CW). MR as a source of carbon was utilised for microbial growth (Chessa, et al., 2016) and plant development. Thus, in the unvegetated controls, microbial activities were higher from no limiting OM substrates. Weerasekara et al. (2017) similarly found decreased FDA in loam and sand-like soils with plant growth.

MR-application to CW increased Technosol WHC (detailed in Figure 41), improved plant growth (discussed in Chapter 4.3.1), and resulted in augmented P, Ca and Mg soil contents (refer to Table 12), that supported microbial activities. This is seen from the results for poor teff growth, low TOC and low microbial activity in 100% CW treatments, and is in agreement with Bandick & Dick (1999) who described higher enzyme activities in soils that received added organic input compared to controls without added OM after plant growth.

FDA results in Figure 45 concur with SIR analysis in Figure 44, where the highest microbial activity was in Technosols amended with 7% (w/w) MR. As previously discussed, the 7 wt.% dosage MR resulted in fungal growth and did not improve soil health and quality.

Soil metal(loid) concentrations are deleterious to microbial enzymes (Yun, et al., 2017), of which the hydrolysis through FDA encompasses the activity of several microbial enzymes e.g., esterases, proteases, and lipases (Schumacher, et al., 2014). Therefore, augmented microbial activity from FDA analysis in

conjunction to microbial biomass from SIR tests and the previously presented ameliorated soil characteristics (increased nutrient content, improved WHC, increased pH and decreased salinity) in CW+MR5%-I suggest reduced metal(loid) solubility in this bioaugmented-Technosol. CW+MR2.5%-I also showed improvements of soil physiochemical properties but did not concur with microbial activity and biomass assessments.

Molecular methods (as detailed in Chapters 2 and 3) were used to infer microbial community development and function in Technosols of the final growth study. The results for quantified double stranded DNA (dsDNA) extracted from each Technosol treatment before and after the final growth trial are shown in Figure 46.

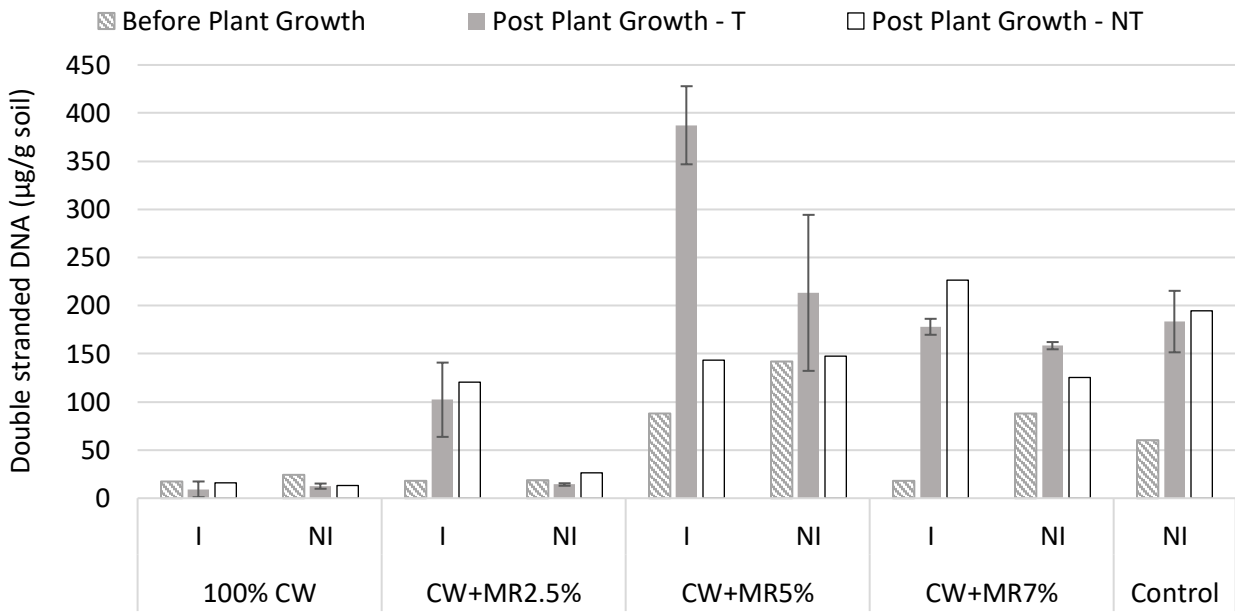


Figure 46: Average concentration and standard deviation double stranded DNA per gram of soil in coal-based Technosols with and without bioaugmentation and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated, NI is non-inoculated, T is with teff growth and NT represents no teff growth.

The greatest amount of dsDNA per gram of soil were quantified in bioaugmented CW+MR5%, with 25-fold more than inoculated 100% CW. Thereby, supporting the previously detailed results for CW+MR5%-I in both plant growth trials. Non-inoculated CW+MR5% had similar quantities of dsDNA to the control, which further motivates the use of 5% MR as amendment to CW. The contribution of bioaugmentation to soil extractable DNA is evident in Figure 46, where all inoculated treatments had more amounts of dsDNA after teff growth than the non-inoculated controls. This contribution is most pertinent in CW+MR2.5%, where the bioaugmented treatment showed a 7.3-fold increase in dsDNA per gram soil relative to the non-inoculated type.

Before plant growth, inoculated CW+MR5% had on average 4.9-fold more dsDNA per gram of Technosol compared to the other inoculated treatments. This is unexpected as all treatments were initially inoculated with the same number of cells per gram of Technosol. A relatively high initial concentration of

dsDNA was also found in both non-inoculated CW+MR5% and CW+MR7%, and the control (potting soil). The results for the control were expected as pedogenesis had already been initiated in the agricultural soil and a higher concentration of initial microbial biomass was evident in Figure 44. Bacterial contamination during DNA extraction could have led to these results; however, the SIR results for initial microbial biomass in non-inoculated CW+MR5% and CW+MR7% coincide with the DNA extraction results. Thus, eliminating the probability of bacterial contamination during the extraction process. Inoculum activation for all soil types were controlled and identical; therefore, the assumption was made that no additional, unexpected benefits (i.e. higher microbial biomass) could have resulted from inoculation of fabricated soils. Hence, it could only be accounted for in the soil fabrication process, suggesting that the higher concentration of labile OM in soils amended with 5% and 7% MR increased the microbial growth during soil incubation (at optimum mesophilic growth conditions) (Schiraldi & De Rosa, 2014). Thereby, corroborating Lebrun et al. (2021) and Bakhoum et al. (2012) who concluded that soil bacterial populations are strongly dependent on the amendments that alter soil properties.

Technosol microbiomes were profiled in terms of bacteria, fungi and archaea, and nutrient cycling genes through qPCR amplification of 16S rRNA genes (as detailed in Chapter 3). The relative abundance of bacteria, fungi and archaea before and after plant growth in each of the Technosols are presented in Figure 47. Refer to Table 25 and Table 26 in Appendix D for gene copy numbers of vegetated and unvegetated soils, respectively. From Figure 47 and Table 25, it is clear that CW+MR5% (both I and NI) had the highest initial and final bacterial populations of the investigated Technosols, suggesting and supported by Weiler et al. (2020) that the bacterial population from inoculation was best able to establish and proliferate in the engineered soils amended with 5 wt.% MR. The high initial soil bacterial abundance in CW+MR5%-NI and CW+MR7%-NI corroborates the SIR and FDA results, and concurs with Shen et al. (2019) who found that bacterial populations increased from OM addition to coal waste in stacks compared to the control soils. In CW+MR2.5% and CW+MR5%, final bacterial abundances were more than archaea abundances, implying that nitrification was governed by bacteria rather than archaea as reported by Hafeez et al. (2012).

It was expected that the highest microbial diversity would be found in Technosols with a higher pH and WHC, and lower metal(loid) availability compared to Technosols with more acidic pH and higher metal(loid) concentrations (Lebrun, et al., 2021). However, there are no apparent trends in the microbial diversity of the investigated Technosols with teff growth. In addition, the effects of increasing amendment dosage or inclusion of bioaugmentation were not clear from the qPCR profiles. However, fungal populations, with respect to bacteria and archaea, were lower in treatments with inoculation before plant growth. This trend does not remain after plant growth. Instead, the relative abundances of fungi increased with plant growth in all soils, including the control.

Additionally, bacterial communities from the inoculum (as outlined in Chapter 3), were only enhanced in CW+MR2.5% and CW+MR5% during vegetation. Of the fungal populations in the aforementioned engineered soils, *Sacchoromyces cerevisiae* were introduced by EM Pro-Soil. This yeast plays an important role in soil aggregation, nutrient breakdown and nutrient cycling (Botha, 2011). Subsequently, supporting the previously presented results on teff growth and physiochemical soil parameters that concluded

promise and best performance (relative to the other fabricated soils) in inoculated CW+MR2.5% and CW+MR5%. Moreover, the enhanced WHC and teff growth (increased growth rates and grain yields) in these Technosols, could be attributed to fungal populations of AMF and EMF that created mycorrhizal networks to support root development, water uptake and carbon sequestration (Tiwari, et al., 2021; Margerison, et al., 2020; Atlas & Bartha, 1998).

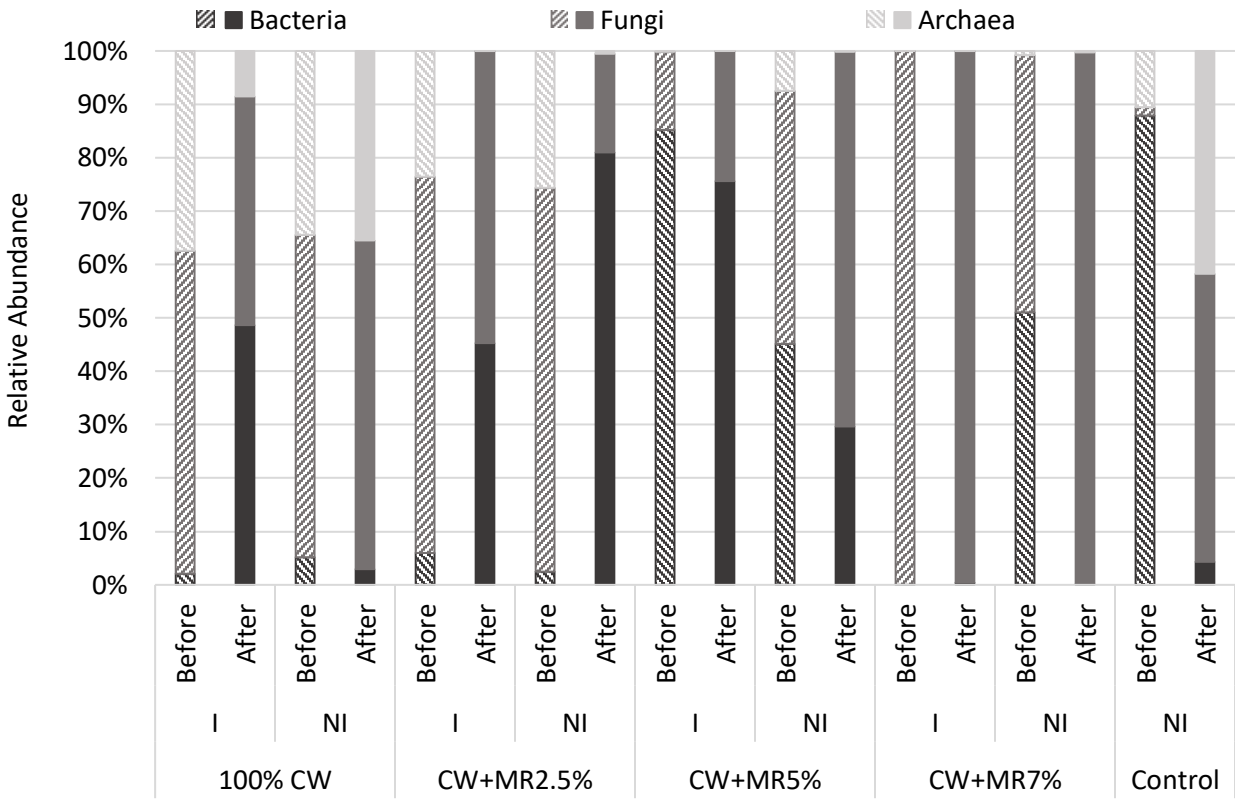


Figure 47: Relative abundance (expressed as percentage) of bacterial, fungal and archaeal gene copy numbers within vegetated coal-based Technosols, with and without bioaugmentation and potting soil as the control, before and after the final plant growth trial in winter. Where, I represents inoculated treatments and NI is non-inoculated soils.

Bacterial growth is deterred by high soil salinity (as seen in all CW+MR7% for both plant growth trials), yet fungi are less affected (Dixon, et al., 1993). Thus, fungi persisted in CW+MR7% as seen in Figure 47 and Table 25 (refer to Appendix D). Suzuki et al. (2010) suggested a positive correlation between soil fungal community abundance and soil fertilization; supporting the high fungal gene copy numbers per gram soil found in Technosols with higher concentrations of OM as MR, especially in CW+MR7%. In corroboration, many studies have shown that fungal biomass and associated activity are higher in substrates with larger soil macro-aggregates (Chiu, et al., 2006; Kirchmann, et al., 2004; Kimura, 2000). Microbial respiration and nitrification decline with an increase in EC (Yan, et al., 2015; Thornton, 1912) (as discussed with SIR results); hence, the reduction seen in bacterial abundance in CW+MR7%. The abundance of nifH genes (genes regulating nitrogen fixation) were expected to be very low in these soil-like mixtures.

Non-inoculated CW+MR2.5% had very small amounts of extractable soil dsDNA in Figure 46; amounts similar to that of 100% CW which could not support teff growth. This was unanticipated as the above and below ground biomass production was exceedingly high in this Technosol (refer to Figure 32), and all of its planted teff survived in the final growth trial during unfavourable growth conditions. Profiling the Technosols' microbiome indicated that bacterial, fungal and archaeal abundances were in the range of that in 100% CW, where, both bacteria and fungi gene copies per gram of CW+MR2.5%-NI were 4 orders of magnitude less, and archaea was 3 orders of magnitude less than in CW+MR2.5%-I. In non-inoculated substrates amended with 5% and 7% (w/w) MR, bacterial and archaeal 16S rRNA gene copies per gram soil were also less than in the inoculated substrates. However, in both CW+MR5% and CW+MR7%, fungi copies per gram soil were one order of magnitude higher in non-inoculated than the inoculated, with the highest of all treatments in CW+MR7% after teff growth. Suggesting not only that soil fungal abundances increased with amendment dosage, but also increased in the absence of inoculation with lower abundances of bacterial and archaeal copy numbers when available OM concentrations are high.

Sun et al. (2020) found that SCG, a chemoautotrophic ammonia oxidising archaea that participates in nitrogen cycling, was the dominant phyla in the coal mining soils, yet 90% of archaea genera in coal soils were unidentifiable. Sterngren et al. (2015) reported that nitrogen fixation through archaea is more significant in poor quality soils with grass compared to fertile soils. This could have played a role in N cycling in CW+MR5%-NI that showed the highest initial archaeal gene copy numbers (3.82E+06) per gram Technosol. Archaeal diversity decreased with teff growth in all but non-inoculated 100% CW and the control. Potting soil had the highest archaeal gene copy numbers (2.43E+06) per gram soil after teff growth.

Non-planted controls showed low microbial diversity with high fungal gene dominances throughout Technosol types after 82 days in the greenhouse (refer to Figure 78 and Table 26 in Appendix D). The results showed low copy numbers of bacteria and archaea genes, especially in CW+MR5% and potting soil without vegetation. Therefore, PGPR and other endophytic microbes were assumed to be less abundant or absent in unvegetated fabricated soils. These microbes synthesize phytohormones and siderophores for metal mobilization (Tiwari, et al., 2021). Hence, in their absence, higher metal(loid) concentrations would be expected in all non-vegetated and non-inoculated Technosols. This was predominantly seen from Cu, Fe, Mg, Mn, Ca and Zn soil content in CW+MR5% and CW+MR7% (refer to Table 10 and Table 24), with negligible differences in soil metal(loid) concentrations for inoculated and non-inoculated CW+MR2.5% after the final growth trial. Thus, as previously proposed, bioaugmentation in a coal-based fabricated soil enhances metal(loid) uptake; however, only when soils are amended with dosages of more than 2.5 wt.% of MR and vegetated. The results are auspicious to research by Nimnoi et al. (2010), Kotani-tanoi et al. (2007) and Costa et al. (2006) who reported that differences in soil bacterial community structure are strongly dependent on plant and soil type, more so than on the applied inoculant.

When considering fabricated soils and the influence of amendments, it is important to understand how the structural modifications (i.e. soil water retention) influence the soil microbial-facilitated functions (Hafeez, et al., 2012), such as nutrient nitrogen cycling. Nitrification (nifH gene) and denitrification (nirS,

nosZ, and nirK genes) are important steps in the nitrogen cycle detailed in Chapter 2. The genes were amplified through real-time qPCR of which the results are graphically presented in terms of relative abundance in Figure 48 below, and gene copy numbers in Table 25 and Table 26 in Appendix D.

As expected, nifH genes were initially more abundant in Technosols before plant growth commenced, of which the relative abundance of this nitrification gene was the highest in bioaugmented CW+MR5% (52.41% rel. abundance) as the inoculum contained *Rhodopseudomonas palustris* and *R. sphaeroides,* known for their capacity to regulate both nitrogen fixation and denitrification (Knowles, 1982).

The N cycling genes diversity in all Technosols differed significantly between the inoculated and non-inoculated treatments. Fabricated soils amended with 5% and 7% MR (wt.%) had the highest copy numbers. This is auspicious to literature on the positive correlation between soil available organic carbon and denitrifier community abundance (Knowles, 1982). Abundances of the nirK gene encoding the nitrite reductase denitrification enzymes were dominant after teff growth with values ranging from 1.63E+09 to 4.09E+09 per gram Technosol. While, nosZ followed by nirS genes copy numbers were slightly lower in CW+MR5% and CW+MR7% (Table 25). This coincides with Hafeez et al. (2012) who found highest copy numbers of nirK genes in wasteland Technosols with alfalfa.

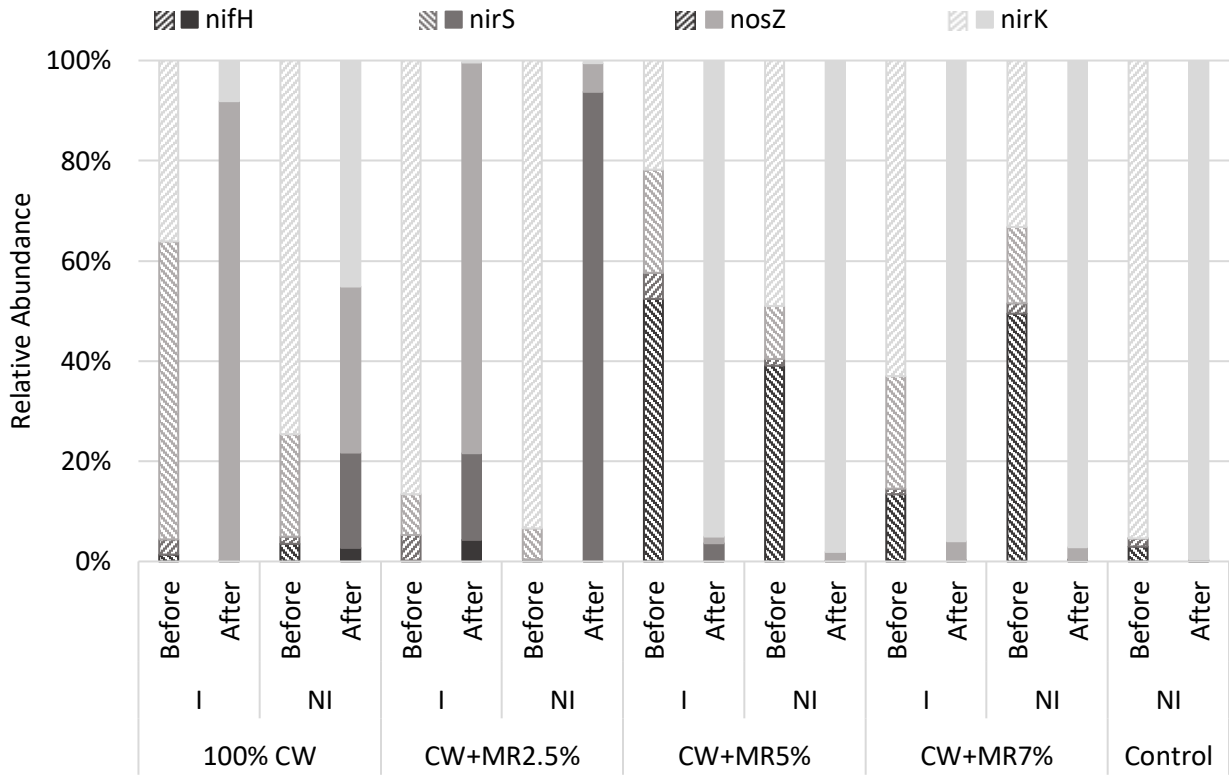


Figure 48: Relative abundances (expressed as percentage) of nitrogen cycling genes (nifH, nirS, nosZ and nirk) in vegetated coal-based Technosols, with and without bioaugmentation and potting soil as the control, before and after the final plant growth trial in winter. Where, I represents inoculated treatments and NI represents non-inoculated treatments.

Enwall et al. (2010) found that the abundance and distribution of denitrifiers were correlated to certain soil parameters; soil pH for nirS and soil copper content for nirK genes, indicating dependence of

denitrifying microbes on soil structure. However, the presented results for this study eluded no similar correlations. Additionally, Enwall et al. reported similar abundances between nirS and nirK (varied within 1 order of magnitude). In the current investigation, this was only seen in the bioaugmented CW+MR5% treatments (before plant growth, nirS: 3.16E+06 copies/g soil and nirK: 1.33E+07 copies/g soil; after plant growth, nirS: 1.42E+08 copies/g soil and nirK: 3.67E+09 copies/g soil) with teff, and in all non-planted controls of 100% CW. Yet, as expected the 100% CW controls demonstrated very low gene copy numbers of denitrifiers, as denitrifying bacteria transform nitrates back into nitrogen by forming asym- and symbiotic relationships with plants (illustrated in Figure 6) which were absent in the pure CW controls. For CW+MR5%-I, it is an indication of effective biostimulation with 5% MR for the specific inoculum to structurally support plant growth (as detailed in section 4.3.1) and the microbial community ecology for N cycling.

Enwall et al. (2010) delineated a negative correlation between the abundance of nirS and nirK genes and extractable soil P and K, but no similar correlation was found in this investigation. CW+MR5% and CW+MR7% had the highest P concentrations (Figure 62) and greatest abundance of denitrifiers, perhaps due to plant-mycorrhizal associations which enhance P and N uptake (Atlas & Bartha, 1998). Furthermore, Enwall et al. (2010) found a positive correlation between clay-like soil structure and nirS gene abundance. This supports the high nirS gene copy numbers in CW+MR7% (I: 1.26E+06 copies/g soil; NI: 4.42E+06 copies/g soil).

In all unvegetated soils, except CW+MR5% and CW+MR2.5%-I, the relative abundances of nifH gene copy numbers compared to nirS, nosZ and nirK decreased tremendously after 82 days, whilst nirS and nosZ gene copy numbers augmented. Similarly to vegetated Technosols, nirK gene abundances in unvegetated treatments were dominant after 82 days in the greenhouse, especially in engineered soils amended with 5% and 7% MR, and the control soil. It is evident from the low abundances of nifH gene copy numbers in the non-planted controls (graphically summarized in Figure 79 in Appendix D), that plant-microbe interactions contributed to nitrogen fixation within the vegetated bioaugmented-Technosols.

CHAPTER 5

5 CONSIDERING FEASIBILITY FOR IMPLEMENTATION

Technosol feasibility must be determined by technical, economical, and environmental criterions (Zongo, et al., 2012). A full assessment is required when considering implementation. For this investigation, initial feasibility was assessed based on the results for seasonal plant growth experiments that were performed to investigate the benefits of including biostimulation and bioaugmentation in coal-based fabricated soils. Technical considerations included, plant growth development, grain yield, biomass production, soil physiochemical quality and soil biological analysis. An economic analysis considers the costs related to the production, implementation and monitoring processes of the Technosols. For this investigation, initial economic predictions based on the amount of resources required, the volume of water required for vegetation, alternative options for amendment materials, and the grain and biomass production per annum were made to consider the implications of scaling up. The environmental criterion refers to the sustainability of the system when implemented. In this study, we briefly addressed the effects of seasonal variation on Technosol performance, the benefits of using otherwise-wasted resources and the influence of Technosols on the surrounding environment and community.

5.1 Estimates for Initial Cost and Material Requirements

Material requirements and cost estimations regarding bioaugmented soil fabrication were performed as part of the techno-feasibility. The average South African market prices in 2022 were used for financial analysis. It should be noted that fertiliser rates and maintenance costs are subjective and dependant variables. Nonetheless, the derived cost estimations provide insight to the economics of mined land rehabilitation. Sample calculations for cost estimations are shown in Appendix F: Economic Analysis Assumptions & Calculations.

Traditional coal mine rehabilitation strategies are focused on revegetation with ‘new’ topsoils at a depth of 50 cm to ensure vegetational growth (Weiler, et al., 2020). Under the experimental conditions, to produce 1 L of bioaugmented CW+MR5%, it was necessary to mix 681 g CW with 82 g MR and 62 mL EM Pro-Soil. The soil-like substrate has an apparent specific weight of 0.76 g/mL (0.94 g/mL for CW and 0.18 g/mL for MR). Therefore, one can estimate that about 3585 tonnes CW, 229 tonnes MR, and 323 kilo-litres EM Pro-Soil would be necessary to produce one hectare of soil with a depth of 50 cm. For one hectare of conventional potting soil, 4450 t are required at an estimated cost of 4.95 million ZAR. Furthermore, high overhead costs for excavation and transportation are associated with the topsoils (Cowan, et al., 2016). In addition, waste disposal and ARD treatment facilities are extremely costly (Kazadi Mbamba et al., 2012). The expense of purchasing natural soil is therefore eliminated when implementing Technosols.

Nevertheless, commercial inoculums are very expensive. The estimated cost of applying activated EM Pro-Soil per hectare Technosol (50 cm depth) is 15.9 million ZAR, based on the desired number of microbial cells per mL soil recommended by Weiler et al. (2020). However, when considering annual chemical fertilization for traditional South African soils at a rate of 72 830 kg/ha (FAO, 2018), overall maintenance costs would increase by approximately 680 130 ZAR per hectare per annum (Agritech,

2013). Chemical fertilizers are deleterious to the environment and soil bacteria communities (Lebrun, et al., 2021), whereas the commercial benign inoculum should sustain soil fertility, quality and health for arable land after mine closure. Further investigation into alternative, commercially available inoculums that are more financially viable with similar plant growth and soil quality enhancing capabilities to EM Pro-Soil must be considered.

The use of amendments in Technosols play a significant role in soil quality and structure (Herran Fernandez, et al., 2016) as shown in the greenhouse studies of this investigation. MR was sourced from SA Breweries Ltd. in the Western Cape, South Africa. SA Breweries Gauteng is the nearest to the eMalahleni area at a proximity of 144 km. Therefore, the use of MR is disadvantageous when considering the costs associated with transportation to the mining site.

The Mpumalanga province is known for its diverse agricultural production, thus, associated wastes are widely available. This is also seen globally. In India, 5 tons of organic manure/ha arable soil are generated per year, which is estimated to be 100 kg NPK/ha/year; however, these wastes are not properly used (Mandal, et al., 2018). Thus, opportunities exist to incorporate animal manure collected from surrounding farms as amendments into Technosol fabrication as suggested by Chessa et al. (2016).

Additional resources in a close proximity to the eMalahleni colliery that have successfully been used as amendments are: biosolids (e.g., sewage sludge (Weiler, et al., 2018; Jordan, et al., 2017)), leaf litter (Lebrun, et al., 2021), urban waste (Prado, et al., 2020) and landfill waste (Weiler, et al., 2020; Herran Fernandez, et al., 2016). Webb (2004) found that sewage sludge as amendment, fertilised the soil and increased the total biomass production of red oat grass (*Themeda triandra*) during a rehabilitation study on open cast coal mines specifically in Mpumalanga. However, sewage sludge is often associated with a high heavy metal content (Herran Fernandez, et al., 2016). Moreover, Truter et al. (2007) investigated soil amelioration with class F fly ash and sewage sludge to disturbed soils of coal mines in Mpumalanga. Results suggested augmented macro- and micronutrients in soils and enhanced growth of indigenous grasses such as, teff.

When considering alternative amendments, domestic waste from the coal mining community must be considered (Prado, et al., 2020; Weiler, et al., 2020). An average of 0.65 kg domestic waste per capita per day with an OM content of 64% (wt.%) is generated throughout Africa (Hoornweg & Bhada-Tata, 2012). Thus, when assuming compost as a substitute for MR with similar effects and a 40% OM to compost transition ratio (Diaz, et al., 2002), the domestic waste of ultimately 3800 people per annum can be used to fabricate one hectare of Technosol amended with 5% of this urban OM. Consequently, the feasibility of using coal-based bioaugmented Technosols as topsoils are further enhanced by optimising amendment material selection to incorporate local urban or agricultural waste sources.

5.2 Water Requirements and Biomass Production

High soil moisture availability enhances photosynthesis and leads to increased teff aboveground biomass (Hilemicael & Alamirew, 2017). Therefore, biomass predictions of teff in Technosols with the associated water requirements are useful for financial feasibility studies. Predictions regarding water requirements for teff growth in the best performing Technosols (CW+MR2.5% and CW+MR5%) were made from data

collected in the final greenhouse study. Initial soil weights, material bulk densities, cumulative water requirements (summarized in Table 27), and above ground biomass and grain yields per Technosol were utilised. The conditions of the final growth trial were most similar to the year-round climate in eMalahleni with an average annual temperature of 16.3 °C and average annual precipitation falls of 760 mm (Climate-Data, n.d.).). The results are presented in Table 14. Sample calculations are summarized in Appendix F: Economic Analysis Assumptions & Calculations.

Table 14: Estimated total volume of water required per teff growth cycle and approximate dry biomass and grain yields per hectare of Technosol at a depth of 50 cm.

Technosol Type	Inoculated (I) or Non-Inoculated (NI)	Water Required (KL)	Above Ground Dry Biomass (t)	Grain Yield (t)
CW+MR2.5%	I	9 494	16.0	2.51
	NI	5 455	20.5	2.48
CW+MR5%	I	9 574	12.2	1.31
	NI	5 943	3.81	0.32
Natural soil	NI	19 368	32.0	6.73

The land-use activities (coal mining, agricultural practices, and energy production facilities) in the area have rendered eMalahleni into a water stressed municipality (Municipality, 2022). Coal mining and associated ARD in the area have prohibited underground water resources exploitation. Thus, to extend the circular economy approach and further reduce the financial impact of the rehabilitation scheme, run-off water from the mine, treated mine waste water, or on-site collected rainwater can be used for plant irrigation. Additionally, from Table 14, implementing bioaugmented CW+MR5% would utilise 49% less water than the agricultural soil.

Teff is known for its agricultural value in the food and beverage industry, and for livestock feed as described in Chapter 2. It was estimated that a total of 16 tonnes of teff dry biomass can be cultivated in inoculated CW+MR2.5% and 12 tonnes in bioaugmented CW+MR5%, compared to 32 tonnes in the control soil. These predictions seemed unrealistic and exceedingly high compared to literature on teff farming that described 4-8 tons of dry teff shoots per hectare per season (AGT Foods Africa Pty Ltd., 2017). This is because optimised yields were obtained in the controlled, small-scale experiment of this study, yet the economic benefit of teff production in bioaugmented-Technosols was clear from the estimated price per hectare of Technosol as shown in Table 15. The financial benefits of teff cultivation in Technosols extend to the coal mining communities for job opportunities (seed sowing, maintenance and biomass processing), empowerment through skills development, increased public welfare, and relationship strengthening between the involved parties.

Table 15: Estimated economic benefit from teff cultivation per hectare of Technosol at a depth of 50 cm.

Technosol Type	Inoculated (I) or Non-Inoculated (NI)	Estimated price (ZAR) of teff hay*	Estimated price (ZAR) of teff grain**	Estimated price (ZAR) of teff flour**
CW+MR2.5%	I	718 972	50 860	60 279
	NI	924 393	50 208	59 506

CW+MR5%	I	549 241	26 495	31 402
	NI	171 638	6 573	7 790
Natural soil	NI	1 441 800	136 185	161 405

*Based on the average market price for teff hay in South Africa (For the Farmer, 2022).

**Based on FAO Analysis of price incentives for teff (FAO, 2015).

As previously discussed, a positive correlation (R^2 = 0.91) was found between grain yields and above ground teff biomass. Therefore, similar trends in biomass production and grain yield, and in associated prices were seen in Table 14 and Table 15. Expectedly, teff cultivated in agricultural soil would result in the greatest financial benefits due to augmented biomass and grain yields within this soil type. However, predications were made from potting soil results for teff biomass. It can be assumed that commercial topsoils that have been exposed for extended periods to chemical fertilisation, would have lower productivities. Furthermore, when considering the cost of 4450 tonnes of agricultural soil per hectare, the 38% increased price margin of teff hay per hectare over that in inoculated CW+MR5% becomes less significant.

5.3 Teff as Cattle Feed

To ensure that the cultivated teff shoots contain the necessary nutritive values for animal feed, teff macro- and micronutrients contents from the best performing Technosols and control soil were compared to literature for teff hay as cattle feed. The results are presented in Table 16.

Table 16: Nutritional values of teff cultivated in Technosols and agricultural soil compared to teff hay for cattle feed by Vinyard et al. (2018).

		CW+MR2.5%		CW+MR5%		Natural Soil	Cattle Feed
Variable	Unit	I	NI	I	NI	NI	Teff Hay
Na	%	0.05	0.05	0.06	0.07	0.03	0.04
Mg	%	0.36	0.40	0.34	0.43	0.34	0.2
Ca	%	0.53	0.62	0.65	0.97	0.36	0.51
K	%	1.49	0.77	1.55	0.78	1.87	2.38
P	%	0.33	0.14	0.26	0.17	0.27	0.27
Mn	mg/kg	202	104	117	98.5	22.2	33
Fe	mg/kg	95.1	84.5	117	172	68.3	230
Cu	mg/kg	14.45	11.9	14.2	13.4	8.10	10
Zn	mg/kg	86.2	39.3	76.0	46.8	81.4	33
Mo	mg/kg	0.74	0.54	0.80	0.69	1.49	2

It was clear that the non-inoculated Technosols resulted in teff shoots with higher Na, Mg and Ca contents compared to the teff grown in inoculated treatments. However, as discussed in Chapter 4.3.2, the above ground dry biomass from the bioaugmented-Technosols CW+MR2.5% and CW+MR5%, were characterised by higher K, P, Mn, Cu, Zn and Mo compared to biomass from non-inoculated fabricated soils. Metal translocation and nutrient cycling were enhanced by exogenous microorganisms (Margerison, et al., 2020) that were more abundant in these soil-like substrates (refer to Table 25). From Table 16, teff cultivated in coal-based Technosols had augmented nutritive values relative to the standard

values for cattle feed. Of the bioaugmented fabricated soils, CW+MR5% showed good prospect for teff hay production, with higher Na, Mg, Ca, Zn, and Fe levels compared to the reference values.

5.4 Environmental Considerations

Research on Technosols as topsoils contributes to the development of mine waste management strategies to include the circular economy principle. However, when considering the sustainability of implementing Technosols, the self-sustaining ability thereof with seasonal changes, and the impacts and advantages on the surrounding environment must be considered.

5.4.1 *Technosol Performance with Seasonal Effects*

Jenny (1941) described climate as one of the five soil formation factors influencing pedogenesis (refer to Equation 1 in Chapter 2). Soil chronosequence studies are often performed to evaluate this change over time (Sauer, 2015). Zhao et al. (2018) concur that research on climate spatial variation of soil nutrients provide valuable information for restoration and revegetation strategies. The feasibility of the biostimulated and bioaugmented fabricated soils are in part determined by whether plant growth is sustained whilst the Technosol consistently demonstrates characteristics of a self-sustaining topsoil in seasonal effects.

The change in temperature, relative humidity and dew point for the initial and final growth trial are presented in Figure 80, Figure 81, and Figure 82 in Appendix E: Plant Growth trials in Comparison. The final percentage of plants that had died in each of the growth trials are presented in Figure 83. Here, it is evident that bioaugmented CW+MR2.5% performed better in warmer conditions (100% survival rate), with only 83% of the cultivated teff remaining by the end of the colder, final growth trial. Whereas, inoculated CW+MR5% presented ideal results during summer and winter with 100% survival rates for teff.

When evaluating soil conditions, final Technosol pH values were all slightly more acidic in summer, compared to winter. The conditions changed with time as suggested by Jenny (1941), as a result of biogeochemical transformations, irrigation with alkaline rain water in the colder season, or more reducing root exudates and abiotically influenced microbial community functions within the Technosols (Atlas & Bartha, 1998). Technosol pH may continue to increase with every cycle of teff growth. Additional research is required to investigate long term implications; however, incorporating the plant biomass produced after every growth back into the soil may reduce rates of nutrient decline and improve water retention.

From soil elemental identification and quantification results in Chapter 4, Technosol iron content was lower in winter compared to summer, suggesting enhanced metal solubility. The water holding capacities of Technosols differed slightly between seasonal growth trials. Higher WHC were reported in winter months for CW+MR2.5% and CW+MR7%, whereas those of CW+MR5% were lower in this colder growth cycle. Nonetheless, bioaugmented Technosols with 5% MR as biostimulation were able to support and sustain Teff growth even in unfavourable conditions and resulted in improved WHC and reduced

evapotranspiration, stabilised soil pH and salinity with an environmentally benign leachate, plant root development and higher levels of K and P in teff above ground biomass.

5.4.2 *Impacts on the Surrounding Environment*

Fabricating soils from CW and otherwise-wasted resources as amendments reduce resource requirements and prevent waste accumulation. Thus, the environmental impact of mining on the mining communities, landscape opportunities and future land use are minimized. Implemented Technosols will accumulate SOM over time (Amaral Filho, et al., 2020) for carbon, nitrogen and sulfur (building blocks of SOM) sequestration. It conserves the structure of the pedoderm (Mills & Fey, 2004), ensures organic nutrient availability (as depicted in Figure 6) and is vital to soil microbial activity (Botta, 2015). Revegetation in bioaugmented-Technosols amended with 5 wt.% MR reduces the probability of acidification by reducing metal(loid) solubility, inhibiting iron leaching and ensuring soil organic sulfur content below the South African maximum screening values for soils. Frameworks on coal rehabilitation provide scope on land and waste management to identify areas or zones specifically vulnerable to degradation, from where, Technosol revegetation strategies can be optimised based on the identified issues or areas of improvement. Various plant species can be cultivated after mine closure for optimal land use, augmented economic benefit and improved local biodiversity.

Revegetation techniques have been reported to improve health concerns associated with untreated coal mining waste dumps, alleviate the aesthetic quality of the community through vegetational growth, increase the local biodiversity of fauna surrounding the mine, and create job opportunities (Hattingh, et al., 2018; Karaca, et al., 2018; Lamb, et al., 2014; Amaral Filho, et al., 2020). The engineered soils align with the SDGs by utilising waste resources, mitigating land degradation and by promoting the development and implementation of environmentally sound technologies (Department of Statistics South Africa, 2019). Thereby, contributing to the social aspect of sustainable mine rehabilitation.

CHAPTER 6

6 CONCLUSIONS & RECOMMENDATIONS

6.1 Research Overview

Coal mining processing contributes to global economics; however, the associated activities and processing are deleterious to the surrounding environment. These impacts can be minimized through the successful implementation of sustainable mine rehabilitation schemes. Current topsoil strategies are mostly unsuccessful as it focuses solely on soil organic content improvements through chemical fertilisation, or soil pH adjustments through lime application; therefore, do not result in sustained soil quality, health and fertility. Due to the interdependence between soil, plants and microorganisms, little research has been done on the effects of microorganisms in conjunction with nutrient amendments to coal waste on plant growth in degraded mine land (Thavamani, et al., 2017).

To investigate coal-based Technosols through different seasonal growth trials of teff cultivation, the physical, chemical and biological properties were assessed. This was necessary to consider the feasibility of implementing bioaugmented and biostimulated Technosols as a mine rehabilitation tool at a South African colliery. Additionally, the interactive relationships between plants and soil microbial ecology were investigated to evaluate the development and to consider the function of the microbiomes in the development of self-sustaining Technosols as topsoils. The project aimed to answer key questions regarding the benefits of microbial inoculation and amendment application when considering temporal changes.

6.2 Research Conclusions

The primary objective for the investigation was to investigate if bioaugmentation of an amended coal-based Technosol improved all aspects of soil performance (plant development, soil fertility, soil physiochemical characteristics). To meet this objective, two seasonal (summer and winter) plant growth studies with teff were performed in a greenhouse. Technosols from CW were amended with different ratios (w/w) of MR (0%, 2.5%, 5%, 7%) and inoculated with a mixed culture inoculum. Favourable plant growth results with supporting soil physiochemical characteristics and soil fertility results showed that CW+MR-based Technosols are capable of supporting plant growth in different seasons, and that bioaugmentation with EM Pro-Soil in a 5 wt.% MR amended fabricated soil improved overall soil health, quality and fertility.

6.2.1 *Trends in Effects of Bioaugmentation and Amendment Application*

It was concluded that bioaugmentation of coal-based fabricated soils resulted in increased soil pH levels. However, the changes of pH during microbial growth are carbon source dependent (Sanchez-Clemente, et al., 2018) and would vary when other amendment types are investigated. Furthermore, Technosol pH levels decreased when MR dosage increased, as MR was initially characterised by an acidic pH. Teff growth is favoured in soils with pH values between 5.5 and 7.5 (Prado, et al., 2020). Therefore, higher amendment dosages negatively influenced teff growth through soil pH reduction, whereas, the alkaline conditions in CW+MR5% enhanced intracellular proton motive forces for microbial geochemical functions

(Neina, 2019). Technosol pH levels increased with teff growth in colder climates compared to warmer conditions, as the consumption of protons during anaerobic microbial respiration resulted in a pH increase (Prado, et al., 2020). This might be specific to teff as a pioneer species.

Expectedly, inoculation slightly decreased redox conditions in Technosols before and after teff growth as the redox potential of a medium decreases during bacterial growth (Reichart, et al., 2007). The depletion of labile OM (from amendment application) and oxygen by microorganisms for respiration, and potential reducing root exudates contributed to the Eh reduction (Husson, 2013). Contradictorily to literature that describes lowered Eh values from enhanced microbial respiration upon increased OM application (Gardiner & James, 2012), redox potentials increased with increasing MR dosage and with evapotranspiration. It was concluded that the anaerobic conditions of coal-based Technosols reduced the probability of pyrite oxidation, sulfuric acid generation, and aluminium cation solubility (Husson, 2013).

It was concluded that initial salinity of coal-based Technosols was directly proportional to the amendment dosage, and decreased with inoculation. Nonetheless, plant-microbe relationships developed with time to release root exudates and enhance cation uptake that stabilised the salinity of all fabricated soils (Cervantes, et al., 2011).

MR acted as a structural ameliorant as WHC increased with higher MR dosages. In addition, bioaugmentation improved soil structure (improved WHC and teff root development) by soil agglomeration (Margerison, et al., 2020; Rivas-Perez, et al., 2016). Moreover, WHC of biostimulated and bioaugmented Technosols were superior to only non-inoculated Technosols due to structural stability introduced by microbes performing OM decomposition and soil aggregation (Atlas & Bartha, 1998). Thereby, emphasizing the significance of inoculation in mine waste based soils to prevent erosion from metal leaching or extensive run-off.

Supplementarily, it was determined that soil organic C, P, K, Ca and metal contents, with leachate TN, TOC and TIC were directly proportional to the weight percentage of MR. Bioaugmentation with nutrient amendments increased soil macronutrient contents through nutrient cycling (Tiwari, et al., 2021), corroborating research by Weiler et al. (2020) and supporting the primary objective of the investigation. It was concluded that bioaugmentation reduced soil metal solubility (Fe, Cu, Zn, B, Mn) to levels below the maximum screening values with negligible iron leaching; however, only with vegetation and dosages more than 2.5 wt.% of MR as amendment to CW. Additionally, carbon and sulfur sequestration occurred in vegetated fabricated soils, as overall percentages of organic carbon and sulfur were higher than in non-vegetated Technosols, supported by Weiler et al. (2018, 2020).

It was concluded that microbial proliferation (biomass, activity, quantified dsDNA) was promoted by incubation, amendment dosage and bioaugmentation. Initially, bacterial growth was reduced and fungal growth enhanced by plant growth and higher dosages of MR from increased salinity and nitrogen levels (Rousk & Baath, 2007). Expectedly, inoculation resulted in enhanced bacterial and archaeal gene copy numbers per gram Technosol.

It was concluded that optimised irrigation strategies with plant growth ensured biogeochemical transformations, as overall microbial biomass and diversity in Technosols achieved in the final trial, were significantly greater than after the initial growth trial. It was assumed that PGPR and other endophytic microbes were less abundant in the absence of vegetational growth; hence, higher metal(loid) (Cu, Fe, Mg, Mn, Ca, Zn) concentrations were reported in non-vegetated and non-inoculated Technosols, especially in CW+MR5%. Correspondingly, fungal abundances increased with plant growth in all treatments. However, archaeal diversity decreased with teff growth in all but non-inoculated 100% CW and potting soil.

It was concluded that nitrification was governed by bacteria rather than archaea from higher final bacterial abundances compared to archaeal abundances (Hafeez, et al., 2012). The abundances of the nirK gene encoding the nitrite reductase denitrification enzymes were dominant in all Technosols and non-inoculated controls after teff growth, especially in CW+MR5%. This coincided with Hafeez et al. (2012) who found highest copy numbers of nirK genes in wasteland Technosols with *Medicago sativa* (alfalfa). Furthermore, vegetation contributed to nitrogen cycling with less nifH abundances in non-planted controls.

6.2.2 *Soil Health, Quality and Fertility in Sustaining Plant Growth*

In conclusion, coal waste was characterised by its poor water retention, carbonaceous nature and high sulfur and metal(loid) content. Initial elements of concern were Mn, Mo, Sr, V and Ba; however, literature suggests extraction of these elements by bacillus microbes (Wood, et al., 2016) present within the applied inoculum. Amending CW with MR (3.5-fold the moisture content of coal tailings) ameliorated the Technosol's water retaining capabilities; altered the soil texture to loam and held significant value for enhanced nitrogen cycling (10-fold more nitrogen than the potting soil) and plant root stimulation from increased phosphorus levels.

From the plant growth trials, it was concluded that a surface sowing technique was 66% more effective in terms of teff seed germination due to structural impedances inferred from MR application. This is advantageous when considering implementation. Increased humidity and reduced temperatures (below 20 °C) within the greenhouse were adverse to seedling survival. Expectedly, biomass generation in all Technosols were higher in summer than winter suggesting favoured teff growth in warmer conditions.

From delayed seedling emergence results and inadequate above ground teff growth rates, it was concluded that pure compost could not support plant growth and was not a good positive control. Levels of phosphorus and potassium were inadequate for teff germination when compared to the potting soil, and excessive K inhibited the uptake of other cations into plant organs (Rawat, et al., 2016). The alkalinity of compost decreased the availability of many soil nutrients that were important for plant development (Prado, et al., 2020). Furthermore, compost was characterised by poor water retention. Conversely, potting soil was a good positive control with an average germination rate of 95% after 5 days. The best shoot growth rate per day was achieved in potting soil, corresponding to the tallest teff (56.3 cm) of teff cultivated in the colder climate study.

It was concluded that pure coal waste cannot sustain plant growth. Although the substrates presented no structural impedances to seed germination, only 4% teff in inoculated pure CW survived after 82 days in the final plant growth trial due to inadequate phosphorus and potassium concentrations. The insufficient K levels delayed plant maturity and increased susceptibility to pestilence (Rawat, et al., 2016), also seen in CW+MR7%. The sand-like texture and small particle size of the tailings presented the lowest initial EC from very low nutrient cation concentrations and poor WHC. It was concluded that implementing 100% CW as a topsoil would result in extensive water run-off, crusting or gully erosion. Thereby, emphasizing the significance of amendments to Technosols and the correct dosage thereof to ameliorate soil structure (Jordan, et al., 2017; Macia, et al., 2014).

Both plant growth trials in summer and winter presented similar results concluding the inadequacy of CW+MR7% as a soil-like substrate. The high salinity, iron levels and OM concentration enhanced fungal growths (specifically mould development) and reduced bacterial respiration (Rousk & Baath, 2007) and microbial community diversity (Firpo, et al., 2014). Only 8% of teff planted in inoculated CW+MR7% survived by the end of the initial growth trial. Teff that did survive were characterised by low growth rates and very low above and below ground dry plant biomass. Soil agglomeration and clumping resulted in saturation, poor water permeability (Prado, et al., 2020) and undeveloped root systems in CW+MR7%. Additionally, the large surface area from the colloid pores limited mass transfer, specifically oxygen diffusion, and resulted in reducing conditions (Sondergaard, 2009) which delayed microbial-associated pollutant degradation (Nannipieri, et al., 2003). The high microbial activity and biomass results from FDA and SIR analysis on these substrates represented fungal abundances which was identified with qPCR analysis in the final growth trial. Suzuki et al. (2010) are in agreement and described a positive correlation between soil fungal community abundance and soil OM content. It was evident that a higher organic material input as amendment did not improve soil health nor soil fertility, coinciding with Bakhoum et al. (2012) who described the success of bioaugmentation as dependent on soil type (i.e. amendment dosage in this investigation).

The investigation of soil physiochemical and biological properties of CW+MR2.5% and CW+MR5% presented favourable results. It was concluded that teff growth was supported in both summer and winter in bioaugmented CW+MR5% and non-inoculated CW+MR2.5% substrates. The similar above ground teff dry biomass results for Technosols amended with 2.5% and 5% MR (w/w) were comparable with those presented by Amaral Filho et al. (2020), Firpo et al. (2015), and Weiler et al. (2018), where the addition of 2-5 wt.% OM enhanced the shoot and root productivity in the coal-based fabricated soils. The earliest signs of inflorescence were in CW+MR2.5% and CW+MR5%; 40 days since planting in summer and delayed by 5 days in winter.

Bioaugmented and biostimulated (with 2.5 wt.% and 5 wt.% MR) Technosols were best able to retain water and remediate soil S to suitable levels with teff growth. Whereas, S in 100% CW and CW+MR7% were 1.7-fold the maximum screening value for South African soils. The abundance of macronutrients Na, Mg, Ca, K and P in inoculated treatments, CW+MR2.5% and CW+MR5%, validated the feasibility of teff cultivation in Technosols as topsoils for nutrient-deficient coal mining waste (Truter, 2007).

It was concluded that metal(loid) solubility of Ni, As, Sr, and Hg were reduced in inoculated CW+MR5% from microbial mechanisms (Hrynkiewicz, et al., 2018) that mobilised these elements to root structures. This was corroborated by increased microbial activity from FDA analysis in these Technosols (Yun, et al., 2017). Soil microbiome analysis concluded that inoculated CW+MR5% contained the most quantifiable double stranded DNA, with 25-fold more than inoculated 100% CW. Inoculation and amending CW with 5 wt.% MR, resulted in rapid bacterial growth during soil incubation at optimum mesophilic growth conditions. This was supported by large bacterial gene copy numbers per gram of inoculated CW+MR5%.

Moreover, the microbiome of vegetated, inoculated CW+MR5% demonstrated the greatest potential for nitrogen cycling, with 52.4% relative abundance of nifH gene copy numbers. Additionally, abundances of nirS and nirK varied within one order of magnitude to suggest plant-mycorrhizal associations (Atlas & Bartha, 1998), linking to the enhanced P and N uptake similarly found by Enwall et al. (2010). The results correspond to the inoculant microbes; *Rhodopseudomonas palustris* and *R. sphaeroides*, known for their soil nitrification capabilities (Knowles, 1982).

6.2.3 ***Feasibility***

This investigation, supported by previous research on coal-based Technosols, inferred the beneficial impact of implementing bioaugmented coal-based Technosols when implemented as a mine rehabilitation strategy. This is further motivated by the reduction in capital costs associated with commonly implemented commercial topsoils, minimised acidification, soil agglomeration, sustained plant growth with temporal effects, economic benefits of teff cultivation, and options for reusing up to 9574 kilo-litres of mine site water for irrigation. Furthermore, it was concluded that teff cultivated in inoculated CW+MR5% were suitable for cattle feed with improved Na, Mg, Ca, Zn, and Fe contents. It should be noted that the suitability of teff as cattle feed does not only rely on the nutrient content but on other quality parameters not addressed in this research. The cost of the commercial inoculum, EM Pro-Soil, negatively impacts the economic feasibility of bioaugmented Technosols. Finally, it was concluded that the biostimulated and bioaugmented fabricated soils target SDGs 8, 9, 11, 12, 15 and 17.

6.3 Research Recommendations

The following recommendations apply to future investigations. When considering implementation of Technosols as a mine rehabilitation strategy, amendment sources in close proximity to the mine site should be investigated. Animal manure as part of agricultural waste and municipal waste were recommended as these wastes would directly contribute to waste management and the social sustainability of Technosols in the eMalahleni area. This research would improve feasibility assessments on South African mine waste based soils. It is recommended to investigate plant growth and soil fertility when multiple amendments are used in conjunction with one another, in addition to investigating amendment application rates. It is recommended to include a negative control such as the native mine land soil when performing the plant growth experiments.

Nimnoi et al. (2010), Kotani-tanoi et al. (2007) and Costa et al. (2006) agreed that differences in soil bacterial community structure are strongly dependent on plant and soil type, more so than on the applied inoculant. Thus, the ability of the coal-based Technosols to support other valuable and viable plant

species should be investigated. It is recommended to evaluate legume growth, that can provide valuable nutrition to local communities whilst requiring limited and decentralised processing. Furthermore, the Technosol quality when mixed plant species are cultivated should be investigated. Future research should investigate long term vegetation (several growth cycles) in coal-based Technosols with seasonal variation, and the effects thereof on sulfur bioaccumulation. It is recommended to perform not only greenhouse studies, but in situ growth studies to refine economic predictions of water requirements and biomass production at the specific implemented mine site. To consider the issues intrinsic to the use of pots in soil experiments (i.e. soil compaction) and to reduce variability in results, plant growth studies for Technosols should also be done on a larger scale with less seeds per surface area, or in fields, or with smaller vegetation such as lettuce. This research would extend the applicability and therefore the feasibility of Technosols.

The commercially available inoculum has multiple applications with a reputation of improved plant growth (Efficient Microbes, 2006). As per the manufacturer's description, the recommended dosage of EM Pro-Soil for soil preparation is 15 mL per square meter of soil. If this dosage was applied, 4.5×10^2 less microbes per mL would be inoculated into the Technosols compared to the dosage used in Weiler et al. (2020). Thus, it is recommended to evaluate the minimum required dosage of EM Pro-Soil for inoculation that would result in an economically viable strategy with sustained soil fertility. Furthermore, less expensive commercially available alternatives to EM Pro-Soil should be investigated.

The effects of bioaugmentation and amendment dosages on the soil biodiversity (i.e. the presence and abundance of invertebrates such as, earthworms) should be investigated along with statistical analysis on identified parameter relationships. Subsequently, the addition of earthworms to coal-based fabricated soils and their corresponding effects on soil structure, microbial biomass and activity, and plant performance should be evaluated. Deeb at al. (2017) described improved soil aggregate stability in Technosols from earthworms, and that the benefits of compost as amendment were only observed in the presence of plants or earthworms.

With regards to soil properties, drainage over time in soil , particle distribution density of tailings, and soil bulk densities in bioaugmented coal-based Technosols with different amendment dosages should be investigated as faster drainage results in less water retention (Prado, et al., 2020), and higher bulk density reduces total soil porosity (Hattingh, et al., 2018). These parameters are valuable for evaluating soil compaction over time that can have adverse effects on plant growth and land use. Technosols under investigation should be characterised before and after plant growth for evaluation of metal(loid) uptake and solubility with plant growth. It would be of value to profile the soil microbiomes of the vegetated Technosols in warmer conditions that were characterised by lower pH to consider soil functions with seasonal changes. Furthermore, research on more accurate soil organic carbon analyses should be done to better distinguish between what is coal-derived carbon and plant-derived carbon, especially when focusing on carbon sequestration.

It is recommended to employ Illumina sequencing of the V4-V5 region of the 16S rRNA gene for taxonomic composition data, followed by phylogenetic affiliation of the sequences as suggested by Sun

et al. (2019) and Lebrun et al. (2021). This would ensure a detailed microbial community profile to evaluate the dominance within the bacterial community most abundant in the bioaugmented-Technosol with 5 wt.% MR and investigate the persistence of a microbial community after inoculation.

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APPENDIX A: FDA OPTIMISATION

Initial concentrations of EM Pro-Soil microorganisms (based on cell counts) were utilised in 1xPBS for evaluation of FDA over time; from no incubation to sixty minutes. From the figure below with the corresponding correlations between fluorescence emitted by microorganisms during hydrolysis and the hydrolysis period, it was determined that a period of 10 minutes resulted in the strongest linearity (R^2 = 1), and that as time proceeded, the linearity became less.

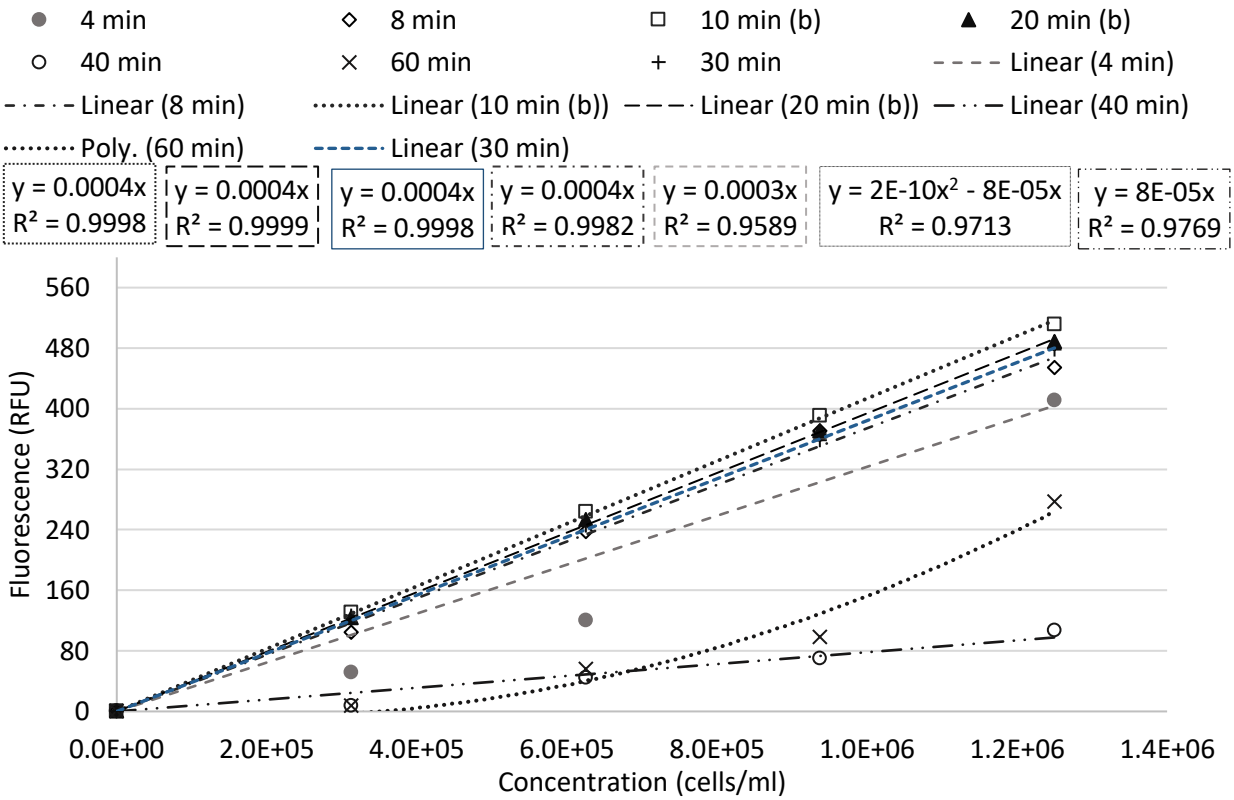

Figure 49: Fluorescence emitted by microorganisms in various concentrations over time for period of hydrolysis optimisation.

This was further validated by performing tests on microbial concentrations between 3.13E+05 and 1.25E+06 cells/mL as shown in the figure (fluorescence over time).

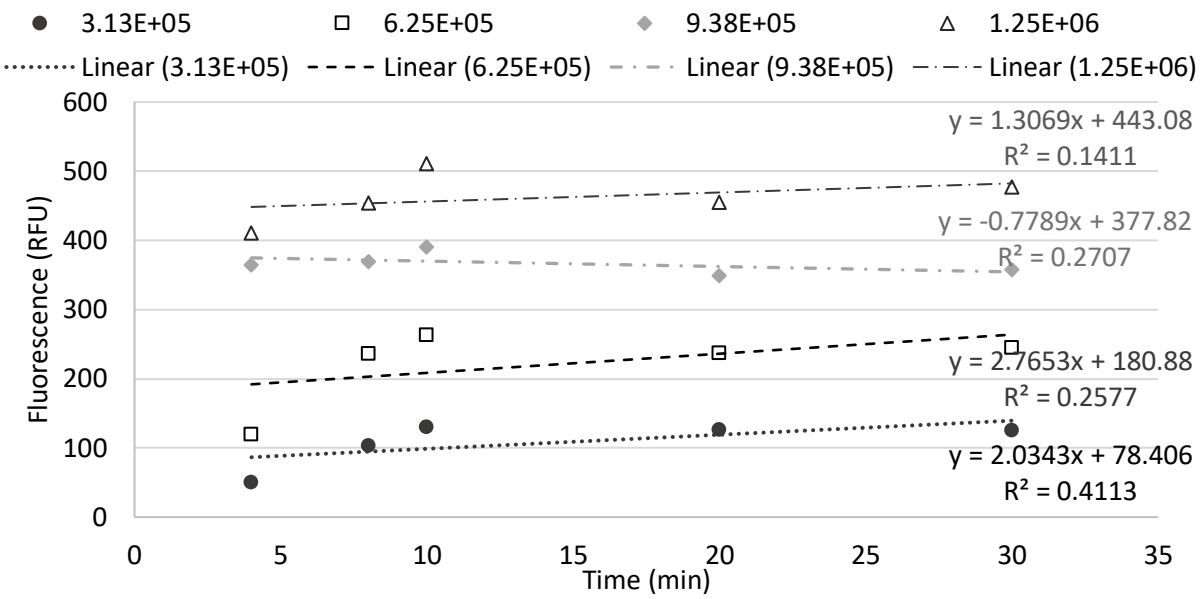

Figure 50: Fluorescence over time for various concentrations of microorganisms from EM Pro-Soil.

It is clear from the figure below, that a linear relationship between fluorescence and time existed between 4-10 minutes of hydrolysis. Ten minutes was used throughout FDA analysis on soil samples as it was assumed that microbial proliferation would take place with plant growth.

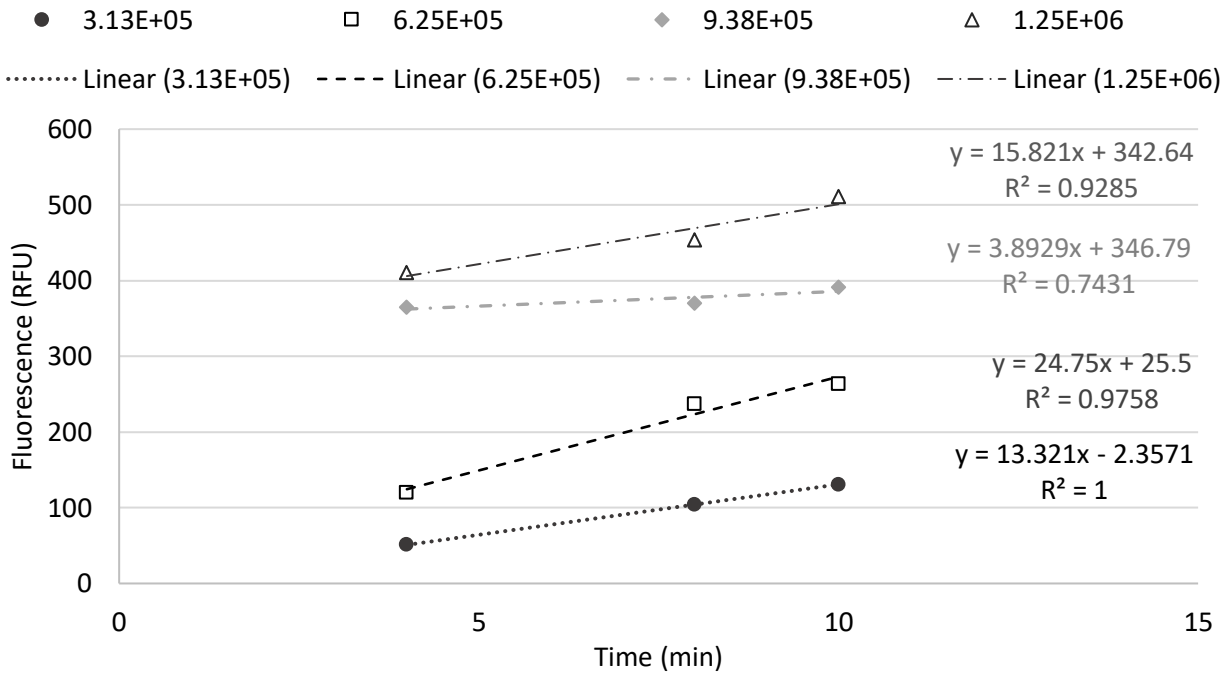

Figure 51: Fluorescence over time for various concentrations of microorganisms from EM Pro-Soil displaying linearity between 4-10 minutes.

To investigate if chloroform is effective in terminating hydrolysis, experiments with 100 times dilutions of EM Pro-Soil were conducted with minimal (A) and vigorous (B) shaking after chloroform was added following incubation of 10 minutes for hydrolysis of fluorescein diacetate. The fluorescence was

measured after centrifugation. After which, all samples (except the controls) were heated for an extra five minutes, and fluorescence was measured again. The results are presented in the table below.

Table 17: FDA optimisation experiments to determine if chloroform is effective in terminating hydrolysis reaction.

Time	0 cells/ml		3.13E+05 cells/ml		6.25E+05 cells/ml		9.38E+05 cells/ml		1.25E+06 cells/ml	
	A	B	A	B	A	B	A	B	A	B
(min)	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU
	53	51	177	176	320	312	447	446	561	553
10	57	51	191	186	315	306	392	390	538	551
	57	49	184	183	323	318	430	432	553	528
Mean	56	50	184	182	319	312	423	423	551	544
Standard Deviation	2	1	7	5	4	6	28	29	12	14
	54	49	184	182	317	316	440	427	549	547
10 + 5 heated	59	50	176	184	310	303	385	382	523	545
	58	51	191	174	322	310	425	441	541	522
Mean	57	50	184	180	316	310	417	417	538	538
Standard Deviation	3	1	8	5	6	7	28	31	13	14

In conclusions, the values for well mixed and minimal mixed samples were very similar. Standard deviations for the samples were also similar, and become even more so after the 5 minutes of reheating. The fluorescence values (in RFU) and standard deviations after the 5 min of reheating were very similar to the controls without the extra 5 min of heat. Therefore, chloroform was successful at terminating the reaction.

To investigate if the microbes or inerts, proteins or other intracellular compounds were emitting fluorescence, experiments with and without the addition of fluorescein diacetate were performed. FDA analysis were initially performed using 100 times dilutions of EM Pro-Soil at 10 minutes incubation (reaction time), and then with 1000 times dilutions thereof. Fluorescence was measured before and after the 10 minute period with chloroform to terminate the reactions. The results are summarized in the tables below.

Table 18: Fluorescence after 10 minutes of diluted EM Pro-Soil concentrations between 0 and 1.25E+06 cells/mL with and without fluorescein diacetate for part of FDA optimisation.

Time	0 cells/ml		3.13E+05 cells/ml		6.25E+05 cells/ml		9.38E+05 cells/ml		1.25E+06 cells/ml	
	no FDA	FDA	no FDA	FDA	no FDA	FDA	no FDA	FDA	no FDA	FDA
(min)	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU
	24	37	167	166	284	320	417	434	512	570
0	23	38	136	174	281	319	421	451	535	577
	23	37	145	175	256	301	401	447	530	566
Mean	23	37	149	172	274	313	413	444	526	571
Standard Deviation	1	1	16	5	15	11	11	9	12	6
Difference	14		22		40		31		45	

	22	73	147	205	268	324	429	477	536	581
10	23	75	141	201	279	347	423	467	535	588
	24	74	176	208	285	344	404	451	517	586
Mean	23	74	155	205	277	338	419	465	529	585
Standard Deviation	1	1	19	4	9	13	13	13	11	4
Difference	51		50		61		46		56	

The difference between the mean values for each microbial concentration is an indication that the microbes were emitting fluorescence. The difference in values for no FDA and FDA are larger than the standard deviation of each mean RFU reading per microbial concentration.

Table 19: Fluorescence after 10 minutes of diluted EM Pro-Soil concentrations between 0 and 1.25E+05 cells/mL with and without fluorescein diacetate for part of FDA optimisation.

Time	0 cells/ml		3.13E+04 cells/ml		6.25E+04 cells/ml		9.38E+04 cells/ml		1.25E+05 cells/ml	
	no FDA	FDA	no FDA	FDA	no FDA	FDA	no FDA	FDA	no FDA	FDA
(min)	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU	RFU
	22	45	38	54	50	68	67	79	81	90
10	24	47	38	57	50	67	65	76	80	83
	21	44	35	55	50	66	63	78	80	86
Mean	22	45	37	55	50	67	65	78	80	86
StdDev	2	2	2	2	0	1	2	2	1	4
Difference	23		18		17		13		6	

The difference between the no FDA and FDA RFU readings are smaller for the 1000 times diluted EM Pro-Soil compared to the 100 times diluted EM Pro-Soil run. Yet, it remains significant (larger than the standard deviation of the respective mean fluorescence values. Therefore, it was concluded that the microbes were fluorescing.

In the process of optimising the FDA analysis in the background of coal waste, it was assumed that sterilisation of malt residue were effective in destroying all microbial activity. Diluted concentrations of EM Pro-Soil ranging between 0 and 1.25E+06 microbial cells/mL were used in the background of ultrafine coal tailings (controls had no added coal waste) to an average of 0.05 g per Eppendorf tube. The results for fluorescence per gram CW was determined from dry weight analysis and are presented in the figure below.

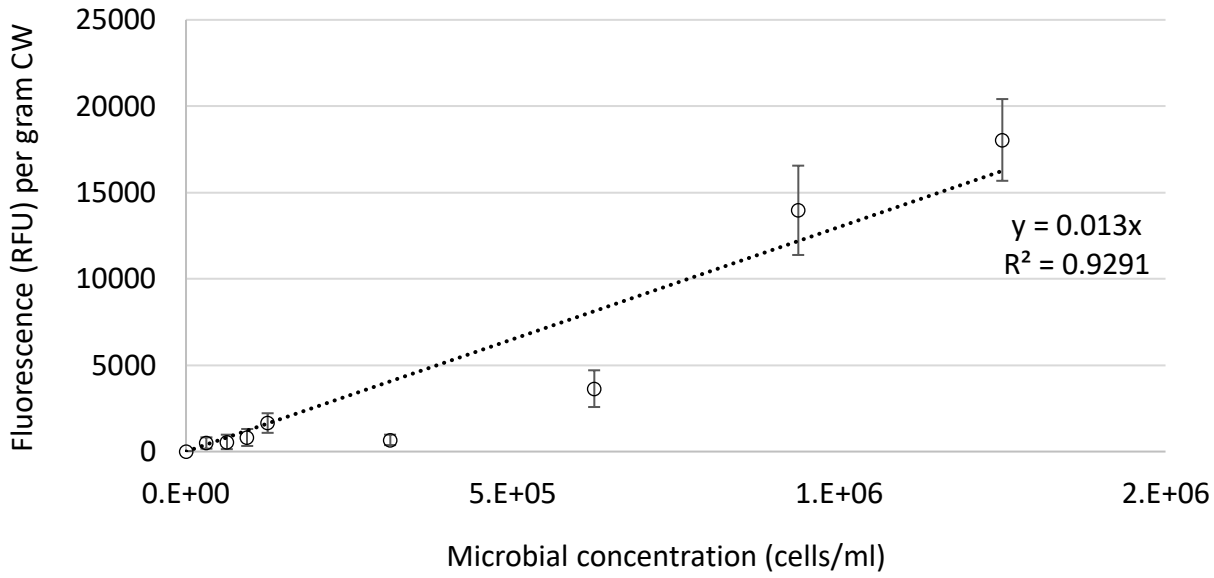

Figure 52: FDA optimisation in the background of CW.

APPENDIX B: FEASIBILITY STUDY ON PLANT GROWTH TRIAL DATA

A preliminary plant growth trial was performed. The results for the germination period (20 days) are presented in the table below. In this plant growth experiments on teff germination in various Technosols, MR dosages more than 2.5% (w/w) delayed and decreased teff germination, whereas the highest germination rate was in pure CW Technosols (refer to the table below). From initial material characterisation, MR application was expected to increase water retention and ameliorate the Technosol structure. However, high MR dosages structurally impeded teff germination. This hypothesis was evaluated by performing a plant growth experiment where teff seeds were sown on the soil surface as opposed to the initial method (at a depth of 1 cm from the surface) in inoculated and non-inoculated MR-amended Technosols with and without bioaugmentation. As expected, results showed improved germination rates in all Technosols, with 100% and 90% germination rates on the third day in CW+MR5% and CW+MR7%, respectively, thereby, supporting the hypothesis. This lead to the development of the single-seed sowing process (illustrated in Figure 14) to eliminate this variable when investigating Technosol health for teff growth in the initial and final greenhouse studies. In support, the surface sowing method can easily be implemented on a large scale.

Table 20: Average rate of teff seedling emergence for 20 days during the preliminary growth trial in coal-based Technosols with compost as the control. Where, I represents inoculated treatments and NI represents the non-inoculated controls.

	100% CW		CW+MR2.5%		CW+MR5%		CW+MR7%		Control
Day	I	NI	I	NI	I	NI	I	NI	NI
0	0%	0%	0%	0%	0%	0%	0%	0%	0%
1	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	33%	43%	26%	23%	5%	6%	2%	4%	8%
4	36%	50%	33%	28%	6%	7%	2%	10%	21%
5	40%	51%	37%	26%	7%	7%	4%	15%	25%
6	42%	54%	38%	27%	8%	7%	4%	17%	26%
7	53%	64%	39%	29%	8%	8%	5%	21%	33%
8	57%	57%	39%	30%	8%	8%	4%	21%	53%
9	61%	60%	39%	31%	8%	6%	4%	21%	65%
10	69%	61%	38%	32%	8%	7%	5%	20%	71%
11	71%	65%	38%	33%	9%	10%	6%	21%	76%
12	73%	65%	37%	33%	10%	8%	5%	19%	76%
13	73%	66%	34%	32%	9%	8%	4%	18%	78%
14	73%	66%	33%	31%	8%	8%	4%	20%	79%
15	73%	66%	29%	27%	7%	5%	3%	17%	89%
16	74%	67%	29%	25%	6%	5%	3%	16%	95%
17	74%	65%	27%	21%	4%	3%	2%	13%	95%
18	74%	65%	26%	20%	4%	3%	2%	12%	95%
19	72%	65%	22%	18%	3%	0%	0%	4%	95%
20	71%	65%	20%	16%	0%	0%	0%	4%	95%

A small sub-test was performed to evaluate the effect of malt residue application on the rate of seed germination. One pot per bioaugmented Technosol type with teff seeds sown on the soil surface (using the single-seed sowing method) was used, with non-inoculated treatments as controls. The results for teff seedling germination (20 day period) are presented in the table below.

Table 21: Teff germination test over a 20 day period to evaluate the effect of malt residue application on teff germination in coal-based Technosols with and without bioaugmentation if seeds are sown on the soil surface.

	CW+MR2.5%		CW+MR5%		CW+MR7%	
Day	I	NI	I	NI	I	NI
0	0%	0%	0%	0%	0%	0%
1	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%
3	0%	50%	100%	90%	90%	50%
4	10%	50%	100%	100%	90%	80%
5	10%	50%	100%	100%	90%	90%
6	10%	50%	100%	100%	90%	90%
7	10%	50%	100%	100%	90%	90%
8	10%	50%	100%	100%	90%	90%
9	10%	50%	90%	100%	100%	90%
10	10%	50%	90%	80%	100%	90%
11	10%	50%	90%	80%	100%	90%
12	10%	10%	20%	40%	30%	90%
13	10%	10%	20%	40%	30%	90%
14	10%	10%	20%	40%	30%	90%
15	10%	10%	20%	20%	30%	90%
16	10%	10%	20%	20%	30%	90%
17	10%	10%	20%	20%	30%	90%
18	10%	10%	10%	10%	20%	90%
19	10%	10%	10%	10%	20%	90%
20	10%	10%	10%	10%	20%	90%

APPENDIX C: INITIAL PLANT GROWTH TRIAL DATA

Table 22: Average seedling emergence as a percentage of the number of seeds planted on day zero in MR amended coal-based Technosols and compost as the control, in the initial growth trial in summer. Where, I is inoculated and NI represents non-inoculated treatment types. Major changes; relocation to the greenhouse on day 8 are represented by dotted lines.

	CW+MR2.5%		CW+MR5%		CW+MR7%		Control
Day	I	NI	I	NI	I	NI	NI
0	0%	0%	0%	0%	0%	0%	0%
1	35%	25%	8%	28%	5%	20%	0%
2	98%	78%	78%	95%	70%	80%	0%
3	98%	88%	90%	98%	75%	85%	13%
4	100%	88%	95%	100%	78%	85%	13%
5	100%	88%	95%	100%	78%	85%	13%
6	98%	88%	98%	100%	78%	90%	13%
7	98%	88%	95%	100%	78%	88%	13%
8	98%	88%	95%	98%	73%	88%	13%
9	98%	88%	93%	98%	73%	80%	13%
10	98%	88%	93%	98%	73%	80%	13%
11	90%	78%	80%	70%	35%	53%	13%
12	88%	75%	78%	58%	30%	30%	13%
13	78%	70%	58%	38%	15%	15%	13%
14	73%	68%	55%	35%	13%	13%	18%
15	70%	65%	50%	33%	10%	13%	38%
16	70%	65%	48%	30%	10%	13%	53%
17	68%	63%	43%	30%	10%	13%	53%
18	68%	63%	43%	30%	10%	13%	50%
19	68%	63%	43%	30%	10%	13%	50%
20	68%	63%	43%	30%	10%	13%	50%

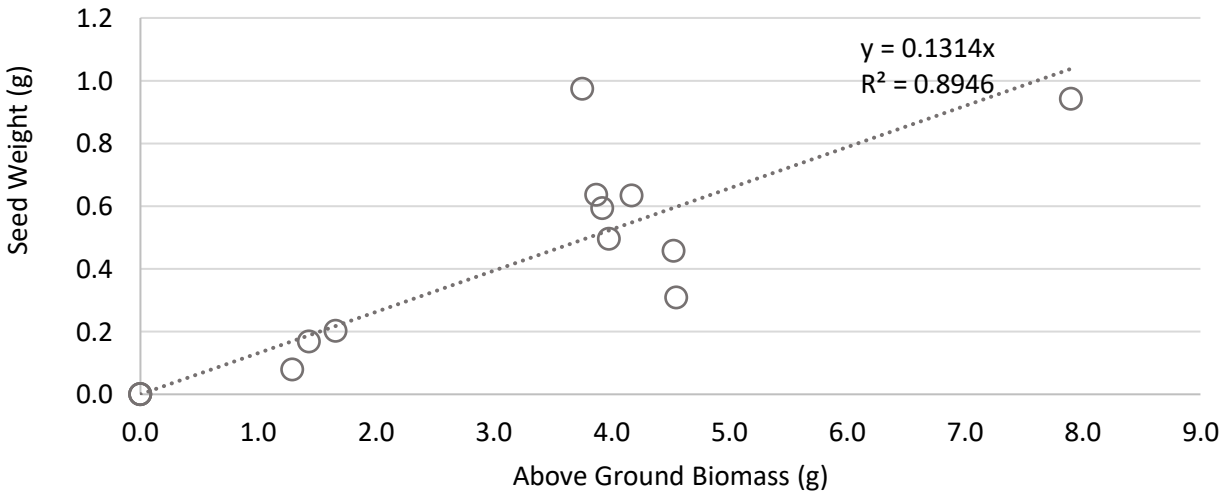

Figure 53: Teff seed productivity as a function of above ground teff dry biomass cultivated in all coal-based Technosols with increasing dosages of MR as amendment (2.5%, 5%, 7%) in the initial plant growth cycle in summer.

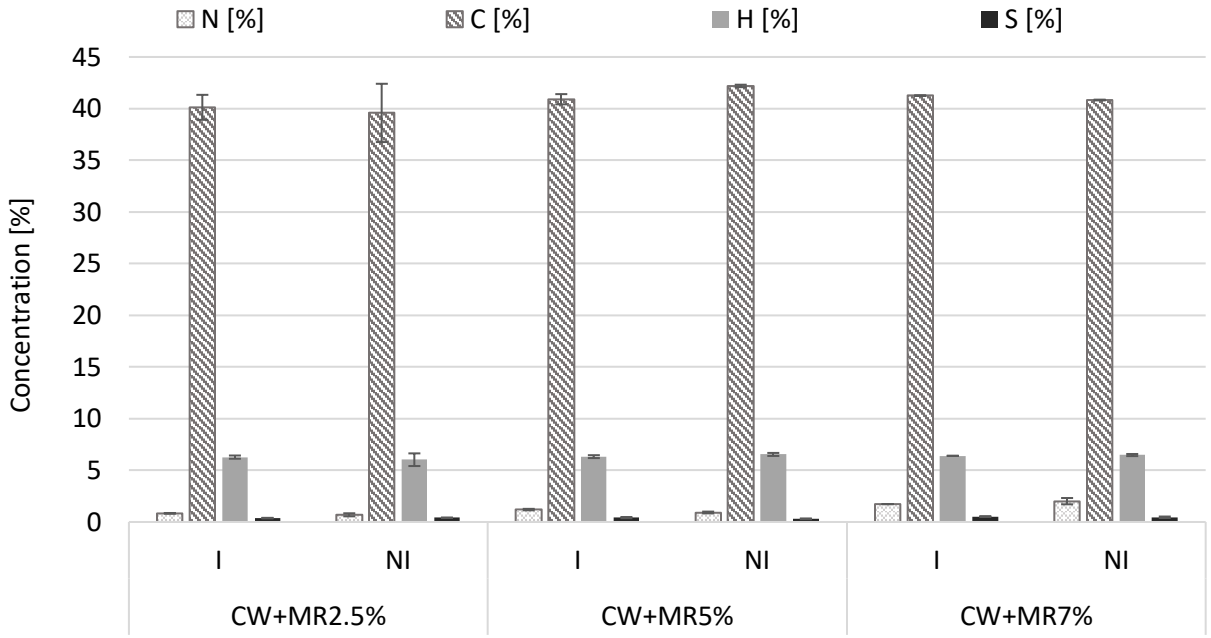

Figure 54: CHNS characterisation (expressed as percentage) in above ground teff dry biomass cultivated in MR amended coal-based Technosols. Where, I is inoculated and NI represents non-inoculated treatment types.

APPENDIX D: FINAL PLANT GROWTH TRIAL DATA

Table 23: Average rate of teff seedling emergence (expressed as percentage) per coal-based Technosol and potting soil as the control relative to the number of seeds initially planted per pot (n=20) during the final growth trial in winter. Dotted lines indicate major changes; the relocation from the laboratory to the greenhouse (day 8) and seedling removal (day 20). Where, I represents inoculated and NI is non-inoculated treatments.

	100% CW		CW+MR2.5%		CW+MR5%		CW+MR7%		Control
Day	I	NI	I	NI	I	NI	I	NI	NI
0	0%	0%	0%	0%	0%	0%	0%	0%	0%
1	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	0%	0%	0%	0%	0%	0%	0%	0%	0%
4	0%	0%	0%	0%	0%	0%	0%	0%	0%
5	0%	0%	0%	0%	0%	0%	0%	0%	0%
6	94%	89%	83%	93%	88%	96%	74%	85%	95%
7	94%	90%	83%	91%	89%	95%	68%	85%	95%
8	91%	76%	81%	86%	85%	95%	51%	84%	95%
9	90%	69%	81%	85%	83%	95%	44%	83%	92%
10	90%	69%	81%	85%	83%	95%	44%	83%	92%
11	90%	69%	81%	85%	83%	95%	44%	83%	92%
12	90%	69%	81%	85%	83%	95%	44%	83%	92%
13	90%	69%	80%	85%	81%	95%	40%	83%	93%
14	88%	69%	74%	85%	79%	95%	36%	80%	93%
15	83%	69%	74%	84%	78%	95%	35%	81%	93%
16	83%	69%	70%	81%	75%	94%	35%	81%	93%
17	83%	66%	70%	81%	75%	94%	35%	80%	93%
18	83%	66%	70%	81%	75%	94%	35%	80%	93%
19	83%	66%	70%	81%	75%	94%	35%	80%	93%
20	83%	66%	100%	100%	100%	100%	88%	100%	94%

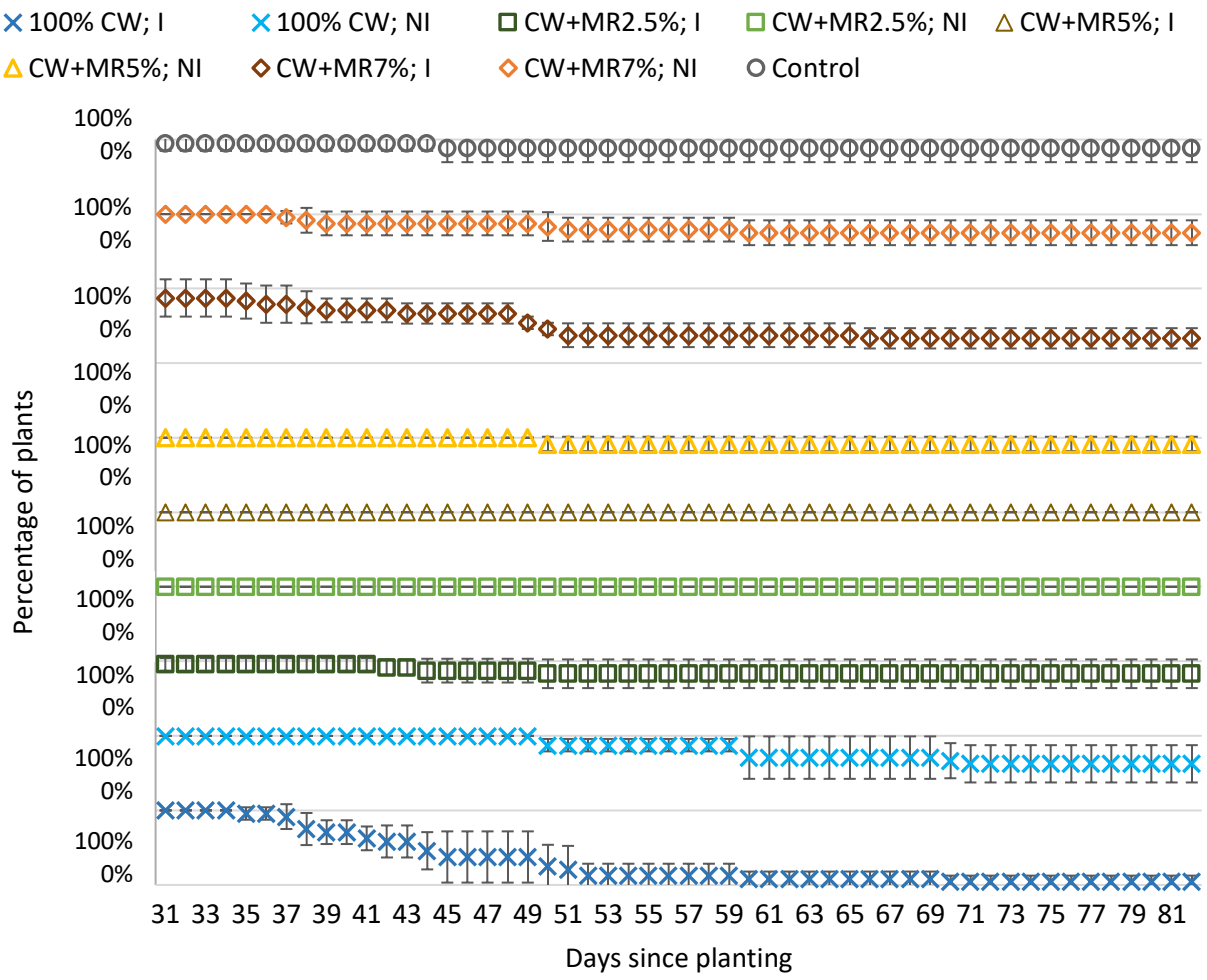

Figure 55: Average percentage of teff plants per pot in each coal-based Technosol and potting soil as the control, during the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

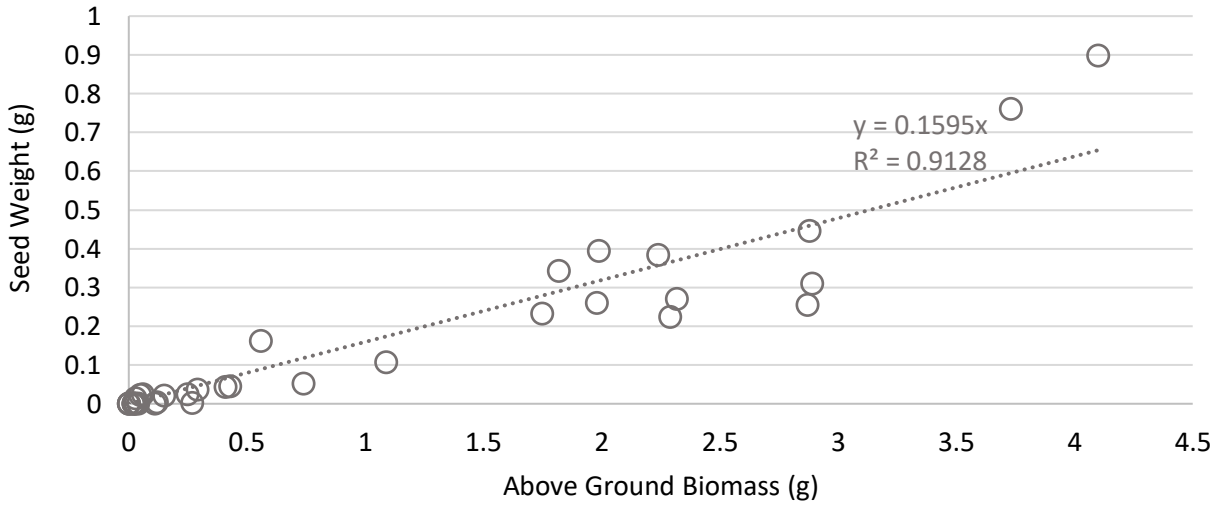

Figure 56: Teff seed production (g) as a function of above ground teff dry biomass in all coal-based Technosols with increasing dosages of MR as amendment (2.5%, 5%, 7%) in the final plant growth trial in winter.

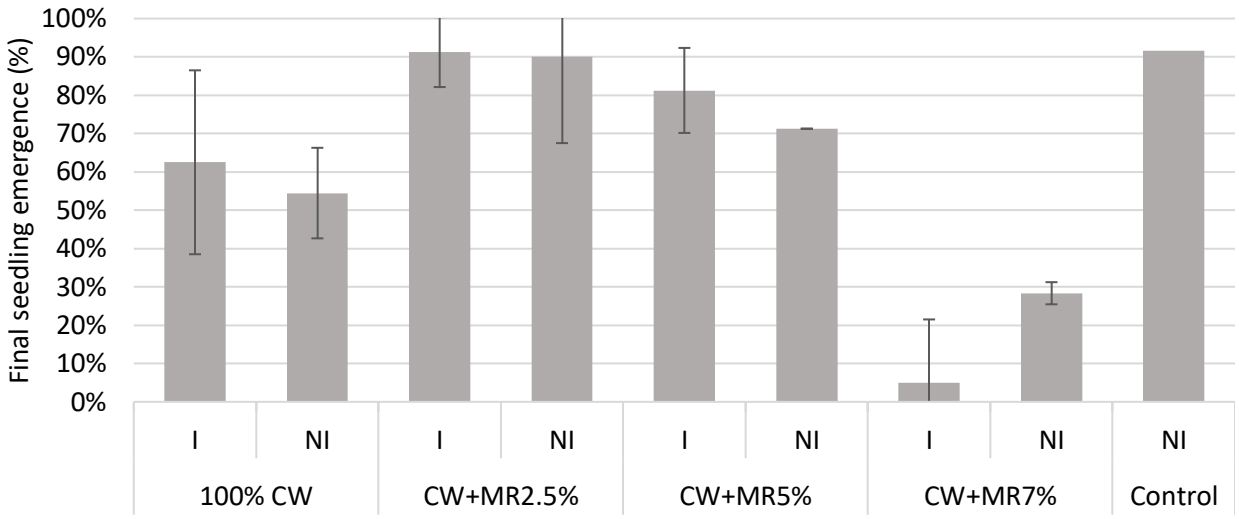

Figure 57: Teff seedling emergence (expressed as percentage) relative to the number of seeds sown that was achieved after 14 days in pure coal waste. Seeds were collected from above ground teff biomass cultivated in all coal-based Technosols in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

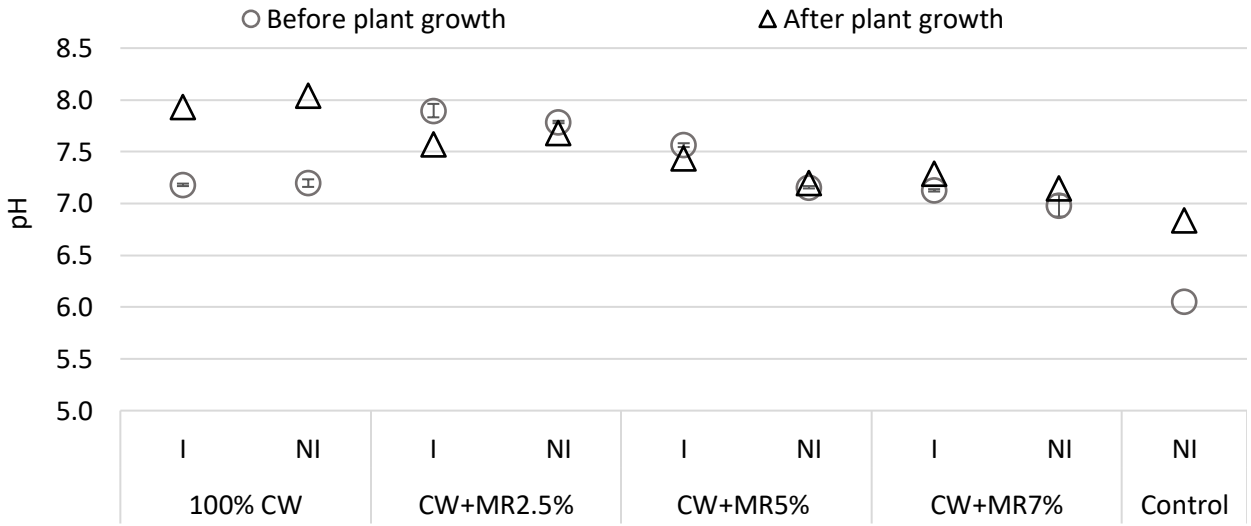

Figure 58: Average pH values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

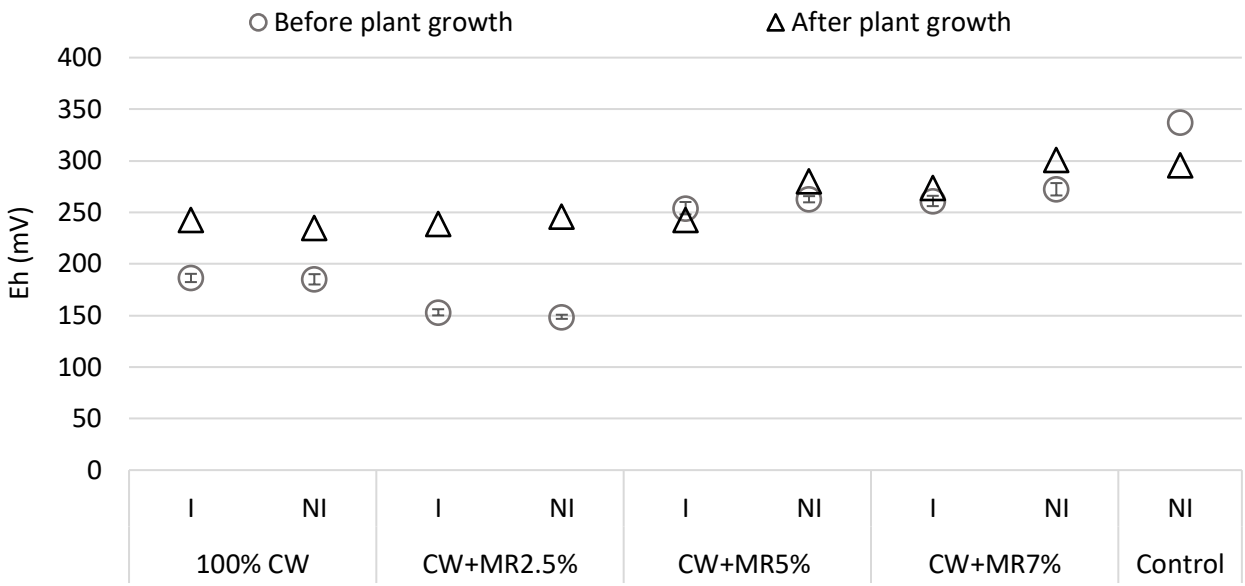

Figure 59: Average redox potential (Eh) values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

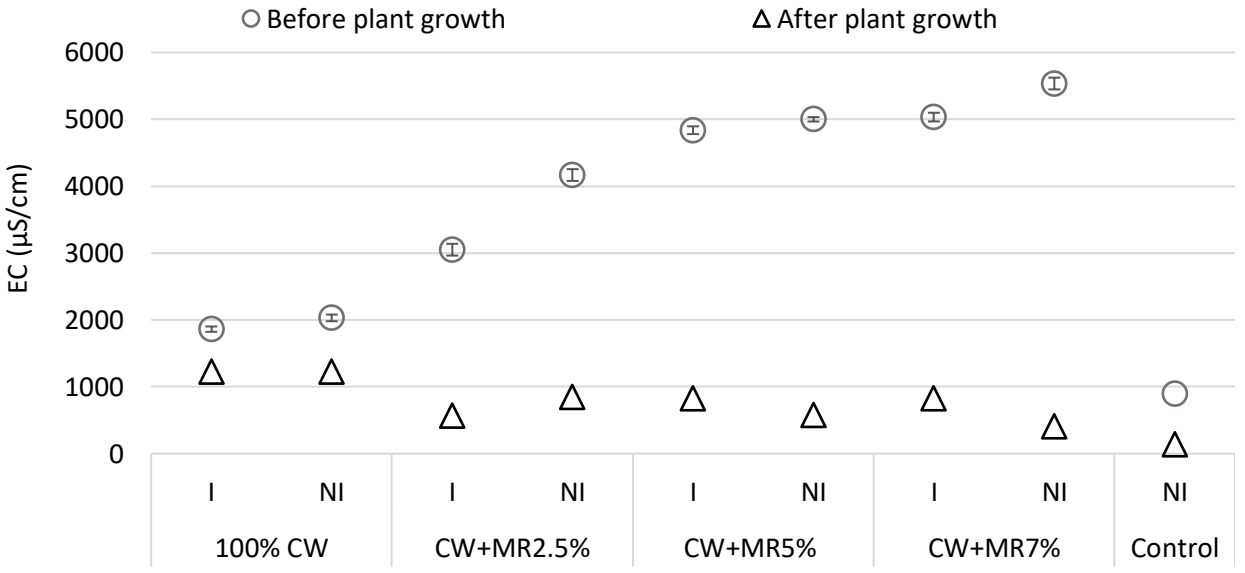

Figure 60: Average electrical conductivity (EC) values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter.

Table 24: Physiochemical characteristics of non-vegetated coal-based Technosols, with and without bioaugmentation, and potting soil as the control, after 82 days in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

		100% CW		CW+MR2.5%		CW+MR5%		CW+MR7%		Control
Parameter	Unit	I	NI	I	NI	I	NI	I	NI	NI
pH	KCl	7.5	7.3	7.2	7.2	6.8	7.3	6.5	7.1	5.8
Resist.	(ohm)	320	400	280	380	310	290	320	310	680
T Value	cmol/kg	36	36	36	37	32	30	27	30	38

CEC	mg/kg	13	10	13	10	17	12	14	14	19
Stone	Vol %	1	1	1	1	1	1	1	1	2
C	%	50	45	47	46	51	54	51	54	35
TN	%	1	1	1	1	1	1	1	1	0
P (Bray II)	mg/kg	13	7	40	39	84	39	116	64	51
K	mg/kg	184	28	210	34	200	47	152	51	387
S	mg/kg	884	1360	768	722	1160	630	1080	677	75
Ca^{2+}	cmol/kg	33.2	34.4	32.4	34.4	29.0	27.6	24.0	28.0	24.6
Mg^{2+}	cmol/kg	1.6	1.7	2.0	1.8	2.3	2.0	1.9	1.8	10.8
K^{+}	cmol/kg	0.5	0.1	0.5	0.1	0.5	0.1	0.4	0.1	1.0
Na^{+}	cmol/kg	0.4	0.2	0.6	0.2	0.6	0.2	0.3	0.2	0.4
Ca	%	93.0	94.6	91.2	94.2	89.6	92.2	90.1	92.9	65.6
Mg	%	4.5	4.7	5.6	4.9	7.1	6.7	7.1	6.0	28.8
K	%	1.3	0.2	1.5	0.3	1.6	0.4	1.5	0.4	2.6
Na	%	1.2	0.5	1.7	0.7	1.7	0.7	1.3	0.7	1.2
Cu	mg/kg	2.0	2.6	3.8	3.1	2.9	3.7	4.1	4.2	4.1
Zn	mg/kg	1.7	1.4	2.6	2.3	3.2	3.1	5.7	5.1	13.7
Mn	mg/kg	23.5	21.9	23.2	23.9	23.5	23.4	23.7	24.6	69.6
B	mg/kg	0.3	0.3	0.7	0.7	0.7	0.8	0.7	0.6	0.3
Fe	mg/kg	28.7	24.3	47.4	32.8	48.7	63.0	63.4	78.7	258.0
S Am.acet	mg/kg	3280	3280	3360	3280	2890	2510	2460	2440	67

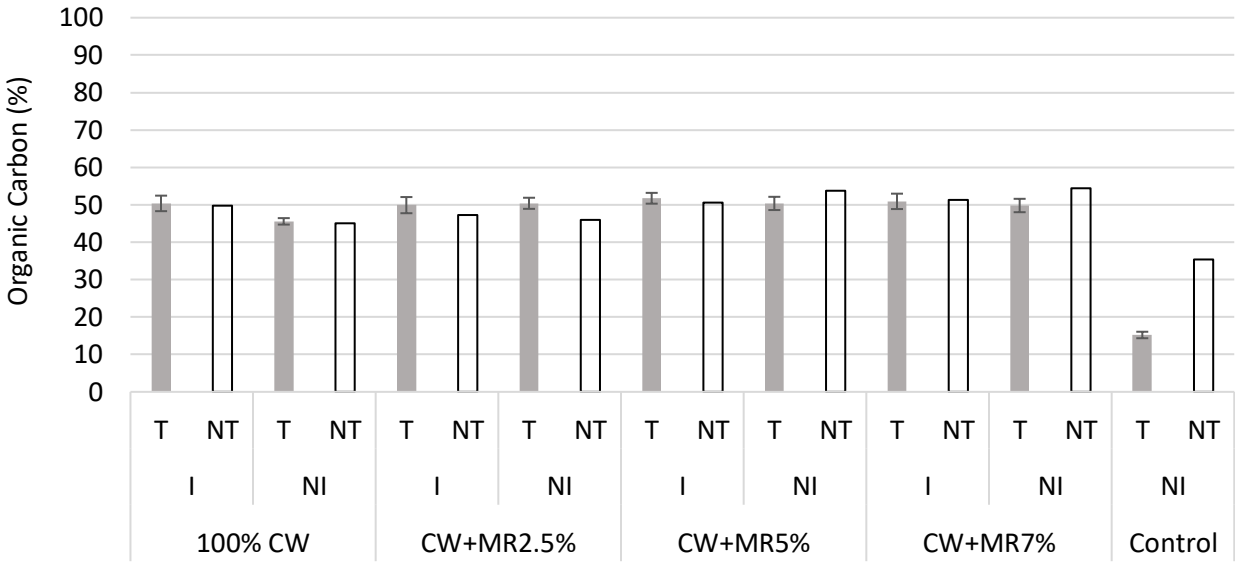

Figure 61: Percentage organic carbon (C) in vegetated (T) and non-vegetated (NT) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

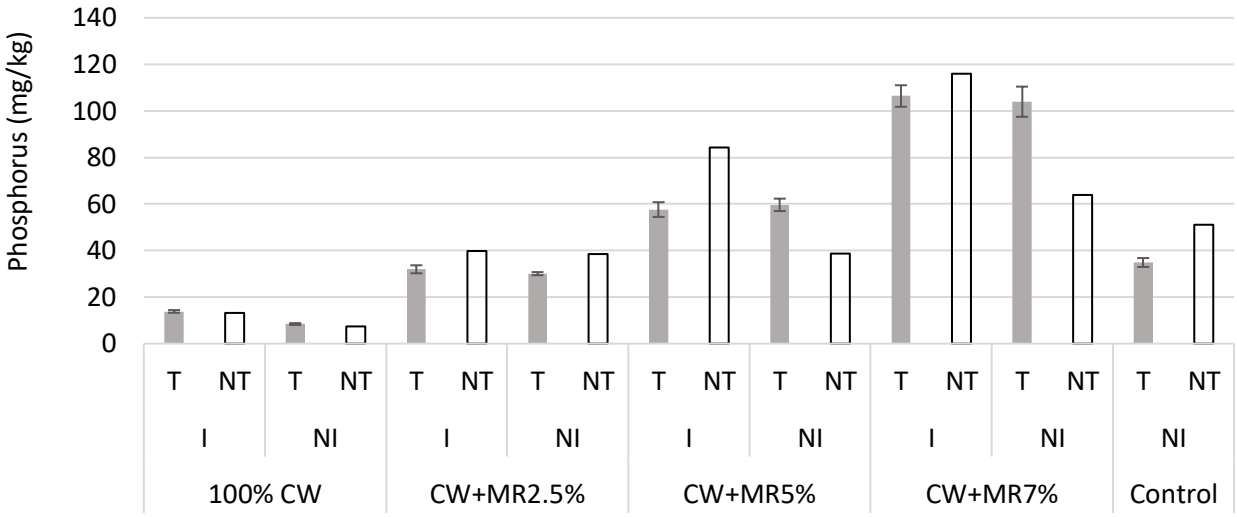

Figure 62: Concentration of phosphorus (P Bray II) in non-vegetated (NT) and vegetated (T) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

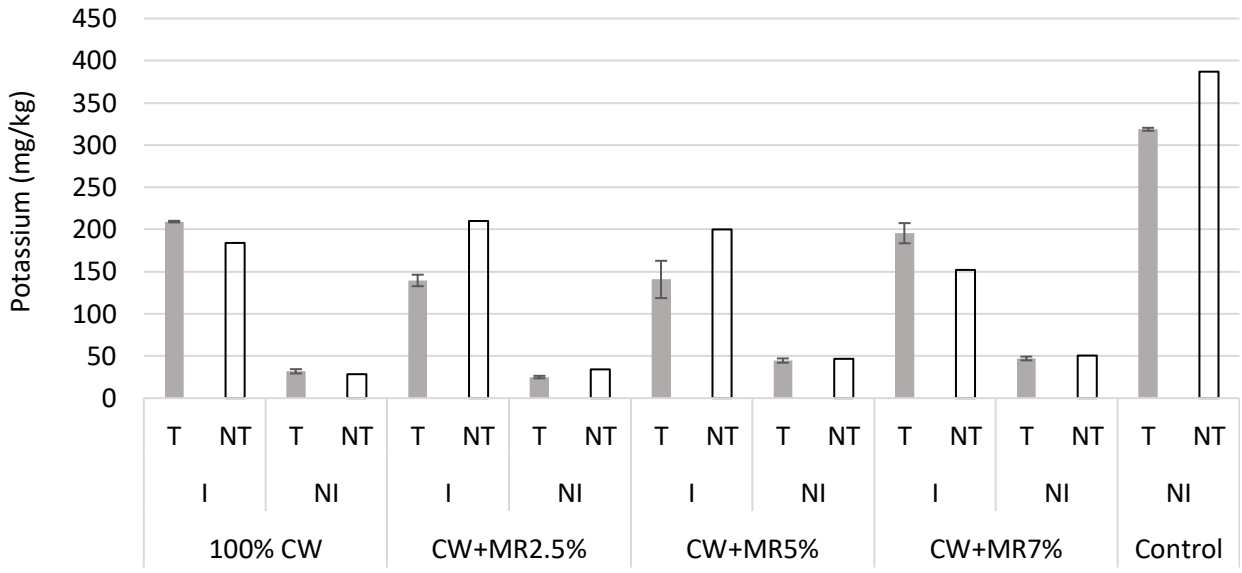

Figure 63: Concentration of potassium (K) in vegetated (T) and non-vegetated (NT) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

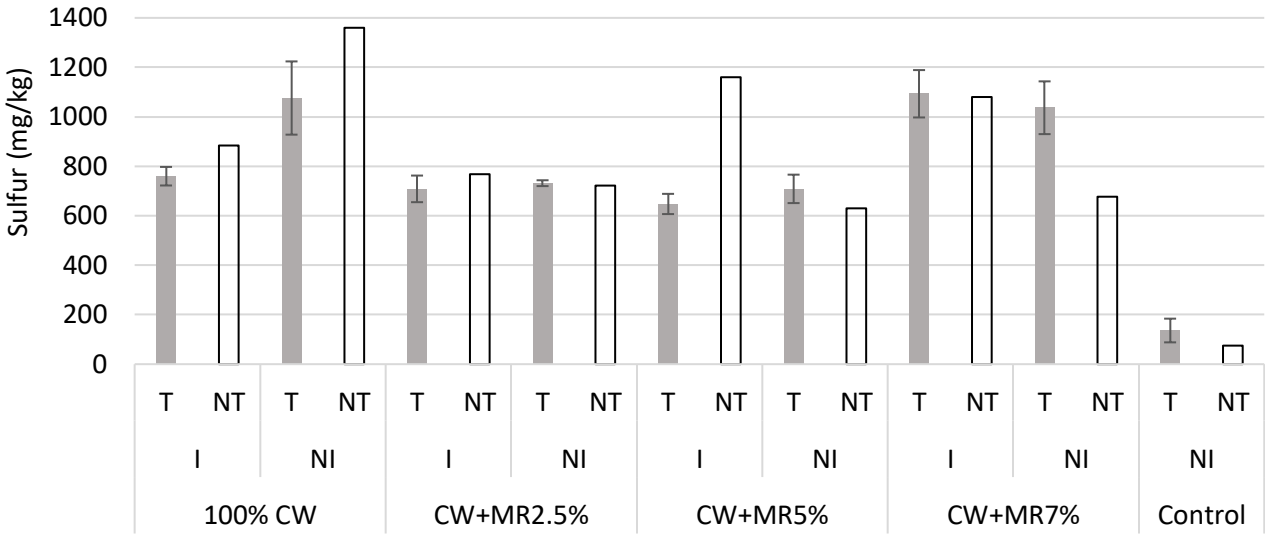

Figure 64: Concentration of sulfur (S) in vegetated (T) and non-vegetated (NT) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

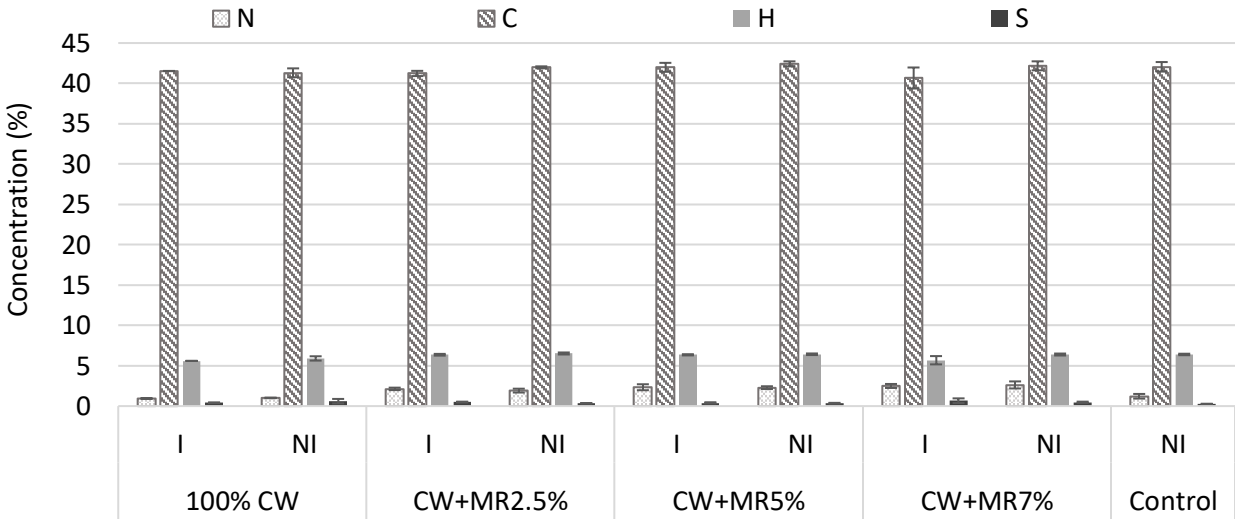

Figure 65: CHNS characterisation (expressed as percentage) in above ground teff dry biomass cultivated in coal-based Technosols and potting soil as the control in the final growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

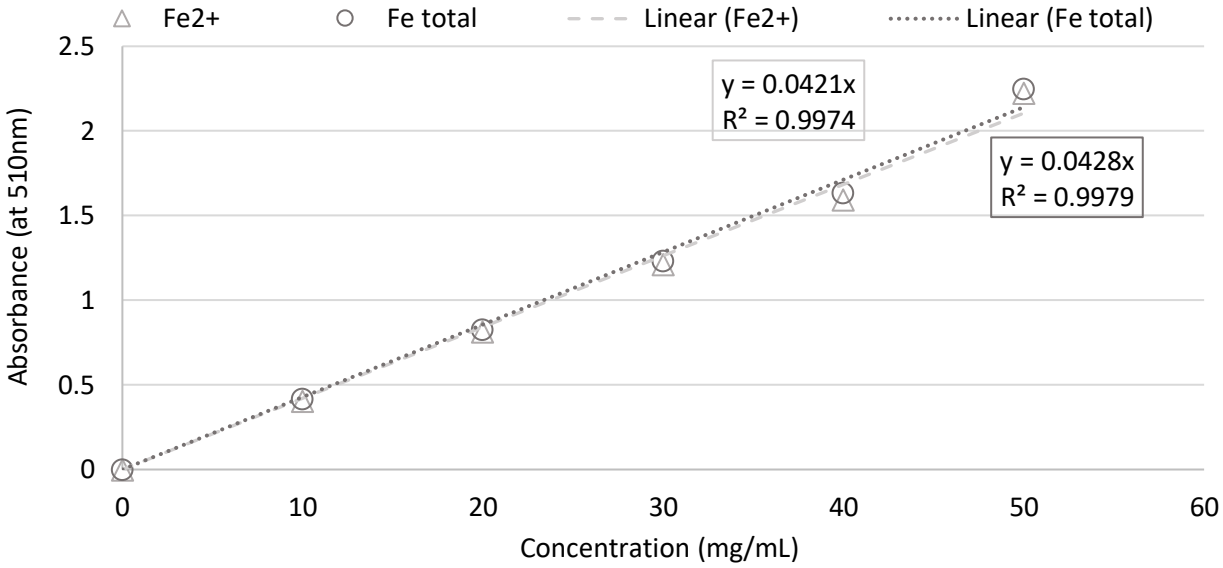

Figure 66: Standard curve for concentration in coal-based Technosol leachates measured through spectrophotometry (absorbance at 510 nm).

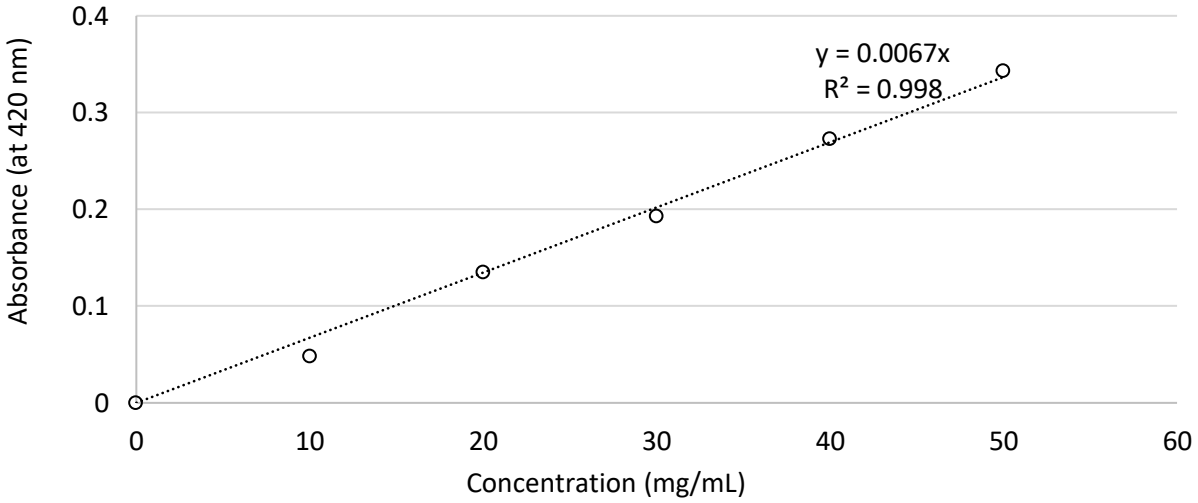

Figure 67: Standard curve for sulfate concentration in coal-based Technosol leachates measured through spectrophotometry (absorbance at 420 nm).

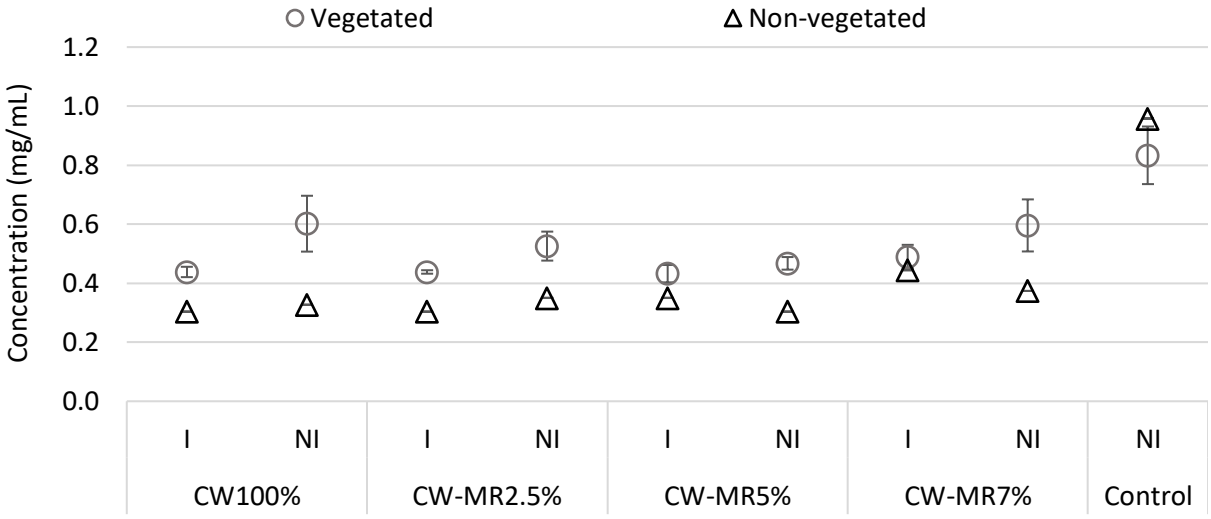

Figure 68: Average concentration of ferric iron (mg/mL) within leachates collected from vegetated and non-vegetated coal-based Technosols and potting soil as the control, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

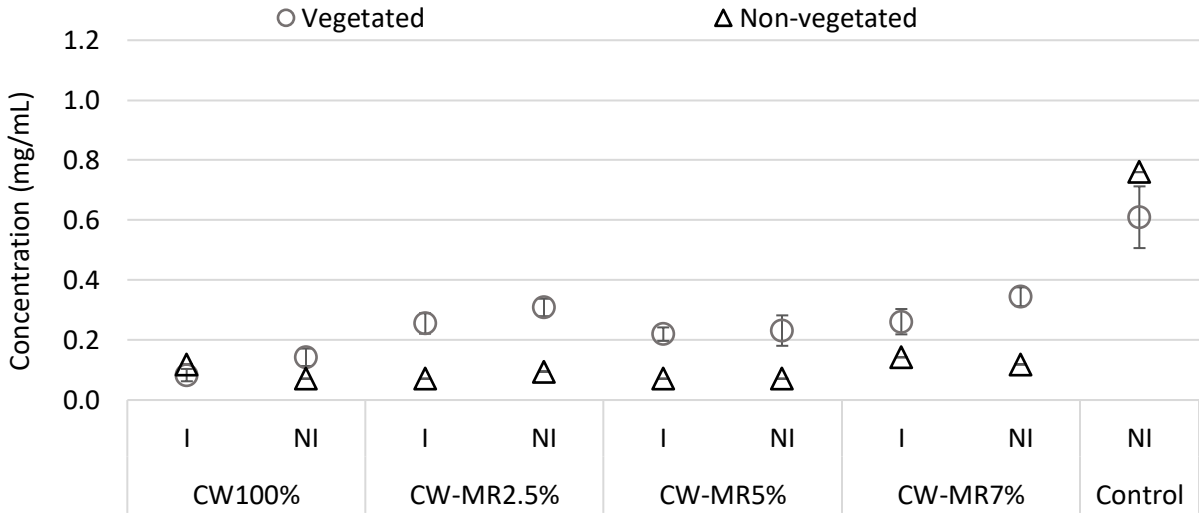

Figure 69: Average concentration of ferrous iron (mg/mL) within leachates collected from vegetated and non-vegetated coal-based Technosols and potting soil as the control, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

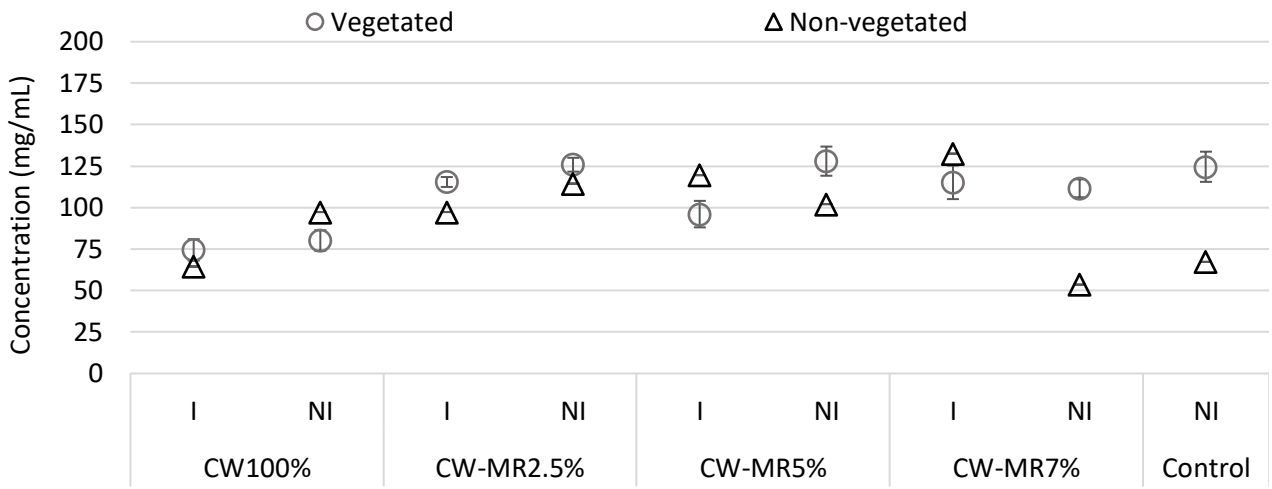

Figure 70: Average concentration sulfate (mg/mL) within leachates collected from vegetated and non-vegetated coal-based Technosols, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

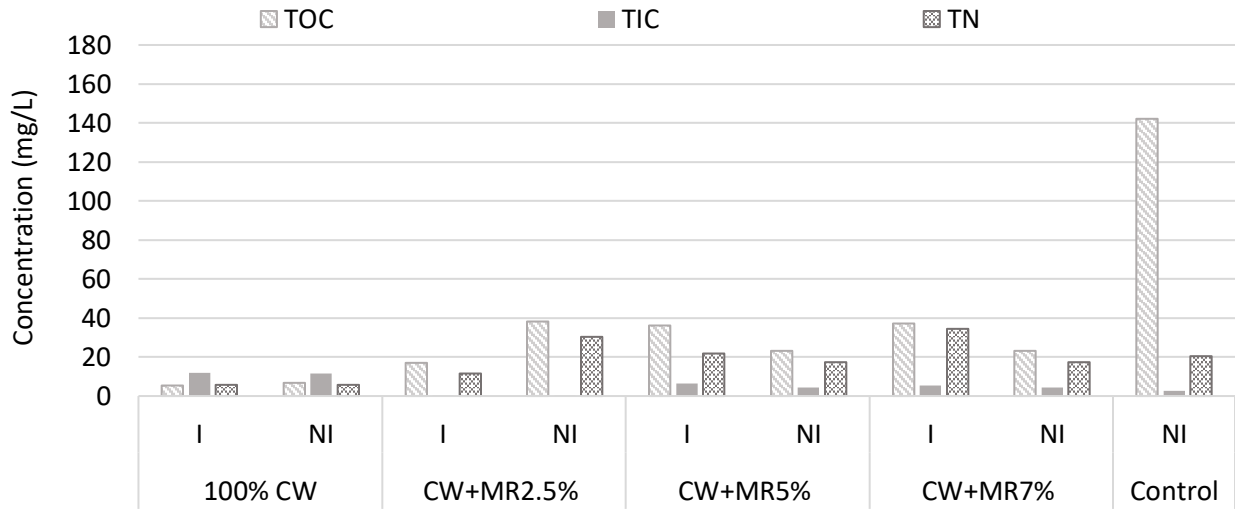

Figure 71: Total organic & inorganic carbon (TOC, TIC) and total nitrogen (TN) in non-vegetated coal-based Technosol leachates in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

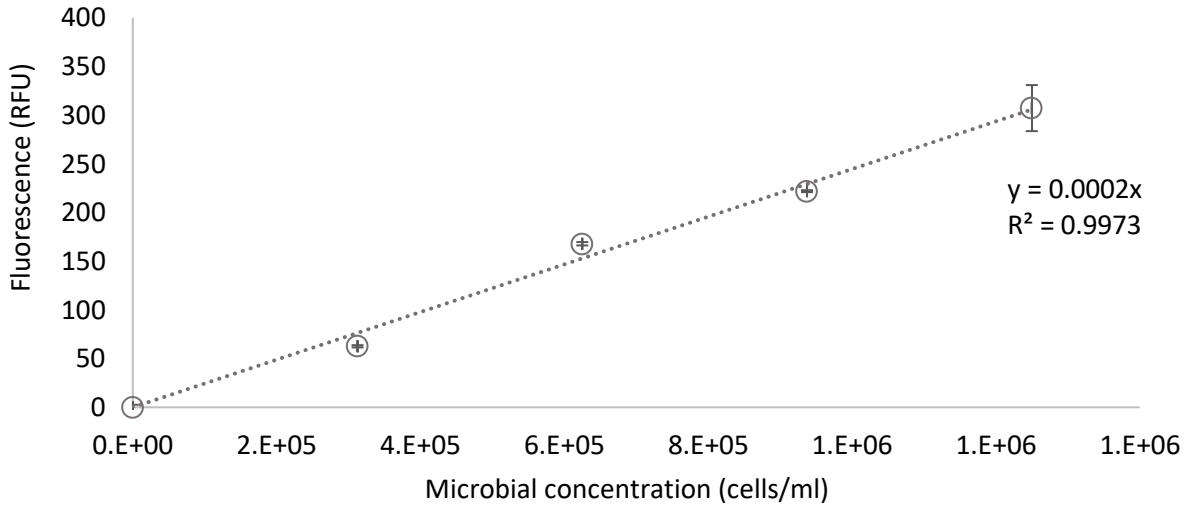

Figure 72: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 0 of inoculum activation as measured through FDA analysis.

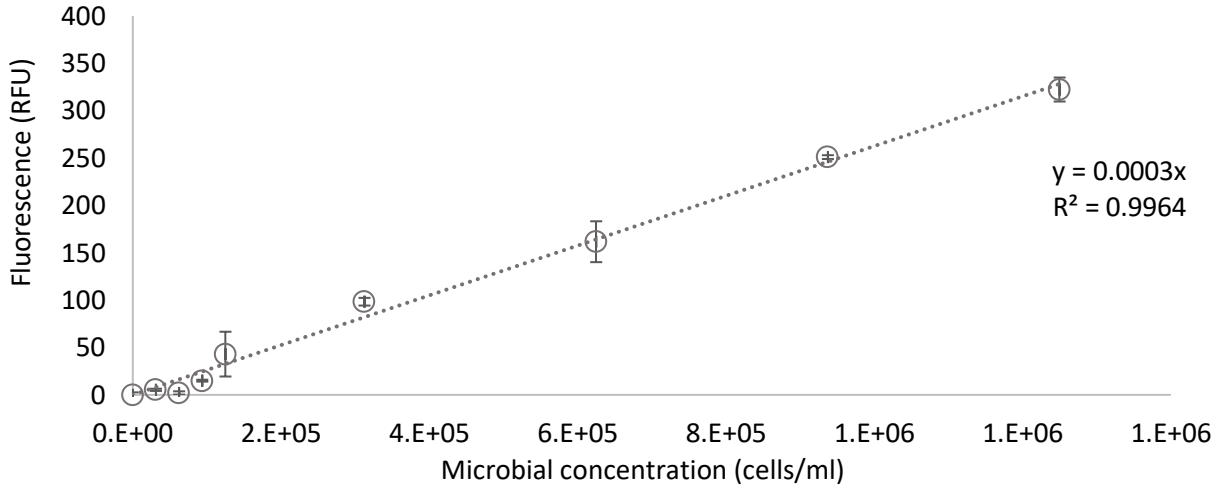

Figure 73: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 1 of inoculum activation as measured through FDA analysis.

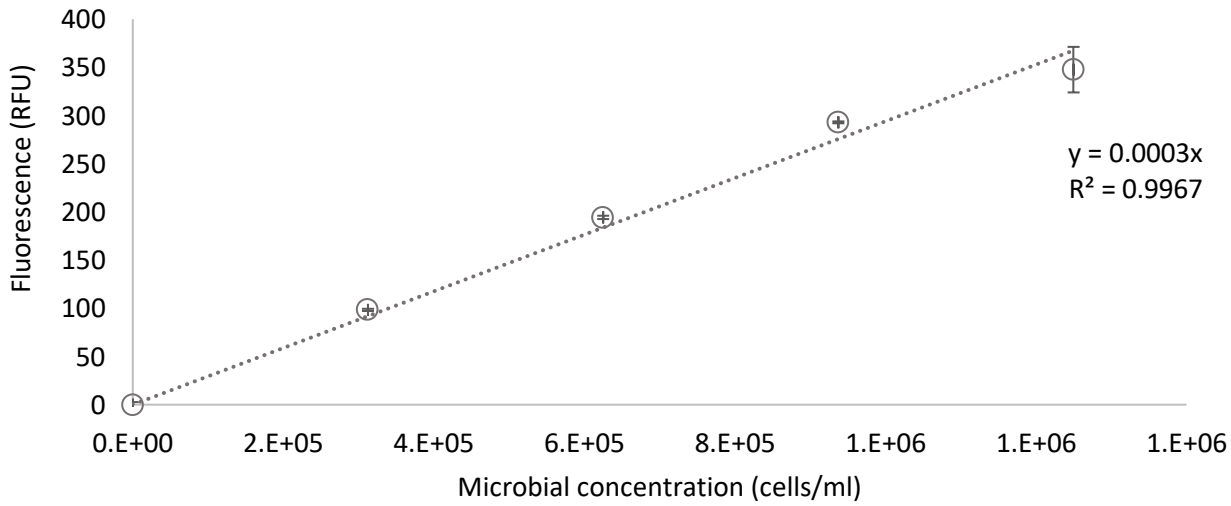

Figure 74: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 3 of inoculum activation as measured through FDA analysis.

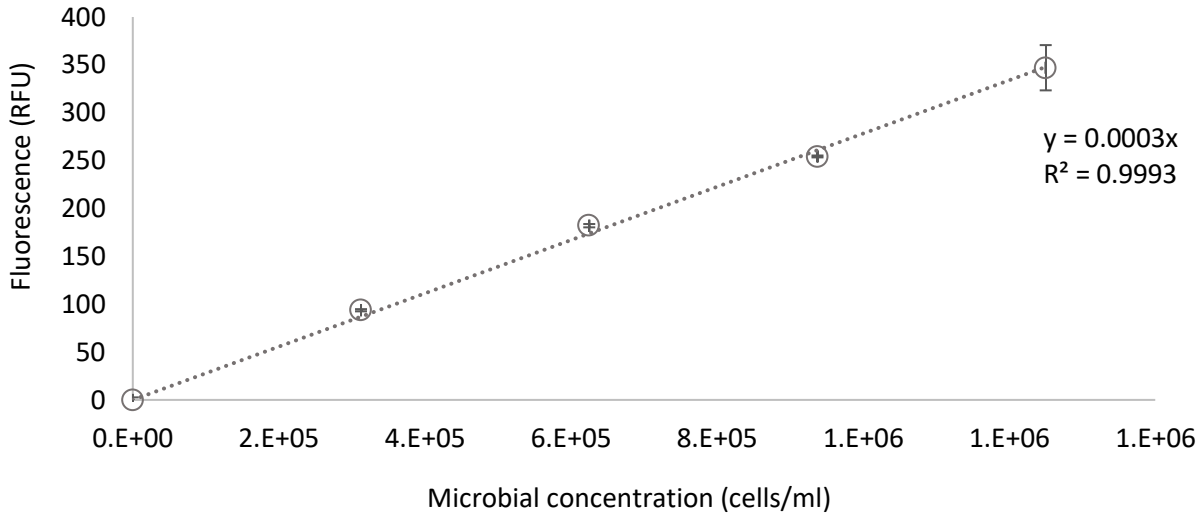

Figure 75: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 5 of inoculum activation as measured through FDA analysis.

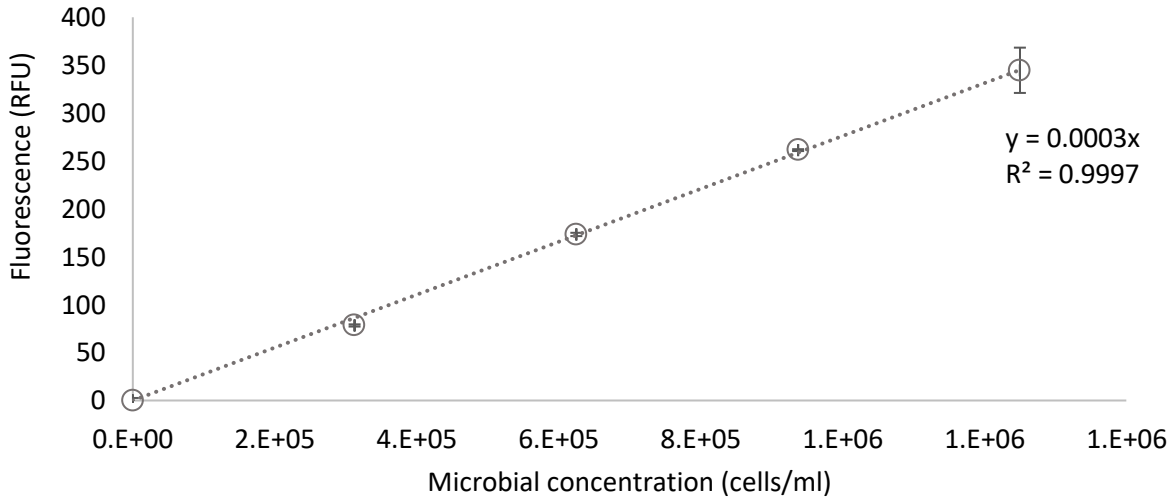

Figure 76: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 7 of inoculum activation as measured through FDA analysis.

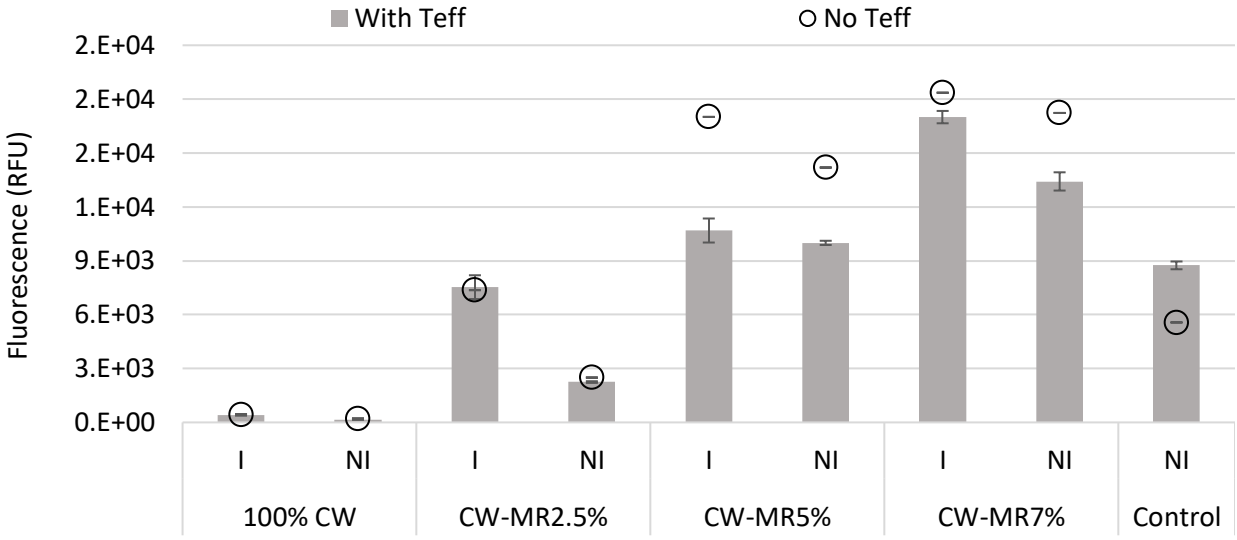

Figure 77: Average fluorescence (RFU) emitted per vegetated and non-vegetated coal-based Technosol and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Sample calculations for eluted DNA aliquot preparation:

From DNA extraction and quantitation, the double strand DNA concentration per soil sample one: c_1 = 5.2 ng/µL. To prepare aliquots for c_2 = 1 ng/ µL and a total volume of 100 µL.

Therefore, the desired amount to extract from the sample is: $m = c \times v = \left(1\frac{ng}{\mu L}\right) \times (100\ \mu L) = 100\ ng$

That amount is contained in: $m = c \times v = (100\ ng) \times \left(5.2\frac{ng}{\mu L}\right) = 19.23\ \mu L$

$\therefore (100 - 19.23)\mu L = 80.77\ \mu L$ deionised water added to make up the total volume.

Table 25: Universal bacteria, archaea, fungal, and nitrogen cycling (nirS, nirK, nosZ, nifH) 16S rRNA gene copy numbers in vegetated coal-based Technosols before and after teff growth in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

			Bacteria	Archaea	Fungi	nirS	nirK	nosZ	nifH
Before plant growth	100% CW	I	2.98E+02	5.36E+03	8.67E+03	1.51E+02	1.81E+03	2.98E+03	6.88E+01
		NI	8.45E+02	5.62E+03	9.86E+03	9.73E+01	5.16E+03	1.42E+03	2.44E+02
	CW+MR2.5%	I	1.23E+03	4.83E+03	1.45E+04	1.57E+03	2.66E+04	2.51E+03	5.30E+01
		NI	4.50E+02	4.64E+03	1.30E+04	2.84E+01	3.59E+04	2.38E+03	1.33E+02
	CW+MR5%	I	1.46E+07	3.33E+04	2.50E+06	3.16E+06	1.33E+07	1.25E+07	3.19E+07
		NI	2.31E+07	3.82E+06	2.42E+07	1.17E+06	4.36E+07	9.40E+06	3.48E+07
	CW+MR7%	I	1.27E+04	9.02E+03	5.37E+07	4.24E+02	2.47E+04	8.82E+03	5.27E+03
		NI	3.75E+06	5.68E+04	3.54E+06	5.79E+03	9.47E+04	4.34E+04	1.41E+05
	Control	NI	1.52E+08	1.82E+07	2.70E+06	1.67E+06	9.79E+07	3.72E+03	3.06E+06
After plant growth	100% CW	I	3.75E+04	6.51E+03	3.30E+04	4.18E+02	3.18E+04	3.58E+05	6.75E+02
		NI	3.64E+02	4.47E+03	7.74E+03	1.06E+03	2.51E+03	1.84E+03	1.51E+02

	CW+MR2.5%	I	1.52E+08	2.16E+05	1.83E+08	5.93E+06	1.14E+05	2.68E+07	1.46E+06
		NI	5.46E+04	3.22E+02	1.25E+04	5.79E+05	3.19E+03	3.51E+04	1.95E+02
	CW+MR5%	I	4.47E+08	3.89E+04	1.44E+08	1.42E+08	3.67E+09	5.08E+07	5.05E+05
		NI	4.77E+07	3.09E+05	1.12E+08	1.92E+06	4.09E+09	7.63E+07	2.05E+06
	CW+MR7%	I	4.83E+06	5.50E+05	1.11E+09	1.26E+06	1.63E+09	6.57E+07	2.14E+06
		NI	7.38E+04	1.18E+06	5.08E+08	4.42E+06	1.91E+09	4.76E+07	4.95E+06
	Control	NI	2.54E+05	2.43E+06	3.15E+06	1.75E+06	5.25E+09	1.81E+07	4.17E+06

Table 26: Universal bacteria, archaea, fungal, and nitrogen cycling (nirS, nirK, nosZ, nifH) 16S rRNA gene copy numbers per gram of non-vegetated coal-based Technosol before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

			Bacteria	Archaea	Fungi	nirS	nirK	nosZ	nifH
Before plant growth	100% CW	I	2.98E+02	5.36E+03	8.67E+03	1.51E+02	1.81E+03	2.98E+03	6.88E+01
		NI	8.45E+02	5.62E+03	9.86E+03	9.73E+01	5.16E+03	1.42E+03	2.44E+02
	CW+MR2.5%	I	1.23E+03	4.83E+03	1.45E+04	1.57E+03	2.66E+04	2.51E+03	5.30E+01
		NI	4.50E+02	4.64E+03	1.30E+04	2.84E+01	3.59E+04	2.38E+03	1.33E+02
	CW+MR5%	I	1.46E+07	3.33E+04	2.50E+06	3.16E+06	1.33E+07	1.25E+07	3.19E+07
		NI	2.31E+07	3.82E+06	2.42E+07	1.17E+06	4.36E+07	9.40E+06	3.48E+07
	CW+MR7%	I	1.27E+04	9.02E+03	5.37E+07	4.24E+02	2.47E+04	8.82E+03	5.27E+03
		NI	3.75E+06	5.68E+04	3.54E+06	5.79E+03	9.47E+04	4.34E+04	1.41E+05
	Control	NI	1.52E+08	1.82E+07	2.70E+06	1.67E+06	9.79E+07	3.72E+03	3.06E+06
After plant growth	100% CW	I	5.00E+02	3.90E+03	8.76E+03	1.97E+02	4.66E+03	2.62E+03	7.28E+01
		NI	3.90E+04	4.12E+02	3.98E+03	2.37E+03	1.14E+04	2.29E+03	7.65E+01
	CW+MR2.5%	I	2.63E+08	1.71E+05	3.15E+08	1.81E+07	2.29E+04	6.06E+07	1.03E+06
		NI	5.08E+06	3.99E+03	2.51E+06	1.05E+05	1.54E+07	4.46E+05	1.35E+04
	CW+MR5%	I	2.06E+08	3.74E+05	1.34E+09	3.86E+06	2.25E+09	6.39E+07	1.58E+06
		NI	6.70E+04	1.58E+05	6.32E+08	1.16E+06	5.08E+09	1.65E+08	1.17E+06
	CW+MR7%	I	1.35E+09	1.59E+06	1.26E+09	8.34E+06	2.28E+09	1.14E+08	2.72E+06
		NI	8.96E+04	5.33E+03	6.77E+08	1.24E+06	3.00E+09	3.38E+04	2.88E+06
	Control	NI	6.87E+04	7.64E+04	3.38E+06	1.83E+06	4.56E+09	2.39E+07	3.27E+06

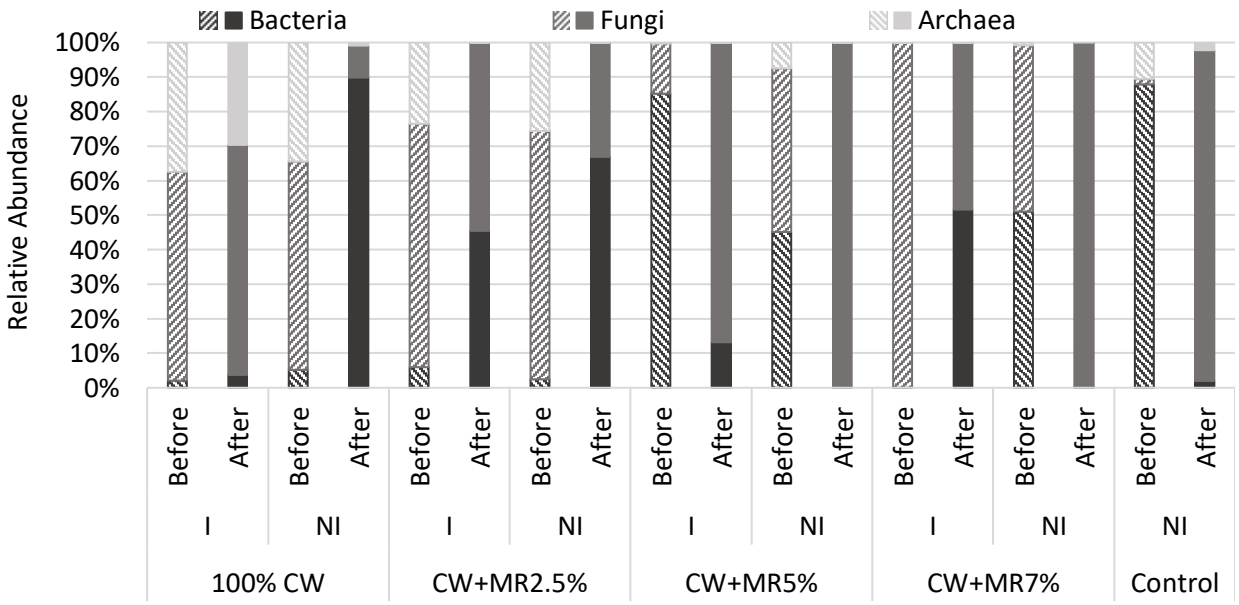

Figure 78: Relative abundances (expressed as percentage) of bacteria, archaea and fungi gene copy numbers in non-vegetated coal-based Technosols before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

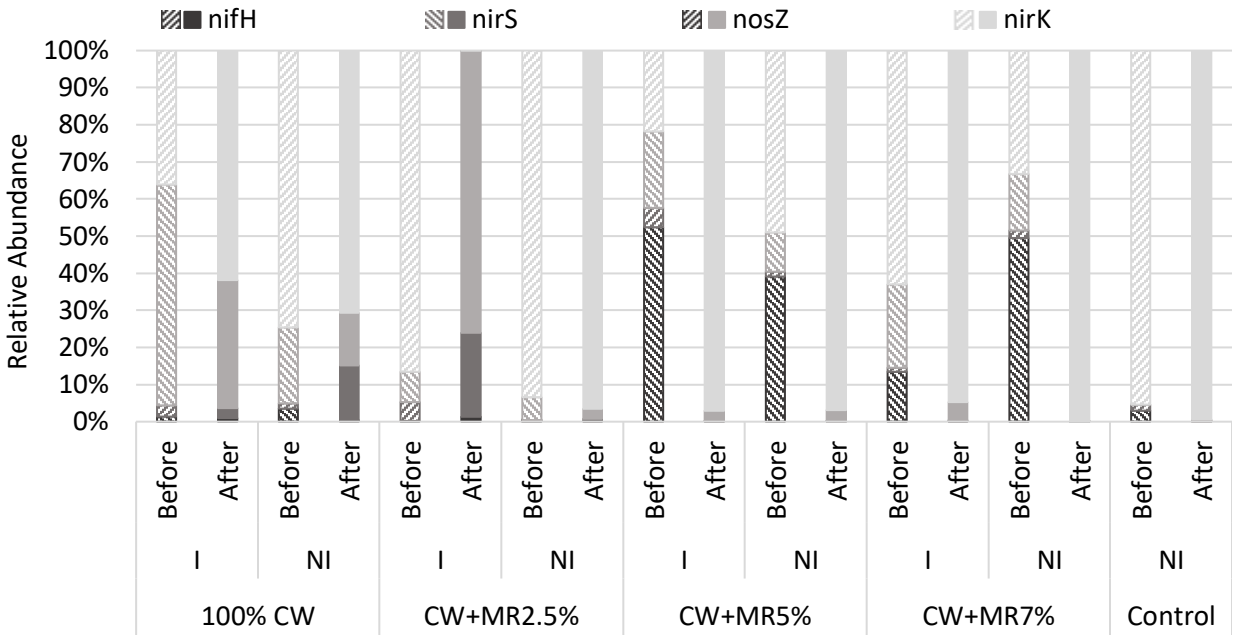

Figure 79: Relative abundances (expressed as percentage) of nitrogen cycling genes (nifH, nirS, nosZ, nirK) in non-vegetated coal-based Technosols before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

APPENDIX E: PLANT GROWTH TRIALS IN COMPARISON

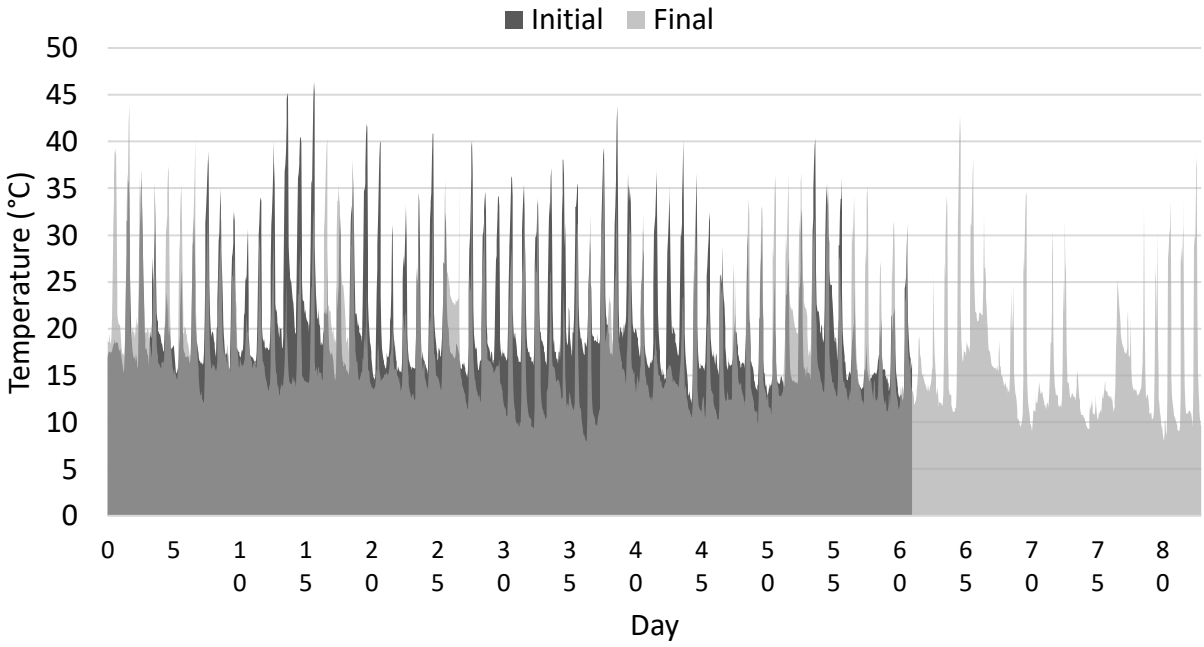

Figure 80: Greenhouse temperature throughout both plant growth trials from day 0 (day of planting) until the last day (day 82) of the final plant growth trial.

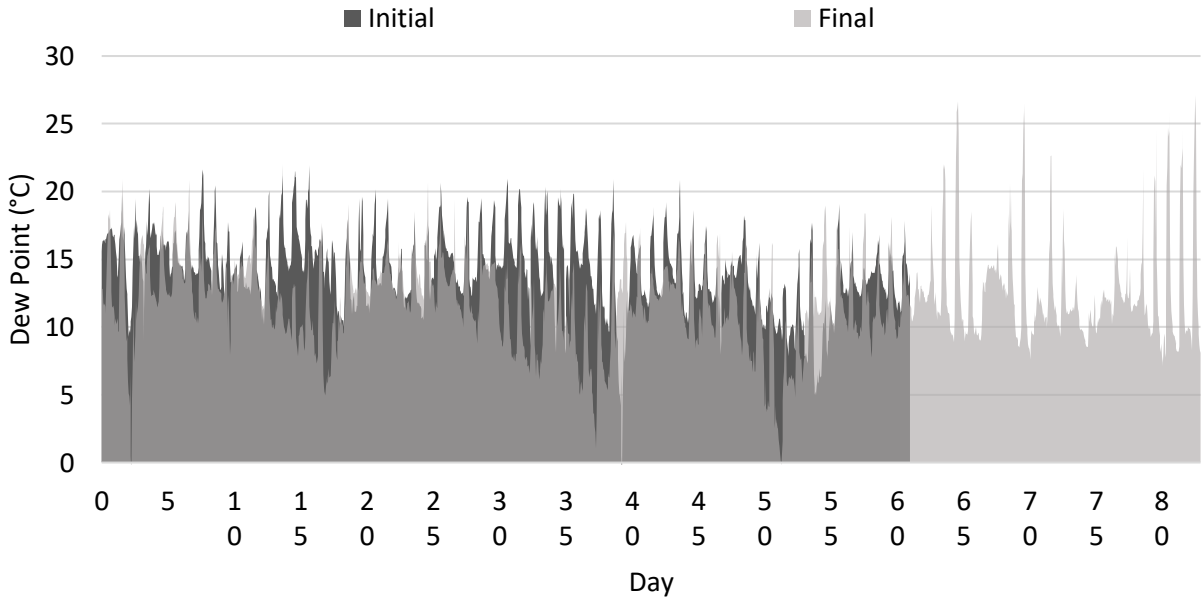

Figure 81: Daily greenhouse dew point conditions throughout both plant growth trials.

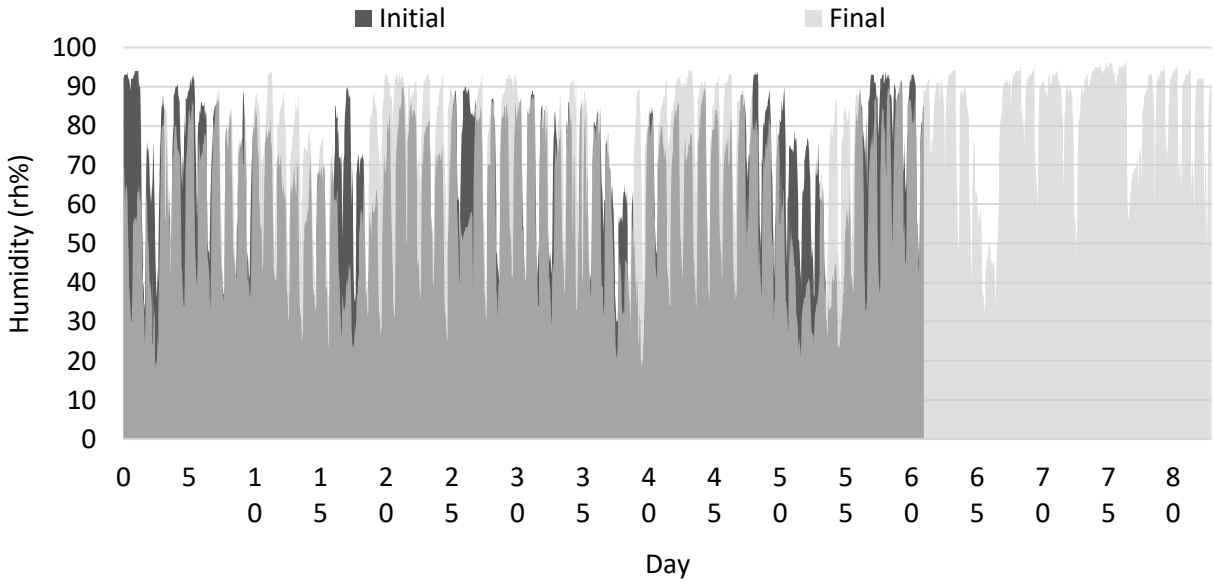

Figure 82: Daily greenhouse humidity conditions throughout both plant growth trials.

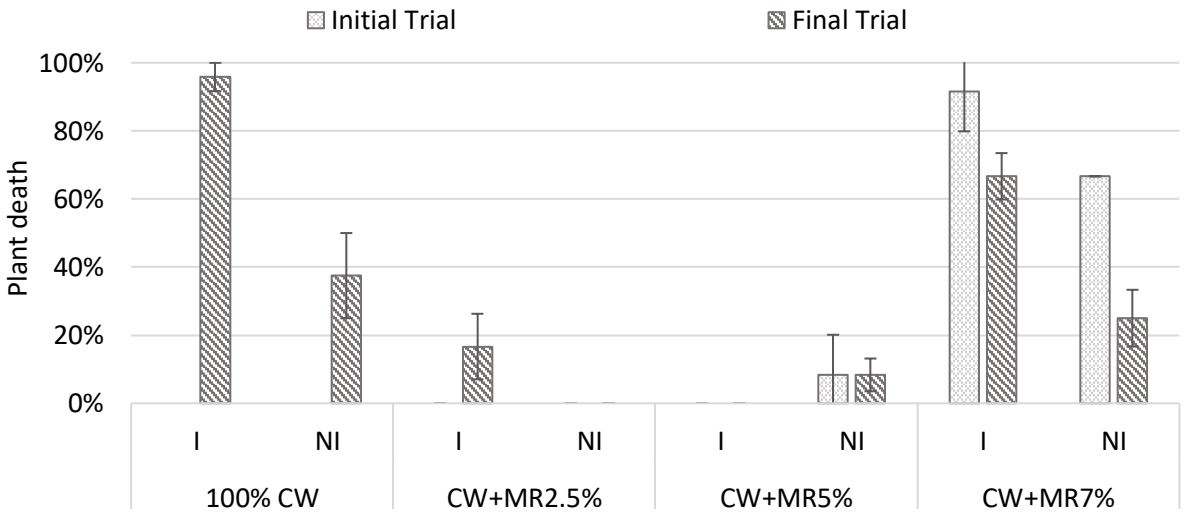

Figure 83: Average plant deaths (expressed as percentage) in coal-based Technosols as effected by seasonal variation for the two plant growth trials.

APPENDIX F: ECONOMIC ANALYSIS ASSUMPTIONS & CALCULATIONS

Table 27: Total volume of water required per kg soil for 90 days.

Technosol Type	Inoculated (I) or non-inoculated (NI)	Teff (T) or no teff (NT)	Trendline Equation	R^2
CW+MR2.5%	I	T	$y = 26.152x + 216.75$	0.968
		NT	$y = 27.635x + 143.5$	0.967
	NI	T	$y = 14.369x + 160.85$	0.960
		NT	$y = 8.6545x - 24.054$	0.844
CW+MR5%	I	T	$y = 27.124x + 172.62$	0.962
		NT	$y = 20.88x + 139.41$	0.971
	NI	T	$y = 16.413x + 130.58$	0.957
		NT	$y = 4.0635x - 30.518$	0.968
Control - Potting Soil	NI	T	$y = 46.896x + 306.6$	0.972
		NT	$y = 30.979x + 157.86$	0.971

Price estimations for teff grain and flour based on recent FAO analysis of teff prices; 1.35 USD/kg for teff grains and 1.60 USD/kg for teff flour (FAO, 2015) in in April 2022. Current conversion rates in in April 2022 between USD and ZAR were used; 1 USD = 15.01 ZAR. Cost estimations on EM Pro-Soil were based on current market prices in in April 2022; 1228.00 ZAR per 25 L of Efficient Microbes Pro-Soil (Efficient Microbes, 2006).

To predict the total volume of water required per kilogram of Technosol when cultivating Teff, the following assumptions were made:

1. Teff plants are grown for 90 days per growth cycle.
2. Four growth cycles per annum (one yield per season).
3. The germination period lasts 14 days.
4. During germination, the water requirement per day is half the volume required per pot per day for the rest of the growth trial.
5. Soils are maintained at 50% field capacity.
6. Seasonal effects are negligible.
7. The number of Teff plants are kept maintained at 14 plants per kilogram soil, or 857 plants per 1 m^2 soil.

Sample calculations for soil requirements (amounts) and associated cost if implemented on a scale of 1 hectare Technosols with a depth of 50 cm:

Sample calculations are based on CW+MR5%.

Apparent density: $\rho = \frac{m}{V} = \frac{619.4\ g}{472.5\ mL} = 0.763\ \frac{g}{mL}$

Thus, for 1 ha CW+MR5%, the total soil weight required: $m_{CW+MR5\%} = V * \rho = (5.0E+09\ cm^3) * \left(0.763\ \frac{g}{mL}\right) * \left(\frac{1g}{1cm^3}\right) = 3.81E+09\ g$

Therefore, mass of CW: $m_{CW} = \rho_{CW} * m_{CW+MR5\%} * weight\ fraction = \left(0.94 \frac{g}{mL}\right) * (3.81E + 09\ g) * 0.95 = 3.59E + 09\ g \approx 3585\ kg$ and the mass of MR added: $m_{MR} = m_{Total} - m_{CW} = 3.81E + 09\ g - 3.59E + 09\ g = 2.29E + 08\ g \approx 228\ kg$

Following, for potting soil: $m_{Control} = 0.89 * 5.0E + 09 = 4.45E + 09 \approx 4450\ kg$

Cost analysis for one hectare of topsoil was based on the average market price for outdoor topsoil; ZAR 990.00 per m^3 of soil. Thus, cost for 1 hectare of conventional topsoil = 4.95 million ZAR.

Chemical fertilisation cost estimations: Based on market prices for NPK fertilisers in April 2022; 466.93 ZAR per 50 kg, with a trend of 72 830 kg/ha in South African soils (FAO, 2018).

Teff above ground dry biomass predictions were based on above ground dry biomass per 100 g soil as previously presented in Chapter 4. Whereas, estimated grain yields for implemented Technosols were based on the correlations between above ground dry biomass and seed weights produced as shown in the figure below.

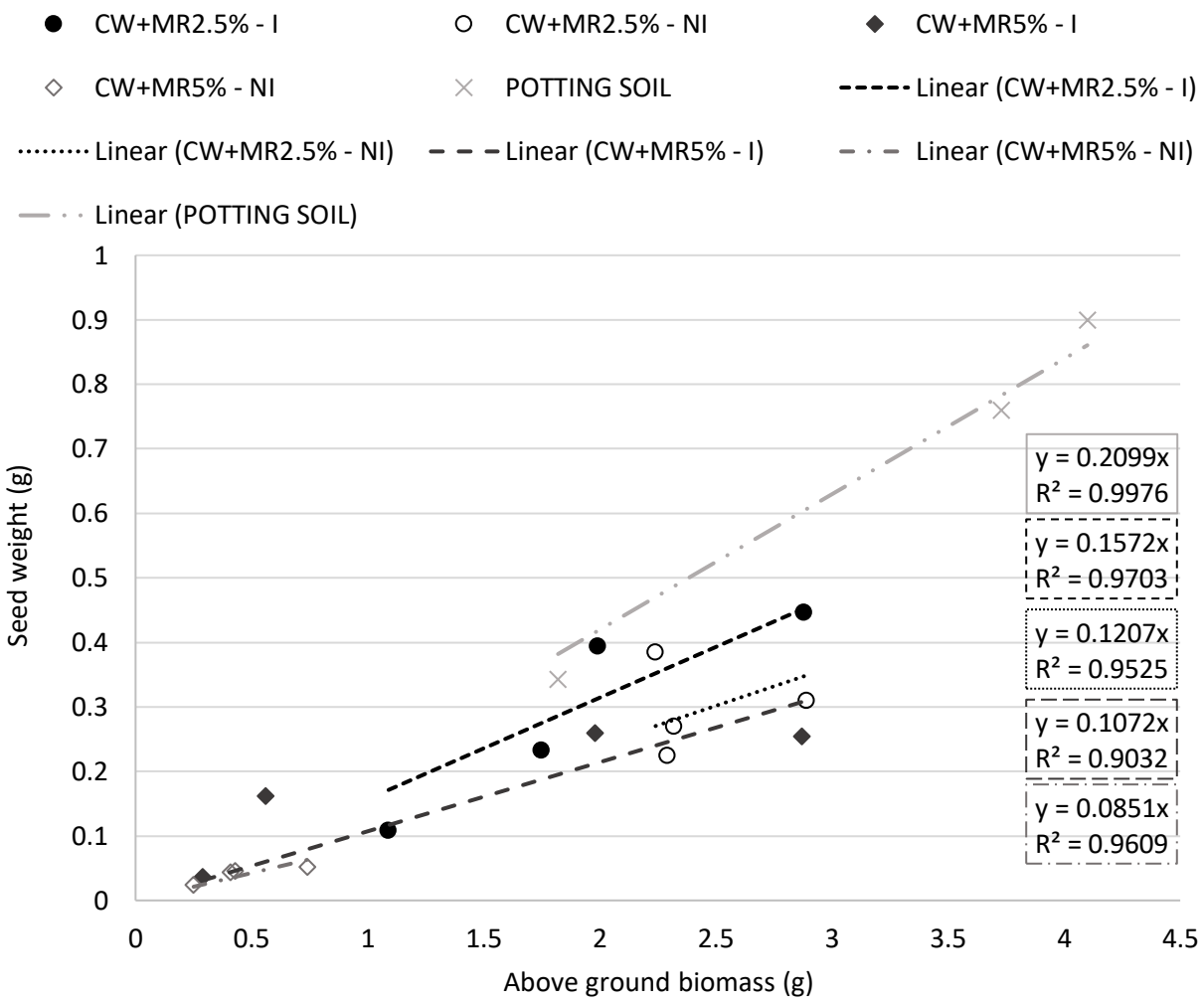

Figure 84: Correlation between seed weight and above ground dry teff biomass achieved in the final growth trial for coal-based Technosols amended with 2.5% and 5% malt residue and potting soil as the control.

APPENDIX G: ETHICS APPROVAL

Application for Approval of Ethics in Research (EiR) Projects
Faculty of Engineering and the Built Environment, University of Cape Town

APPLICATION FORM

Please Note:
Any person planning to undertake research in the Faculty of Engineering and the Built Environment (EBE) at the University of Cape Town is required to complete this form **before** collecting or analysing data. The objective of submitting this application *prior* to embarking on research is to ensure that the highest ethical standards in research, conducted under the auspices of the EBE Faculty, are met. Please ensure that you have read, and understood the **EBE Ethics in Research Handbook** (available from the UCT EBE, Research Ethics website) prior to completing this application form: http://www.ebe.uct.ac.za/ebe/research/ethics1

APPLICANT'S DETAILS		
Name of principal researcher, student or external applicant		Cari van Coller
Department		Chemical Engineering
Preferred email address of applicant:		carivancoller@gmail.com
If Student	Your Degree: e.g., MSc, PhD, etc.	MSc
	Credit Value of Research: e.g., 60/120/180/360 etc.	180
	Name of Supervisor (if supervised):	Dr Mariette Smart
If this is a researchcontract, indicate the source of funding/sponsorship		BASS Fellowship
Project Title		Microbiome function and structural dynamics of a fabricated Technosoil.

I hereby undertake to carry out my research in such a way that:

- there is no apparent legal objection to the nature or the method of research; and
- the research will not compromise staff or students or the other responsibilities of the University;
- the stated objective will be achieved, and the findings will have a high degree of validity;
- limitations and alternative interpretations will be considered;
- the findings could be subject to peer review and publicly available; and
- I will comply with the conventions of copyright and avoid any practice that would constitute plagiarism.

SIGNED BY	Full name	Signature	Date
Principal Researcher/ Student/External applicant	Cari van Coller	Signed by candidate	**06 Feb 2020**

APPLICATION APPROVED BY	Full name	Signature	Date
Supervisor (where applicable)	Mariette Smart	Signed by candidate	**06 Feb 2020**
HOD (or delegated nominee) Final authority for all applicants who have answered NO to all questions in Section1; and for all Undergraduateresearch (Including Honours).	Click here to enter text.		Click here to enter a date.
Chair : Faculty EIR Committee For applicants other than undergraduate students who have answered YES to any of the above questions.			