COMBINED EFFECT OF VERMICAST-*TRICHODERMA*-SAWDUST ON ----KALE, SWISS CHARD, AND PAK CHOY GROWTH

By

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DEDICATION

This thesis is dedicated to God the Almighty source of divine wisdom and to all in pursuit of academic excellence.

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ABSTRACT

Nowadays, organic amendment has been used to address soil fertility problems and improve crop production. Vermicast-sawdust mixed plant growing media are low-cost and rich in beneficial microbes and essential nutrients but not extensively studied. The present study focused on confirming the best of vermicast-*Trichoderma*-sawdust combination. The mixed proportion of the vermicast-sawdust comprised (1) 80% vermicast+20% sawdust; (2) 60% vermicast+40% sawdust; (3) 40% vermicast+60% sawdust; (4) 20% vermicast+80% sawdust; and (5) sawdust alone (control) added without or with 10^5 spores/g *Trichoderma viride*. The nutrient-release pattern and mineralization analysis suggested that more vermicast leads to more nutrient-release pattern speed and minerals, and *T. viride* in the growing media did not show a positive effect. Active microbial community structure showed the mixed media with the 40% vermicast-60% sawdust or the 60% vermicast-40% sawdust gave the highest diversity of active microbial community composition and the highest overall mineral nutrients content compared to the other media. The40% vermicast+60% sawdust mix media was the best combination media on the growth and development of three different microgreen plant species, kale (*Brassica oleraceae var. sabellica L.*), Swiss chard (*Beta vulgaris* Subspecies *Maritima*), and pak choy (*Brassica rapa* ssp. Chinensis).

Keywords: microgreens, nutrient release, vermicast, organic amendment, PLFA

LIST OF ABBREVIATIONS AND SYMBOLS USED

BAME	Bacterial Acid Methyl Ester
CL	cardiolipin
FA	fatty acid
FAME	Fatty Acid Methyl Ester
FID	flame ionization detector
GC	gas chromatography
LC	liquid chromatography
MS	mass spectrometry
MTBE	methyl tert-butyl ether
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PI	phosphatidylinositol
PLFA	phospholipids fatty acids
PS	phosphatidylserine
RDA	redundancy analysis
TMSH	trimethyl sulfonium hydroxide

Chemical Compounds and Ions

$-NH_2$	amino
%	percentage(s)
°C	degree Celsius
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
S	Sulphur
Fe	Iron
Zn	Zinc
Mn	Manganese
B	Boron
K^+	Potassium
NO_3^-	Nitrite-N
NH_4^+	Ammonium

Measurements

S	second
Min	minute
Hr	hour
D	day
Cm	centimetres
G	grams
Ml	microliter
Ml	milliliters
M	meters
Mm	millimetres
Ppm	parts per million

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CHAPTER 1 INTRODUCTION

In this chapter, we will provide a brief synopsis of the project through the thesis overview, objectives, and thesis outline, as well as thesis organization.

1.0 THESIS OVERVIEW

Traditionally, organic amendments (also known as organic fertilizers) have been used as sources of plant nutrients and soil conditioners. Recent advancement in agricultural science have helped in the understanding of the roles of organic amendments on soil bio-physicochemical improvement and plant growth and harvest quality (Scotti et al., 2015; Abbey et al., 2018). The different soil organic amendments materials can be grouped essentially in five categories: animal manure, municipal biosolids, green manure and cover crops, waste from manufacturing processes, and compost (Goss et al., 2013).Using organic amendments instead of synthetic chemical fertilizers and pesticides reduce environmental pollution, production cost to farmers as well as restore or reclaim degraded soils (Diacono & Montemurro, 2011; Quilty & Cattle, 2011).

It is acknowledged that vermicast is one of the most promising organic amendments in agriculture. Vermicast is a stabile and fine peat-like material derived by earthworms from organic matter. Vermicast contains reduced levels of contaminants and a higher saturation of nutrients than the product of vermicompost (Ndegwa et al., 2000). Vermicast has a higher porosity and good water-holding capacity and thus, makes nutrients and water more readily available to plants (Weber et al., 2007). Vermicast is used as a growing medium amendment to improve soil physical properties characterized by low bulk density and enhancement of soil particle aggregation to improve soil structure. It is also relatively high in organic matter, macro- and micro-nutrients with a low C:N ratio (Sanchez et al., 2016; Sriakilam et al., 2016; Lv et al., 2018). Vermicast contains enzymes

such as amylase, lipase, cellulase and chitinase that break down the organic matter in the soil to release essential plant nutrients and make it more available for plant uptake and utilization (Chaoui, 2003).

Many different growing medium substrates such as peat moss, coir, rock wool and sawdust are used in the horticultural crop production industry. Sawdust, a waste product of the timber and wood industries, is widely used as a growing medium substrate for greenhouse plants (Cheng, 1987). Sawdust is an appropriate growing medium substrate in the Atlantic Maritimes of Canada because of its characteristic low cost, local availability, and good water-holding capacity. Since plant roots and soil microbes often compete for N, one way to at least temporarily reduce N availability to plants is to add a carbon source that increases microbial growth and N uptake (Schimel et al., 1989; Marrs, 1993). Sawdust contains a high amount of carbon (C) that has more negative effects on the non-natives than on the native's shrubs (*Lupinus arboreus* Sims) in a community (Alpert & Maron, 2000). Moreover, the levels of the microbial load reduced as the increase of quantity of sawdust (Mensah et al., 2013). The C/N ratio is related to the behaviour of saprophytic microorganisms in soil. The microbes consume organic matter requiring organic C and N in a relatively fixed stoichiometric ratio. Therefore, sawdust can help to reach the C:N ratio to microbe consumption (Scotti et al., 2015).

Trichoderma as fungi presents in rhizosphere, which joining in plant debris decomposition in the soil (Topolovec-Pintaric et al., 2013). Some *Trichoderma* strains can inhabit the rhizosphere, control plant growth, and protect plants from pathogens (Shoresh et al., 2010). Many research papers mentioned about the positive influence of *T. harzianum* and *T. viride* on lettuce, cucumbers and bell peppers growth (Bal and Altinatas, 2006; Bal and Altinatas, 2008; Poldmaat al., 2000; Yedidia at al., 2001). Indigenous *T. viride* have the antagonistic ability to is isolate against some

of important plant pathogenic fungi like *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Botrytis cinereal* (Topolovec-Pintaric et al., 2013).

Past studies have shown that vermicast, as an organic amendment, can enhance plant nutrient acquisition and tissue nutrient density. However, no published literature has mentioned a combination of vermicast-*Trichoderma*-sawdust as amendments for kale (*Brassica oleracea* var. *sabellica*). Therefore, it is necessary to investigate properties exhibited by vermicast-*Trichoderma*-sawdust mixed medium and the impact on plant growth, development and harvest yield and quality.

1.1 THESIS OBJECTIVES

The long-term objectives are to develop a highly efficacious soilless medium comprising compost, sawdust and *Trichoderma* to grow nutrient-rich and healthy organic vegetables and to understand nutrient-release patterns and active microbial community structure dynamics in the formulated media.

The short-term objectives are to:

1) investigate nutrient mineralization dynamics, nutrient release patterns, and active microbial community structure in different proportions of vermicast-*Trichoderma*-sawdust mixed media.

2) evaluate the morpho-physiological response of plants to different proportions of vermicast-sawdust mixed media.

1.2 OUTLINE OF THESIS AND ORGANIZATION

The thesis is divided into six chapters, including the present chapter. Chapter 2 provides a review of the relevant works in the literature done in the field of my study. It discusses the importance and

vermicast and sawdust as soilless growing medium components, different effects of vermicast and sawdust on plants the functions of *Trichoderma* on plants and microbial community structure and importance in growing medium quality. Chapter 3 examines the nutrient-release pattern and mineralization of vermicast-*Trichoderma*-sawdust mixed media. Chapter 4 examines the active microbial composition of vermicast-*Trichoderma*-sawdust mixed media. Chapter 5 examines the effect of vermicast-sawdust mixed media on kale (*Brassica oleraceae var. sabellica L.*), Swiss chard (*Beta vulgaris* Subspecies *Maritima*), and pak choy (*Brassica rapa* ssp. Chinensis) growth. Finally, Chapter 6 summarizes the study and offers insights for future research.

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CHAPTER 2 LITERATURE REVIEW

In this chapter, we will discuss the effects of vermicast, sawdust, and *Trichoderma* on plants, separately, through pass literature papers.

2.1 EFFECTS OF VERMICAST ON PLANTS

2.1.0 Nature of Natural Amendments

The organic amendments application is reliable and useful to improve growing media chemical and biological fertility of soils, as well as to suppress soilborne pathogens (Ros et al., 2003; Scotti et al., 2013; Zaccardelli et al., 2013). The most common soil organic amendments are compost, animal manure, peat moss, wood chips, straw, sewage sludge, sawdust (Goss *et al.,* 2013; Scotti et al., 2015). Microorganisms, organic matter, mineral nutrients, humic substances, and macromolecules play essential roles that are enriched in natural amendments where they play essential roles in its functionality upon application. For example, the partial replacement of synthetic chemical fertilizers with organic amendments had a significant impact on soil microbial community, and increased phosphorus (P) uptake and yield of sweet corn (*Zea mays* L.) (Lazcano et al., 2013). The use of organic amendments also increased microbial activity in soils by 20% as compared to synthetic chemical fertilizers (Dinesh et al., 2010; Gonzalez et al., 2010). Nonetheless, the optimization of the use of organic amendments is vital for sustainable agriculture by improving natural soil fertility and minimizing any harmful environmental effects such as reducing nitrogen (N) losses to the environment (Masunga et al., 2016).

2.1.1.1 Microorganisms

Microorganisms are a crucial part of biogeochemical cycles on the earth and represent the largest genetic reservoir with the longest evolutionary history. Microorganisms regularly live as communities running essential functions of an ecosystem, such as primary production and remineralization of biomass (Friedrich, 2011). Soil microorganisms contain bacteria, actinomycetes, fungi, algae and protozoa. Each of them has its functions in the soil. For example, the first ancient bacteria and microorganisms could fix nitrogen, in time multiplied, and as a result, released oxygen into the atmosphere (Farquhar et al., 2000; Canfield, 2014). More advanced microorganisms can affect soil structure and fertility.

The application of effective microorganism significantly enhanced shoot and root biomass in *Trifolium alexanrinum* L. crop residues (TCR)-amended soil. Soil amendment and plant growth stage make the effective microorganism application different on nutrient uptake. Generally, effective microorganism application can enhance plant nitrogen (N), phosphorus (P), and potassium (K) nutrition in organic amendments (Javaid & Bajwa,2011).

Microbes must reproduce and respond to a unique set of environmental constraints to sustain life. The environmental constraints of microbes range from strict aerobic to anaerobic, from high water requirements to low water requirements, and from autotroph to heterotroph lifestyles, consume from simple inorganic to complex organic substrates (Paul, 2014). This has an impact on the surrounding environment and is later discussed as a crucial component of soil (Paul, 2014). Microbial processes are vital for plant nutrient supply from organic matter decomposition and nutrient dynamics (Paul, 2007). An organic amendment, such as vermicast, typically increases soil microbial biomass by supplying C-rich organic compounds. This is important because many

microbial communities within the cultivated soil are C-limited (Knapp et al., 2010). Additionally, specific microbial groups, which feed primarily on organic compounds, need additional C to change the microbial community compositions (Hu et al., 2011). A better understanding of the microbial processes that take place in soil under the organic amendment could help identify the main drivers determining nutrient bioavailability and plant nutrient bio-accessibility (Lazcano et al., 2013).

2.1.1.2 Organic matter

Organic matter is the accumulation of organic residues, which have undergone biological decay (decomposition) (Paul, 2014). The organic matters such as proteins, nucleic acids, fats, carbohydrates are converted into stable vermicast during the vermicomposting (Bhat et al., 2018). This stabilization process provides vermicast with high exchangeable cations, humic substances, and other organic matter contents (Fernández-Bayo et al. 2008). Humus is the final state of decomposition of organic matter. It is composed of very stable lignin (about 30%), complex sugars (30%), proteins (30%), and fats that are resistant to breakdown by microbes (Henkel, 2015). The chemical components are C (about 60%), N (5%), some oxygen and various amounts of hydrogen, sulphur (S), and P (Henkel, 2015).

2.1.1.3 Mineral nutrients

Generally, the contents of total N, available P, organic matter, and exchangeable Calcium (Ca) in the soil are higher with vermicompost application than with farmyard manure on Celery (*Apium graveolens* L. var. *dulce* Mill), particularly in the second growing season when weather conditions are more favourable (Ilker et al., 2016). Vermicompost has a higher potential to reproduce

microorganisms involved in C and P cycles, which may also be evidence for their cumulative effect on soil microorganisms (Ilker et al., 2016). N mineralization is a critical biological process for plant growth and development. The amount of N released to plants depends on the chemical composition of organic matter and the physical, chemical, and biological properties of microbes in the soil (Mohanty et al., 2011). There have been some studies on the dynamics of N mineralization from organic amendments in Agri-ecosystems. Nevertheless, there are no reported studies on the vermi*cast-Tricho*derma-sawdust mixed medium effect on nutrient mineralization dynamics and microbial community.

2.1.2 Relationships Between Organic Amendments and Microbial Communities

Organic amendments maintain organic matter and sustain soil fertility for agricultural production, particularly in the long-term, by slowly releasing nutrients for plant use (Mohanty et al., 2011). Enzyme activities engaged in the breakdown of organic amendments help to release nutrients for the plants (Dinesh et al., 2010). Soil microbial biomass and their activities increased rapidly after the application of organic amendments, but there were no significant changes in the soil microbial community structure in the short term (Dinesh et al., 2010; Diacono & Montemurro, 2011). Thus, organic amendments, like vermicast, can stimulate soil microbial processes and increase crop yields. It also suggested that changes in the microbial community structure do not always change the function of the microbial community or the availability of plant nutrients and the productivity of plants (Franco-Otero et al., 2012). According to Lazcano et al. (2013), the relationship between soil microbial community structure and function is not clear because of the complexity of soil systems and the existence of several microbial groups. Furthermore, agricultural management practices mainly impact soil and crop health and productivity through changes in the composition

and function of soil microbial communities (Chaparro et al., 2012; Franco-Otero et al., 2012).

2.1.3 Earthworms and Their Functions

Vermicast, the pure product of vermicomposting, involves the non-thermal decomposition of organic waste through the interaction between organic waste, earthworms, and microorganisms (Márquez-Quiroz et al., 2014). The activities of earthworms, such as is red wigglers (*Eisenia fetida*), including their excreta (vermicasts) could benefit the environment and Agri-ecology. Earthworms during their feeding and digestion processes convert biodegradable materials and organic wastes into nutrient-rich vermicast. These have many benefits, including soil development and maintenance of bio-physicochemical properties such as higher nutritional value, water holding capacity, and acts as excellent soil ameliorating media (Singh et al., 2016).

2.1.4 Nutritional Quality and Functional Property

Sudies on nutritional quality and functional property of foods are important for commercialization and to convince consumers for their acceptability (Kolsi et. al., 2017). The nutritional quality of plant food can be measured by their contents of chlorophylls, carotenoids, essential mineral elements and fatty acids. This project mainly investigated the quality of growing media, and plant health and nutritional quality (i.e. anthocyanin content and chlorophyll fluorescence indices) as influenced by the mixed growing media. On the other hand, functional properties can be measured through hydration properties (such as water holding capacity), total phenolics, flavonoids and condensed tannins, antioxidant and enzyme activities (Kolsi et. al., 2017). The continuation of experinments to assess the nutritional and functional indices of target crops grown in selected

vermicast-sawdust mixed media will be done in Dr. Abbey lab in next two years. Plants will be grown to collect more data about nutritional and functional indices by measusing essential nutrients and vitamins (vitamins A, C, D, E, and K), carbohydrates, underivatized mono and disaccharides, total protein and amino acids, antioxidants, hydrophilic, lipophilic and total antioxidant activities, intact lipid analysis as well as fatty acid methyl esters analysis.

2.1.5 Vermicast as A Suitable Nutrient Source for Crops

The most used soil amendment is the compost, decomposition of organic wastes, which represents a waste recycling management (Pérez-Piqueres et al., 2006). One of the promising composts is vermicast. Vermicasts are treated as a miracle plant growth enhancer (Guerrero, 2010). Many agricultural problems could be avoided by vermicast application, such as soil structural degradation, erosion, nutrient loss, nutrient toxicity and salinity attributable to its physicochemical nature, microbial richness and properties.

Generally, Sphagnum peat moss was used as a soilless potting substrate in horticulture due to its desirable physical characteristics and high nutrient exchange capacity (Raviv et al., 1986). However, a recent rise in environmental and ecological concerns about harvesting and destruction of endangered wetland ecosystems worldwide, many people are now looking for an alternative substrate to replace peat (Buckland, 1993; Robertson, 1993). Similarly, vermicast leachates or vermicast water-extracts showed to be effective amendments or foliar sprays by promoting the growth of sorghum (*Sorghum bicolor* (L.) Moench), strawberry (Fragaria× ananassa Duch.), and tomato (*Lycopersicum esculentum* cv. Momotaro) (Gutiérrez-Miceli et al., 2008; Singh et al., 2010; Tejada et al., 2008). The effectiveness of using vermicast is higher than only using synthetic chemical fertilizer in the field (Mahmud et al., 2018). Compared to regular supplementation with chemical fertilizer, the vermicompost application had significantly ($p<0.05$) increased the soil pH

and was able to retain the soil nutrients content.

Regarding the direct effects on plant growth, vermicast is rich with plant macronutrients and micronutrients. Even though some nutrients are released in inorganic forms that are readily available to plants, most are released slowly through mineralization of the organic matter. As a slow-release fertilizer, it supplies the plant with a continuous and constant source of nutrients (Chaoui et al., 2003). Vermicasts can also increase the macromolecules (proteins, carbohydrates, and lipids) content in plants. Due to its characteristic as a slow-release fertilizer, it does not require frequent incorporation into the soil (Mahmud et al., 2018). The irrigation and pest control cost in vermicast-treated areas is estimated to be significantly lower because of the excellent water-holding capacity of the vermicast and its pest-repelling benefits (Adhikary, 2012). Vermicast enhances the 'biological resistance' in plants and protects them against pests and diseases either by repelling or by suppressing them (Sinha, 2009).

Vermicast stimulates the growth and development of a wide range of plant species such as pepper (*Capsicum annum* L. var. California), strawberry, sweet corn, and tomato (Arancon, 2004; Zaller, 2007; Singh et al., 2010; Lazcano et al., 2011; Lazcano et al., 2013). Vermicast is also suitable for some aromatic and medicinal plants such as forage sorghum and rice-straw, fruit such as banana and papaya, and ornamentals such as geranium, marigolds, petunia, chrysanthemum and poinsettia (Arancon et al., 2008). Vermicast positively impacts vegetative growth, stimulates shoot and root development, and alters seedling morphology by increasing leaf area and root branching (Edwards et al., 2004; Kumar et al., 2018). It also stimulates plant flowering, increases the number and biomass of flowers produced as well as increases fruit yield (Singh et al., 2016). Vermicast can also increase the nutritional quality of some vegetables such as lettuce, spinach, and Chinese cabbage (Abbey et al., 2018; Vidal et al., 2018; Wang et al., 2010). Vermicast also positively effects

forestry species, such as acacia, eucalyptus, and pine tree (Lazcano et al., 2010a; 2010b). Therefore, vermicast helps to increase crop productivity, quality and phytochemicals that can benefit human health.

When vermicast is applied to fruits, studies have shown that they have a higher fat content (23.86%) and protein content (19.86%) when compared with those grown with synthetic chemical fertilizers (Ansari & Ismail, 2010).

2.2 EFFECTS OF SAWDUST ON PLANTS

Sawdust is commonly used as growing media for the germination and seedling production of the greenhouse industry due to its high moisture retention, low cost, and ready availability (Sawan & Eissa, 1995). For example, oak tree (*Quercus*) sawdust is a potential substitute for peat moss as a container substrate for seedlings production of Chinese cabbage (*Brassica campestris* L.), because its total carbohydrate content of lignocellulosic substrates (above 30.3 g/100 g) is higher than that of peat moss (about 23.9 g/100 g) (Jung et al., 2015).In the paper of Dzurenda et al. (2010), they concluded that dry-thermally-modified oak sawdust is finer than unmodified oak sawdust. Therefore, in this project, the thermally treated sawdust was selected.

2.2.1 Effect of Non-treated Sawdust on Plant Growth

Sawdust is a suitable growing medium substrate when incorporated with suitable amounts of clay, ammonium nitrate (NH_4NO_3) and organic amendments (Cheng, 1987). A mixture of sawdust at 30% of soil volume with NPK (nitrogen, phosphorus, and potassium) fertilizers added to soil

produced the highest yield on tomato (*Solanum Lycopersicum*) compared to sawdust alone. In another study, biochar obtained through fast pyrolysis of pine sawdust under laboratory experiments improved the quality of soil and enhanced plant growth in Kubuqi Desert, China. (Laghari et al., 2016). Untreated sawdust, coconut fibre, bark, and perlite are widely used in North America (Vano et al., 2011). Some mill residues, such as sawdust and bark, are the cheapest source of biomass and have been widely used in commercial plant production in Canada for decades (Bradley & Solutions, 2007; Sawan and Eissa, 1995). Despite high saturated hydraulic conductivities and good air contents, these wood industry by-products have low water retention capacities. Limitations to plant growth and fruit production with sawdust are attributed to low water availability or inappropriate particle-size distribution, and nutrient immobilization as well as adverse effects due to salt and toxic compound accumulations (Vano et al., 2011). Even when mixed with peat, sawdust can still have some negative impacts on fruit production compared to peat and peat-bark substrates (Jarosz & Konopińska, 2010). For example, compared to plants that grew in peat or peat mixed with pine bark (1:1), plants grown in peat mixed with sawdust (1:1) had significantly smaller fruit unit weight (10.7 g).

Additionally, sawdust can reduce the use of NH_4NO_3 fertilizer in food production. For instance, using 25% sawdust containing urea-formaldehyde to grow sweet corn showed similar results when compared the control that consisted of preplant NH_4NO_3 fertilizer only (Brass et al., 2004). However, replacing NH_4NO_3 fertilizer with 100% sawdust resulted in decreased growth and yields. Therefore, using around 25% -30% sawdust may have better plant growth than 100% sawdust.

2.2.2 Effect of Thermally Treated Sawdust on Plant Growth

Usually, thermally treated sawdust has higher glucose content than non-treated sawdust. For example, the content of hemicellulosic sugar (arabinose, xylose, mannose and galactose) in oak sawdust was higher than that in steamed oak sawdust while the highest glucose content was observed in steam-exposed oak sawdust (Jung et al., 2015). The presence of high levels of micronutrients (K, Ca, Mg and P) or low levels of toxic elements such as Zn and Fe in steam-exposed oak sawdust would be beneficial to horticultural media preparation (Jung et al., 2015). Regarding physical properties, steamed oak sawdust (84.9% water holding capacity) and steam-exposed oak sawdust (92 %) had significantly higher total porosity than non-thermally treatment oak sawdust (82.5%) (Jung et al., 2015). Moreover, through thermal treatment, the porosity can be significantly increased by the removal of lignin and hemicellulose and the reduction of cellulose crystallinity (Kumar et al., 2005). Steam-exposed oak sawdust can be used effectively in the horticultural medium by affecting seed germination, stem length, and leaf area (Jung et al., 2015). The 90% steam exposed oak sawdust+10% perlite in the media positively affected seed germination (87%), stem length (3.0 cm), and leaf area (2.2 cm^2), which works like peat moss (Jung et al., 2015).

2.3 *TRICHODERMA*

Free-living fungi of the genus *Trichoderma* are ubiquitous in different soils and root ecosystems. Some *Trichoderma* strains inhabit the rhizosphere and control plant growth. *T. virens* can directly modulate plant growth by releasing of indole-3-acetic acid (IAA) and be related to indole compounds with auxin activity, which promotes root hair and lateral root development, increasing

the total absorptive capacity of the root system (Contreras-Cornejo et al., 2009). *Trichoderma* can protect plants from pathogens by activating immunity mediated through the canonical defence signals jasmonic acid and salicylic acid (Shoresh et al., 2010). For example, the pool of volatile organic compounds produced by *T. viride* promoted plant growth, increased lateral root formation, plant height and flowering in Arabidopsis (Hung et al., 2013). Particularly, 4-*phosphopantetheinyl transferase1* from *T. virens* plays an essential role in antibiosis, induction of salicylic acid, and camalexin-dependent plant defence responses (Velázquez-Robledo et al., 2011). In this project, *Trichoderma* will be used as the pathogen controller for the combination of vermicast-*Trichoderma*-sawdust.

2.4 MIXTURE OF VERMICAST-*TRICHODERMA*-SAWDUST

Vermicast produced from fermented pig manure with sawdust was reported to significantly increase the bulk density, particle density, pH, electrolytic conductivity, ash, total N, cation exchange capacity, available phosphorous and exchangeable cations compared to the use of only peat moss on the growth of leaf beet, young radish, spinach, and lettuce. There is also no literature on the effect of combining vermicast-*Trichoderma*-sawdust. Therefore, it is hypothesized that the combination of vermicast and sawdust may lead to increased growth of plants enhance plant nutrients uptake and reduce production cost.

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CHAPTER 3 NUTRIENTS MINERALIZATION AND NUTRIENT-RELEASE PATTERNS OF VERMICAST-*TRICHODERMA*-SAWDUST MEDIA

3.0 ABSTRACT

Vermicast-*Trichoderma*-sawdust application has multiple benefits for soil quality and plant productivity improvement. Moreover, vermicast can act as a source of nutrients and sawdust can act as sequester carbon in the soil. Herein we sought to determine nutrients mineralization and nutrient-release patterns of varied proportions of vermicast-sawdust growing media. The treatments consisted of (1) 80% vermicast+20% sawdust; (2) 60% vermicast+40% sawdust; (3) 40% vermicast+60% sawdust; (4) 20% vermicast+80% sawdust; and (5) sawdust alone (control) saw dust without or with 10^5 spores/g *Trichoderma viride*. Total dissolved solids, electrical conductivity and salinity following submergence of the mixed growing medium treatments in deionized water gradually increased to a peak before reaching a stable phase. Nutrients released from all the treatments without *T. viride* were significantly higher ($p<0.0001$) than the corresponding treatments with *T. viride*. The lower the proportion of vermicast, the lower the nutrient released from the medium. Thus, the mineral nutrient contents of the media followed the trend: 80%> 60% > 40% > 20% > 0% of added vermicast. The nutrient-release pattern was represented by a nonlinear regression equation using the Michaelis-Menton model as nutrient-release = θ1 * Time / (θ2 + Time). A repeated measures analysis showed that Time**T. viride***Treatment for total dissolved solids was significantly different ($p=0.0102$). Nitrate contents of all the media treatments were reduced with increased incubation time. In conclusion, are duction in vermicast content of the mixed media significantly reduced mineral nutrients and other chemical

indices, while incubation for four weeks seemed adequate for nutrient mineralization. Further investigation is in progress to assess growing media microbial dynamics and plants growth performance.

3.1 INTRODUCTION

Almost all plant growth and developmental processes can be associated with growing medium properties. Important growing medium processes that impact plants include organic matter decomposition, ammonification, nitrification, denitrification, nitrogen (N) fixation, phosphate (P) mineralization and sulphur (S) transformations (Paul, 2014). These growing medium processes affect nutrients availability for plant uptake and utilization. Typically, nutrients mineralization efficiency is increased when the concentration of a specific element exceeds the needs of the microbial decomposer for biosynthesis or storage. For example, the mineralization of N depends on the carbon:nitrogen (C:N) ratio of the decomposing organic matter. Mineral Nis immobilized once the ratio exceeds 30:1 (McLaren & Cameron, 1996). As a result, the decomposing microbes may absorb N in mineral form (i.e. ammonium or nitrates) leading to reductions in inorganic N in the growing medium (Beare et al., 1994; White, 2013; Paul, 2014).

It is universally acknowledged that natural substances such as compost and vermicast improve growing medium quality and increase plant growth and productivity. Some studies indicated that under controlled environmental production conditions, the performance of these natural amendments is better than synthetic chemical fertilizers (Lazcano & Domínguez, 2011). In this study, we focused on nutrient-release pattern and mineralization ofsoilless mixed media comprising different proportions of vermicast and sawdust with or without the addition of

Trichoderma viride. Vermicomposting using worms is a low-cost, clean and sustainable technology. The worm castings termed as vermicast is popularly used as growing medium amendment because of its richness in beneficial microorganisms, nutrients, humic and non-humic substances with desirable physical properties (Lazcano & Domínguez, 2011; Pathma & Sakthivel, 2012; Bellitürk, 2017; Sinha, 2019; El-Goud &Amal 2020).Furthermore, it was confirmed that vermicast contains growth-promoting hormones (Nagavallemma et al., 2004) and various nutrients except for zinc and iron (Abbey et al., 2018) required by plants. Application of vermicast alone (100%) or 75% vermicast + 25% soilless peat moss with added *Mycorrhizal* fungi were found to be toxic to plants due to the high chemical concentration compared to the addition of 25% or 50% vermicast (Abbey & Appah, 2016). It was also reported that the large surface area of vermicast granules provides more microsites for microbial activity and nutrients retention (Shi-wei &Fu-zhen, 1991) associated with the vermicast richness in nutrients and beneficial microbes. Nutrients released from organic amendments including vermicast may be relatively slow, but not much literature information exist on nutrient-release and nutrient mineralization patterns of vermicast-based growing media.

Sawdust is a forest by-product with high carbon contentand expectations for the improvement of growing medium physical properties.It was reported that sawdust increases growing medium porosity and water retention and can be an option or supplement for traditional soilless growing medium substrates (Agboola, et al., 2018; Palaniappan et al., 2018).Previous studies showed that the application of sawdust-vermicompost extract (1:10 v/v; 1000 mg/L) to the foliage of *Syngonium* plants increased mineral nutrients uptake, particularly N (Khomami et al., 2019). Therefore, an appropriate proportion of vermicast and sawdust mixed medium is expected to have a profound and positive impact on the growing medium biological, physical and chemical properties for the

enhancement of plant nutrients uptake and productivity. Recent development showed increased use of natural additives such as specific microbes, to enhance the functional properties of growing medium amendments. One such microbe is *Trichoderma spp.*

Trichoderma spp. is a free-living fungus, found to have beneficial association with organic and inorganic substances such as humic acids, protein hydrolysates, seaweed extracts and silicon (Fiorentino et al., 2018). *Trichoderma* are ubiquitous in different soils and root ecosystems. They have been reported to protect plants from pathogens by activating immunity mediated genes through the canonical defence signals i.e. jasmonic acid and salicylic acid pathways (Shoresh et al., 2010). Therefore, we postulate that vermicast-*Trichoderma*-sawdust mixed medium can be a holistic approach to provide the essential plant nutrients and increase plant resilience. However, there is limited literature information on vermicast-*Trichoderma*-sawdust mixed medium, and their proportion has not been reported. Based on the available literature information, we hypothesized that 60% vermicast + 40% sawdust with the addition of *Trichoderma* will have the best effect on nutrients mineralization and nutrients release for plant use. Therefore, the objective of the present study was to investigate nutrient mineralization and nutrient-release patterns in different proportions of vermicast-*Trichoderma*-sawdust mixed media.

3.2 MATERIALS AND METHODS

The study was carried out at the Compost and Biostimulant Laboratory and the research greenhouse located in the Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University from March 2018 to September 2019. Vermicast i.e. earthworm castings excreted by Red wiggler worms (*Eisenia fetida*) was obtained from Pagonis Live Bait, ON, Canada;

and heat-treated maple tree sawdust was obtained from Thermal Wood Canada, NB, Canada for the experiments. Promix-BX (Premier Horticulture Inc., Quakertown, USA), a general-purpose peat-based substrate consisted of 75%-85% sphagnum peat moss, horticultural-grade perlite and vermiculite, chemical fertilizer, dolomitic and calcitic limestone, a wetting agent, and mycorrhizal fungus (*Glomus intraradices*) was purchased from Halifax Seed Inc., Halifax, NS, Canada.

3.2.1 Media Preparation

The two treatments were five levels of vermicast-sawdust mixed media (weight by weight)as follows: (1) 80% vermicast+20% sawdust, (2) 60% vermicast+40% sawdust, (3) 40% vermicast+60% sawdust, (4) 20% vermicast+80% sawdust, and (5) sawdust alone (control); and two levels of *Trichoderma viride* treatments as follows: without (A) or with 10^5 spores/g of *T. viride* (B). The microbial treatment was prepared by adding 5 ml of cultured *T. viride* to 400 ml of sterilized distilled water to get 10^5 spores/g by Fisher brand Finnpipette II (https://www.pipette.com/21377822-Fisherbrand-Finnpipette-II-200-1000-uL). The treatment combinations were coded as A1-A5 and B1-B5 (e.g., A1 represented 80% vermicast+20% sawdust without adding *T. viride*; B1 represented 80% vermicast+20% sawdust with added *T. viride*; Table 1).

Table 1. Combined growing media treatments and codes for the mineralization and nutrient-release pattern experiments during December 2018 to February 2019 incubation period.

Code	Vermicast (%)	Sawdust (%)	*T. viride*
A1	80	20	Absent
A2	60	40	Absent
A3	40	60	Absent
A4	20	80	Absent
A5	0	100	Absent
B1	80	20	Present
B2	60	40	Present
B3	40	60	Present
B4	20	80	Present
B5	0	100	Present

A, no added *T. viride*; B, added *T. viride*.

Distilled water (2.5 l) was added to every 1-kg sawdust-vermicast mixed medium and stirred for 1 min to increase the moisture content to approximately 40%, and to sustain microbial activity during incubation. This was necessary because the heat-treated sawdust was dry.

3.2.2 Growing Media Incubation

The individual mixed medium was incubated in the dark at room temperature (approximately, 22^{o}C) for 90 days. Samples were taken at (1) 0 (just before incubation), (2) 30, (3) 60 and (4) 90 days after incubation. The samples were coded A1-1 to A5-4 and B1-1 to B5-4. For example: A1-1 represented treatment A1just before incubation; and B1-4represented treatment B1 at the fourth

sampling time or day 90.100-g samples of each media treatment were kept in a plastic bag and stored in a 20℃freezer (Whirlpool, Mississauga, ON, CA) until ready for analysis. Thus, a total of 160 mixed media samples were collected every 30 days during the incubation period.

3.2.3 Incubation and Nutrients Mineralization

Samples (100 g) collected from the individually incubated growing medium was kept in plastic bag and stored at -20^{o}C. Samples were taken from the freezer and thawed at room temperature for 24 hr before sending them to the Nova Scotia Department of Agriculture Laboratory Services, Truro, NS, Canada for nutrients analyses. These samples were treated as greenhouse soil paste according to standard laboratory protocol (AOAC, 2003). They were tested for nitrate-N, total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), chloride (Cl^{-}), sulphate (SO_4^{2-}) and aluminum (Al). They were determined using the AOAC-968.08 inductively coupled plasma (ICP) spectrometer method (AOAC, 2003).

3.2.4 Nutrient-release

Briefly, 20-g samples of each mixed medium was tied in a rosin press nylon bag (i.e. dimension 2.5x10.16 cm and mesh-size 160 µm)and submerged into 500 ml deionized water contained in a glass column measuring 35-cm height and10-cm inner diameter. The bags were weighed down with washed pebbles as described by Abbey et al. (2013) with slight modification. The test solution was placed in a sample cup (a minimum of 2.5cm in dept is required). The chemical indicesi.e. total dissolved solids (TDS), electrical conductivity (EC), salinity and pH were used to determine

nutrients released into solution using an ExStik ® EC500 instrument (Extech Instruments Corporation, NH, USA). These chemical indices were recorded every 15 min up to 30 min; followed by every 30 min up to 1.5 hr; every 1 hr up to 3 hr; 3 hr; 5.5 hr; 9 hr; 12 hr; and then every 24 hr for 7 days after submergence.

3.2.5 Statistical Analysis

The experiment was arranged in a5×2 factorial design with three replications. Analysis of variance (ANOVA) was performed with Minitab 18.3statistical software (Minitab Inc., PA, USA) to determine the significance of the treatments. Treatment means were compared using Fisher's least significant difference (LSD) test at $\alpha = 0.05$ when the ANOVA model statistics indicated differences between treatments.

Additionally, a nonlinear regression model was used to explain the pattern of nutrient release. Nonlinear regression model is a powerful tool in the mechanical analysis of a system and is represented by $y_i = f(x_i, \theta) + \varepsilon_i$; where θ represents a parameter vector that has more than one value $(\theta 1, \theta 2, \theta 3 \ldots, \theta k)$; y_i is each response i.e. i = 1, …;and n and x_i represents the independent variables. In this experiment, we used the Michaelis-Menton and asymptotic models to explain the nutrient-release patterns. The repeated measures analysis refers to several measurements made on the same experimental unit over different time or spatial points. The primary objectives for repeated measures data analysis are to examine simple factor effects (main effects) and the interaction effects between them, and it also reduces variability among subjects. In this experiment, we selected unstructured (UN) covariance structures in the mixed model.

3.4 RESULTS AND DISCUSSION

The trend for total dissolved solids (TDS), electric conductivity (EC) and salinity following submergence of the growing media treatments in deionized water showed an initial steep rise before gradual and continuous increase and reaching a stable phase (Figure 1). The EC, salinity and TDS increased slowly after 24 hr. The pH of the media that had no *T. viride* (i.e. group A) remained unchanged over time and were within the range of 6.48 and 7.59. There was close similarity in the pH curves of treatments A2 and A3 after 24 hr that were below the curve for treatment A1. The pH of A4 was close but slightly (<0.2) below the pH value of A2 and A3. Similar observations of pH were made for the EC, salinity and TDS. For the treatment that had sawdust alone (A5), the pH was the lowest compared to the other treatments. The pH of A5 reached a peak (around 7.4) between 48 – 72 hr (Figure 1) after which it drastically declined to constant level of 6.8. These variations can be ascribed to low H^+ ion concentration and exchanges for the sawdust alone treatment.

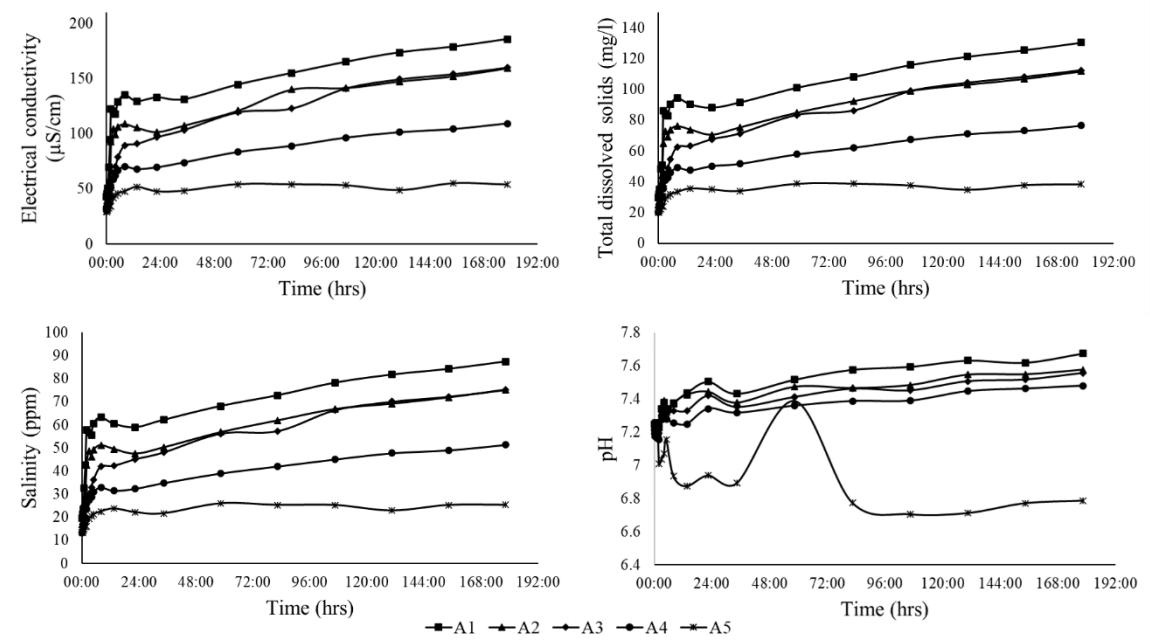


Figure 1. Changes in electric conductivity, salinity, total dissolved solids, and pH of different combinations of vermicast-sawdust media (group A) during December 2018 to February 2019 incubation period. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control).

The pH trend for all the growing media that were added with *T. viride* (i.e. group B) was like that of the group A treatments, except A5. The pH of A5 was reduced and remained constant after 96 hr. There was an increase in the pH of the other treatments but dipped at 36-hr before rising again (Figure 2). Overall, the changes in water quality indices for group B were slower but gradual compared to group A. This can be attributed to the presence of *T. viride* in group B media. Treatment B5 had a lower pH than the other treatments. The pH of B5did not reach a peak but reduced within the first 72 hr and remained constant at an approximate pH of 6.4. The pH of B5 was lower than A5, which can be attributed to the presence of *T. viride*. For most crops, the optimum pH is between 6.0 and 7.8 (Deina, 2019). Usually, 65% of the applied nutrients are available for plants at pH levels below 5.9, while 35% may be available below a pH of 5.5. Thus, except A5 and B5, all the other mixed media treatments were suitable for plant growth.

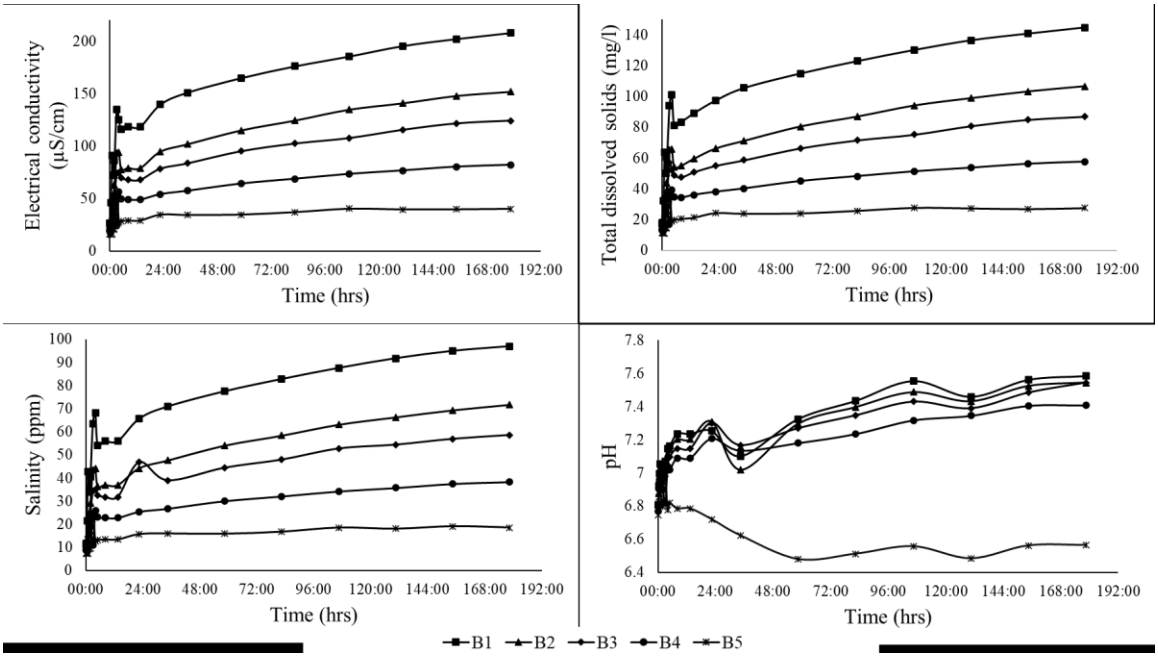


Figure 2. Changes in electric conductivity, salinity, total dissolved solids and pH for different combinations of vermicast-Trichoderma-sawdust media (group B) during December 2018 to February 2019 incubation period. B1, 80% vermicast+Trichoderma+20% sawdust; B2, 60% vermicast+Trichoderma+40% sawdust; B3, 40% vermicast+Trichoderma+60% sawdust; B4, 20% vermicast+Trichoderma+80% sawdust; B5, Trichoderma+100% sawdust.

Consistently, pH did not vary amongst the treatments whether *T. viride* was added or not, except for A5 was higher than B5.

The water quality indices exhibited small continuous fluctuations in the first 5 hr for both group A and group B treatments (Figures1 & 2). The EC, salinity and TDS of all the treatments increased slowly. The curves for A1 and B1 treatments were consistently the highest. These results suggested that the values for the nutrient-release indices were higher for group A treatments compared to those for group B treatments. Therefore, it can be said that the addition of *T. viride* had slight influence on media, but it did not have significant effect on the different media in terms of EC,

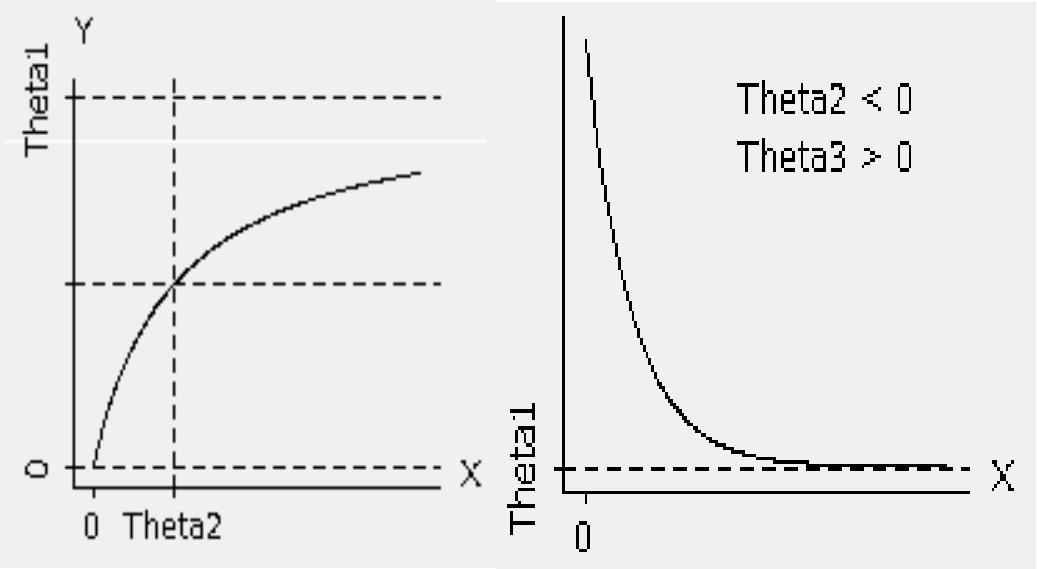
salinity, TDS and pH. A t-test showed that the pH for group A treatments were significantly ($p<0.05$) higher than the pH for group B (Table 2). Apart from the EC values for treatments A4 and A5 that were significantly ($p<0.05$) higher than the EC values for treatments B4 and B5, all the other group A treatments had higher EC values than their corresponding group B treatments. However, TDS and salinity values for A1 were slightly lower but not significant than those for B1; while values for A2, A3, A4 and A5 were respectively higher than values for B2, B3, B4 and B5. Therefore, the mixed media without *T. viride* was found to be likely more efficacious than those media added with *T. viride*.

Table 2. Two-sample t-test for comparing the pH, electrical conductivity (EC), total dissolved solids (TDS) and salinity for the mixed media without (A) and with (B) *T. viride* during December 2018 to February 2019 incubation period.

	pH	EC	TDS	Salinity
A1	7.41±0.16	122.4±43.9	84.6±31.5	57.2±20.8
B1	7.21±0.23	125.4±56.7	88.6±39.8	59.4±26.9
p-value	0.003	ns	ns	ns
A2	7.37±0.13	101.8±38.0	71.0±26.2	47.5±17.7
B2	7.18±0.23	88.3±41.1	61.9±28.7	41.3±19.5
p-value	0.002	ns	ns	ns
A3	7.35±0.12	88.7±43.2	62.0±30.2	41.6±20.2
B3	7.16±0.21	73.0±33.2	51.4±23.2	34.7±16.1
p-value	0.002	ns	ns	ns
A4	7.30±0.10	67.4±25.4	47.0±17.9	31.4±12.1
B4	7.09±0.19	49.5±21.9	34.6±15.3	23.0±10.2
p-value	<0.001	0.026	0.028	0.027
A5	7.00±0.20	44.07±9.37	30.99±6.80	20.46±4.48
B5	6.72±0.16	30.30±10.50	21.00±7.22	13.91±4.93
p-value	<0.001	<0.001	<0.001	<0.001

Values are means ± standard errors; ns, nonsignificant at P>0.05; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

The nonlinear regression model developed for the nutrient-release pattern using Michaelis-Menton model was nutrient-release = [θ 1 * Time / (θ 2 + Time)] (Figure 3A).The pH model for A5 and B5 was the asymptotic model [θ1 - θ2 * exp (- θ3 * Time)] (Figure 3B). Table 3 shows all the parameters used in the nonlinear regression model. We can use the regression model to predict the trend of nutrient-release pattern and extrapolate it to outside the observed range.



(A) (B)

Figure 3. Model for nutrient-release pattern for mixed growing media. (A) is the model applied to the electric conductivity, salinity, total dissolved solids and pH of all the treatments; and (B) is the model used for the pH of the sawdust alone.

Table 3. Nonlinear regression models on electric conductivity (EC), salinity, total dissolved solids (TDS) and pH of inoculated without (A) or with (B) *T. viride* at different vermicast-sawdust ratio during December 2018 to February 2019 incubation period.

Media	EC		Salinity		TDS		pH		
	θ1	θ2	θ1	θ2	θ1	θ2	θ1	θ2	θ3
A1	159.08	1.04	74.58	1.06	111.42	1.16	7.47	0.01	
A2	134.54	1.22	62.76	1.21	93.37	1.18	7.42	0.01	
A3	138.07	3.63	64.65	3.63	96.51	3.64	7.39	0.01	
A4	91.46	1.62	42.93	1.67	64.13	1.64	7.33	0.01	
A5	51.00	0.43	23.79	0.45	36.12	0.47	6.86	-0.34	0.14
B1	178.43	1.89	83.82	1.77	124.96	1.76	7.30	0.02	
B2	127.82	2.18	60.10	2.24	89.63	2.18	7.27	0.02	
B3	105.03	2.11	50.64	2.26	73.44	2.00	7.24	0.02	
B4	71.08	2.21	33.20	2.27	49.83	2.23	7.16	0.01	
B5	37.21	0.73	17.12	0.74	25.71	0.70	6.52	-0.35	0.03

A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

It was found that unstructured (UN) covariance was appropriate for TDS, EC, salinity and pH. Consequently, all the TDS, EC, salinity and pH values were used in the UN covariance structure. The multiple means comparison was done once the repeated measures analysis (RMA) (Proc

MIXED) showed a significant difference ($p<0.05$) (Table 4).The RMA results showed that the interaction effect Time*_Trichoderma_*Treatment of total dissolved solids was significantly different ($p=0.0102$) (Table 4) and therefore, the rest of the 2-way interactions and main effects were ignored. The MMC comparison result found the 34.5 hr, with *T. viride*, A1 and B1 combination had the highest TDS least-squares mean of 10.18. The lowest TDS least-squares mean was at 0.5 hr, with *T. viride*, A5 and B5 of 3.35 (Table S1).

Table 4.Repeated measures analysis for total dissolved solids, electrical conductivity, salinity and pH using unstructured Covariance Structure during December 2018 to February 2019 incubation period.

RMA outcome	Num DF	Den DF	F-Value	P-value
Total dissolved solids				
Time	12	390	361.46	<0.0001
T. viride	1	390	150.27	<0.0001
Time**T. viride*	12	390	3.57	<0.0001
Treatment	4	390	414.98	<0.0001
Time*Treatment	48	390	13.94	<0.0001
*T. viride*Treatment	4	390	8.48	<0.0001
Time**T. viride*Treatment	48	390	1.59	0.0102
Electrical conductivity				
Time	12	390	228.08	<0.0001

T. viride	1	390	75.97	<0.0001
Time**T. viride*	12	390	1.57	0.0978
Treatment	4	390	279.63	<0.0001
Time*Treatment	48	390	16.26	<0.0001
*T. viride**Treatment	4	390	2.23	0.0655
Time**T. viride**Treatment	48	390	1.25	0.1345
Salinity				
Time	12	390	231.42	<0.0001
T. viride	1	390	70.31	<0.0001
Time**T. viride*	12	390	2.01	0.0225
Treatment	4	390	303.74	<0.0001
Time*Treatment	48	390	16.76	<0.0001
*T. viride**Treatment	4	390	3.56	0.0073
Time**T. viride**Treatment	48	390	1.25	0.1325
pH				
Time	12	390	70.08	<0.0001
T. viride	1	390	3052.65	<0.0001

Time*_T. viride_	12	390	17.46	<0.0001
Treatment	4	390	434.12	<0.0001
Time*Treatment	48	390	27.18	<0.0001
_T. viride_*Treatment	4	390	0.61	0.6530
Time*_T. viride_*Treatment	48	390	3.83	<0.0001

DF, degrees of freedom; numerator df = k–1; denominator df = N–k.

The RMA for EC showed a significant ($p<0.0001$)2-way interaction i.e. Time *Treatment, and a significant ($p<0.0001$) main effect of *T. viride* (Table 4). For *T. viride* main effect on EC, it was confirmed that the media treatments without *T. viride* (i.e. group A) had better performance than when the *T. viride* was added (Table 5). It was found that 34.5 hr, A1 and B1 combination had the highest EC least-squares mean (141.14). The least EC least-squares mean was at 0.25 hr, A5 and B5 (22.67) (Table S2).

Table 5. Least-squares mean of electric conductivity for *T. viride* main effect during December 2018 to February 2019 incubation period.

Effect	Estimate	P-value
Without *T. viride*	68.4188	<0.0001
With *T. viride*	56.4762	<0.0001

For salinity, all the 2-way effects were significant i.e. Time**T. viride* (p=0.0225), Time*Treatment (p<0.0001) and *T. viride**Treatment (p=0.0073) (Table 4). For Time**T. viride* interaction on salinity, it was found that 34.5 hr, with *T. viride* combination had the highest salinity least squares mean (43.40). The least salinity least-squares mean was at 0 hr, with *T. viride* (22.67) (Table S3). For Time*Treatment interaction on salinity, it was found that 34.5 hr, A1 and B1 combination had the highest salinity least squares mean (66.51). The lowest salinity least-squares mean was at 0.25 hr, A5 and B5 (10.41) (Table S4). For *T. viride**Treatment interaction on salinity, it was found that media without *T. viride*, A1 and B1 combinations had the highest salinity least squares means (47.31). The lowest salinity least-squares mean was *T. viride*,A5 and B5 (12.11) (Table S5).The 3-way effect of pH i.e. Time**T. viride**Treatment interaction was significant (<0.0001) (Table 4).For Time**T. viride**Treatment interaction effect on pH, it was found that 22.5 hrs, without *T. viride*,A1 and B1 combinations had the highest pH least squares mean (7.51). The lowest pH least-squares mean (6.62) was the A5 and B5 released for 34.5 hrs and A2 and B2released for 2 hrs (Table S6).

Particularly, EC was found to be the best estimator for N compounds and K. In this experiment, treatments with lower EC were always accompanied by lower N and K contents (Table 6). Martínez-Suller et al. (2008) reported that there was a high positive correlation between EC and nutrient concentration. As such, EC was used to estimate mineral nutrients content of the media.

Table 6. The selected mineral nutrient content in vermicast-sawdust mixed media without (A) and with (B) adding Trichoderma during December 2018 to February 2019 incubation period.

Treatment	N (%)	EC (mmhos)	Ca (mg/L)	K (mg/L)	Mg (mg/L)	P (mg/L)	Na (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	B (mg/L)
A1	0.9a	3.4a	136.4a	353.6a	97.8a	22.4a	234.3a	303.7a	405.5a	ND
A2	0.8b	2.4b	84.7b	274.9b	57.5b	18.9a	156.8b	184.3b	310.0b	ND
A3	0.5c	1.6bc	52.3bc	215.3bc	34.1bc	14.4b	97.6c	132.7bc	286.0b	0.10b
A4	0.4d	1.1cd	42.3bc	199.4c	23.8bc	12.0b	61.8d	107.1cd	150.3c	0.13b
A5	0.2e	0.6d	14.4c	166.9c	4.3c	6.7c	12.1e	38.6d	24.0d	0.23a
B1	1.0a	3.5a	128.2a	356.3a	90.5a	20.0a	223.1a	273.1a	453.3a	ND
B2	0.8b	2.5b	93.7ab	306.0b	61.4ab	18.9ab	171.9b	193.1b	405.5a	0.10c
B3	0.6c	1.6c	65.7b	237.2c	42.0bc	16.2bc	115.2c	156.7bc	286.0b	0.11bc
B4	0.4d	1.1cd	50.3bc	213.9c	27.9cd	12.7c	69.1d	116.5c	107.2c	0.12b
B5	0.2e	0.5d	15.5c	166.3d	4.6d	6.6d	11.8e	34.4d	19.0d	0.25a

Means that do not share the same letter are significantly different at $P<0.05$; ND: data were not detected; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control).The corresponding treatments B1-B4 contained *T. viride*; N, nitrogen; EC, electric conductivity; Ca, calcium; K, potassium; Mg, magnesium; P, phosphorus; Na, sodium; $SO4_2^-$, sulphate; Cl^-, chloride; B, boron.

The differences in nutrients contents between the growing medium with *T. viride* (group B) and those without *T. viride* (group A) were not significant ($P>0.05$). In the present study, we found that A2 had the highest Nin the initial sampled media compared to A1 and B1, but the initial observation was reversed after the third sampling at 60 days with the B1 treatment having the highest N (Figure 4). The EC of A1 and B1 slowly decreased from 0 to 60 days and remained around 3 mmhos. The

EC of treatments A1-A4 and B1-B4 declined faster than those of A5 and B5. The EC values of declined in A1 by 42.4%, A2 by 41.2% and B1 by 50.4% compared to a reduced proportion of decline by 37.8% in A3, 23.2% inA4, 33.4% in B2, 28.6% in B3 and 35.7% in B4. The EC of A5 and B5 fluctuated between 0.5 and 0.65 mmhos. As vermicast was reduced in the medium and sawdust was increased, the EC values reduced as incubation time progresses (Figures 4). The nitrate (NO_3^-) contents of all the treatment samples were reduced to near zero after 60 days of incubation, irrespective of the media treatments (Figure 4). The Zn content of most of the medium treatments declined in the first 30 days and then increased again to the level observed at the first sampling.

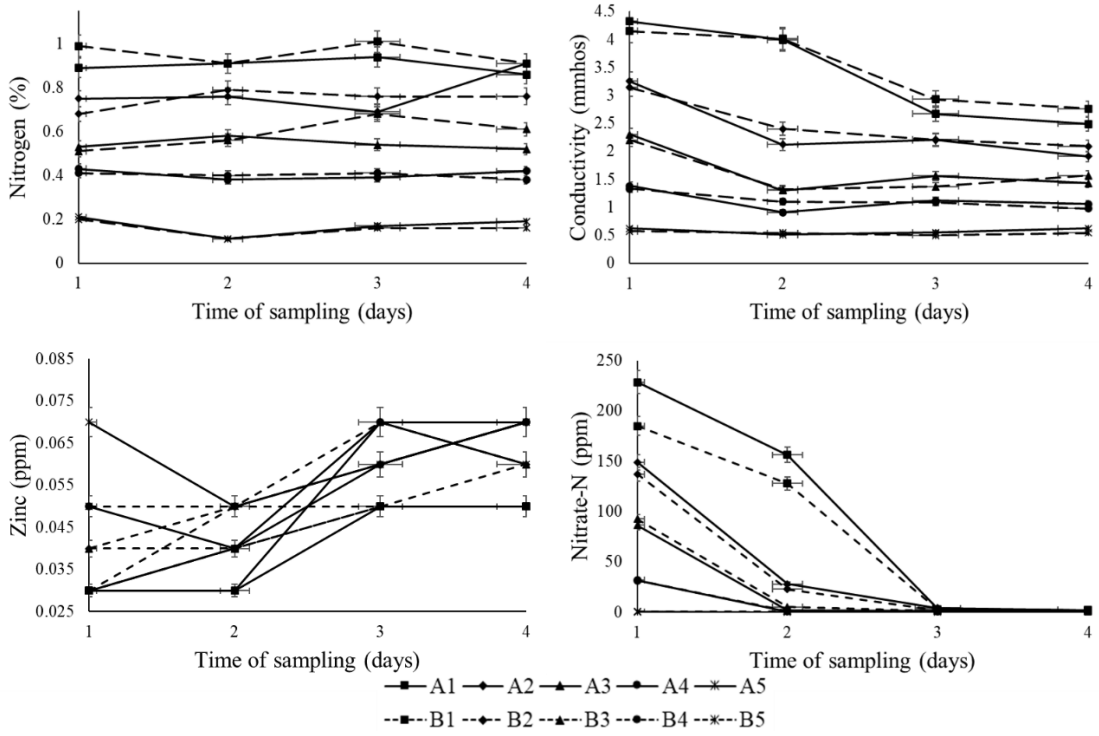


Figure 4. Percentage changes in total nitrogen, conductivity, zinc and Nitrate-N in different proportions of vermicast-sawdust mixed media with or without *T. viride* during December 2018 to February 2019 incubation period. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

The P contents of A5 and B5 were lower compared to the other treatments (Figure 5). The contents

of Ca, P, Mg and S were similar among the different treatments from the third sampling i.e. 60 days after incubation (Figures 5 & 6). However, the nutrients contents of A1 and B1 were reduced within the initial 60 days and remained the same afterwards.

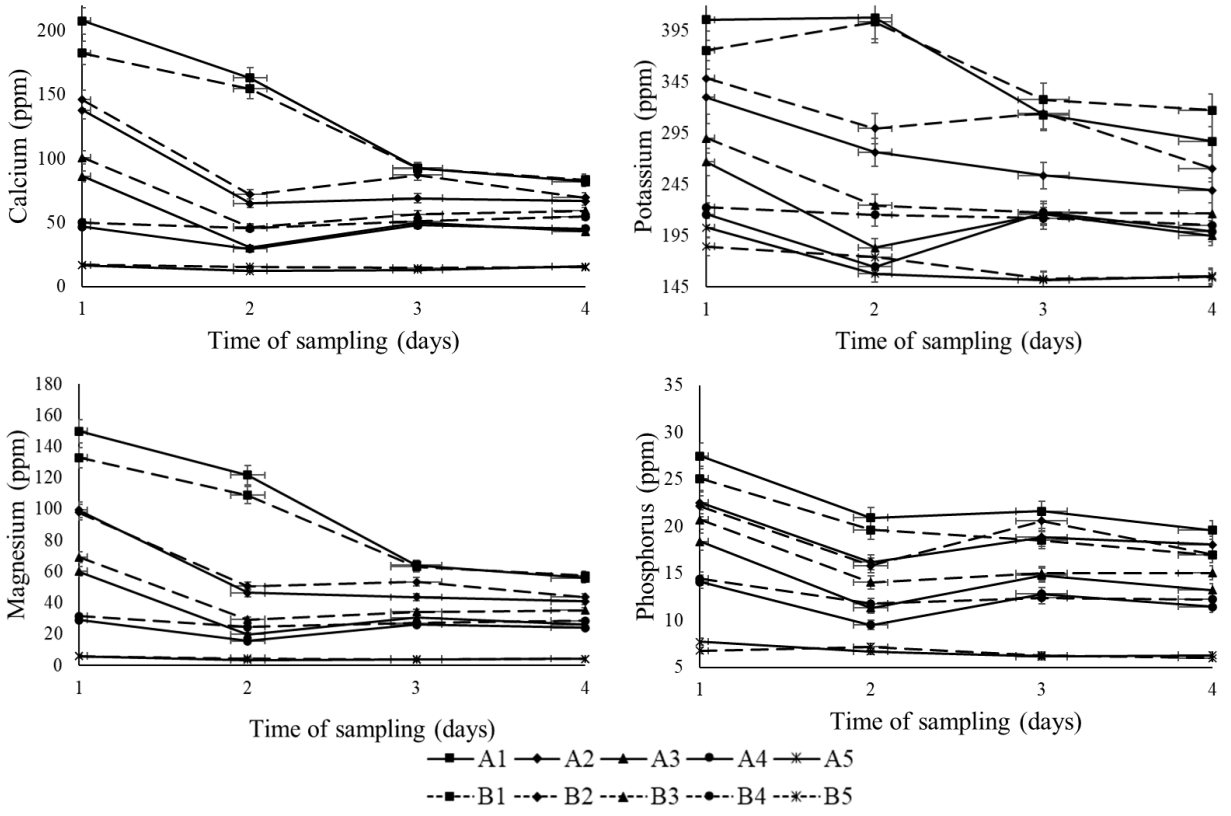


Figure 5. Changes in calcium (Ca), potassium (K), magnesium (Mg) and phosphorous (P) in different proportions of vermicast-sawdust mixed media with or without *T. viride* during December 2018 to February 2019 incubation period. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

The N, Ca, K, Mg, Na, S, and B contents of A1 and B1 were higher than the rest of the treatments (Figures 6 - 9). A similar finding was reported by Jadhav et al. (1997) who showed an increase in the uptake of N, P, K, and Mg by field-grown rice (*Oryza sativa*) when fertilizer was combined with vermicompost. Treatments A1 and B1 were higher in Na content than the other treatments (Figure 6). The Cl^- content of A2 and B2 increased as time progressed, but it did decline in the

other media treatments, which might be caused by microbial activities in the various media (Figure 6).

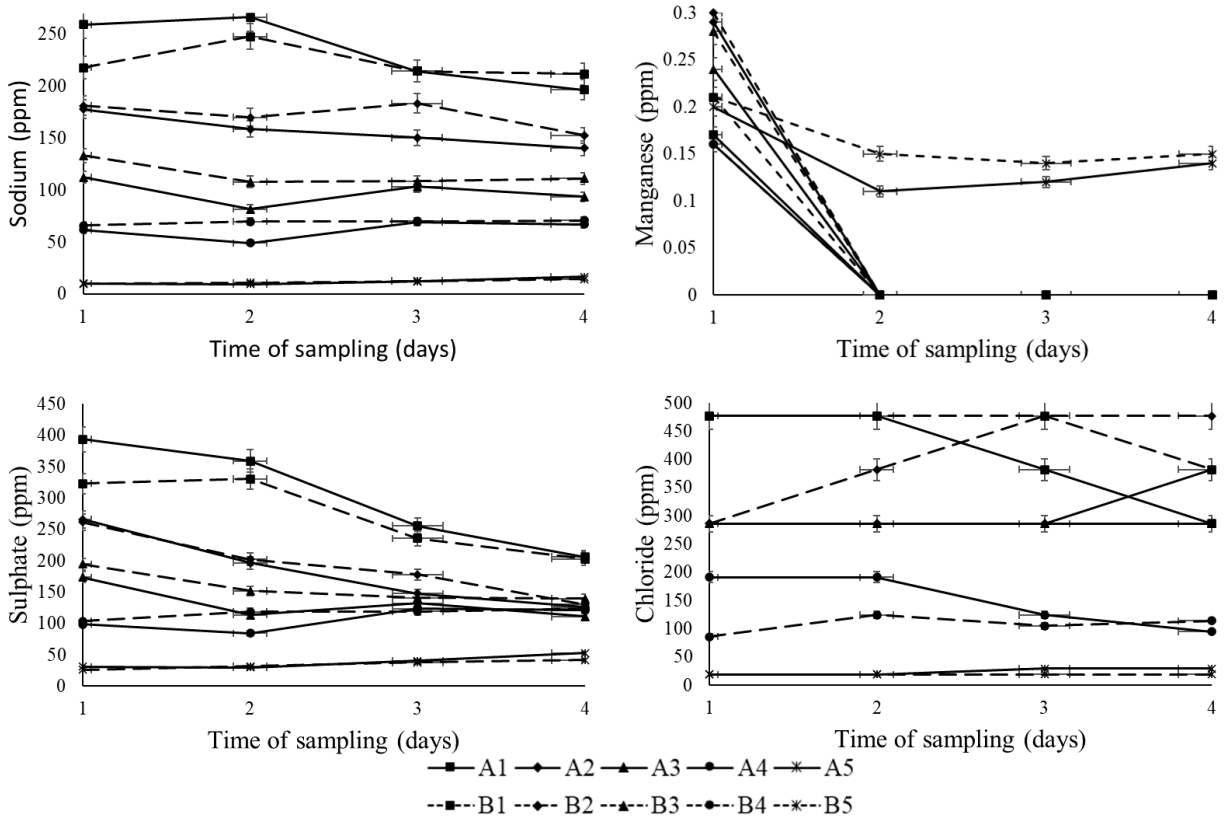


Figure 6. Changes of sodium (Na), sulphate (SO42-), manganese (Mn), and chloride (Cl-) in different proportions of vermicast-sawdust mixed media with or without *T. viride* during December 2018 to February 2019 incubation period. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

The contents of Al, B, Cu, Fe, Mg and Zn were all less than 0.5 mg/L (Figures 7& 8). An increase in growth medium Ca^{2+}, Mg^{2+} and K^{+} beyond certain concentration can inhibit root growth and water and nutrients uptake (Matúš, 2007). From Figure 7, the content of Cu in all the growing media increased as incubation time increased. Therefore, the more the vermicast and less incubation time the better the nutrient status of the growing medium and the more mineral nutrients available for plant use.

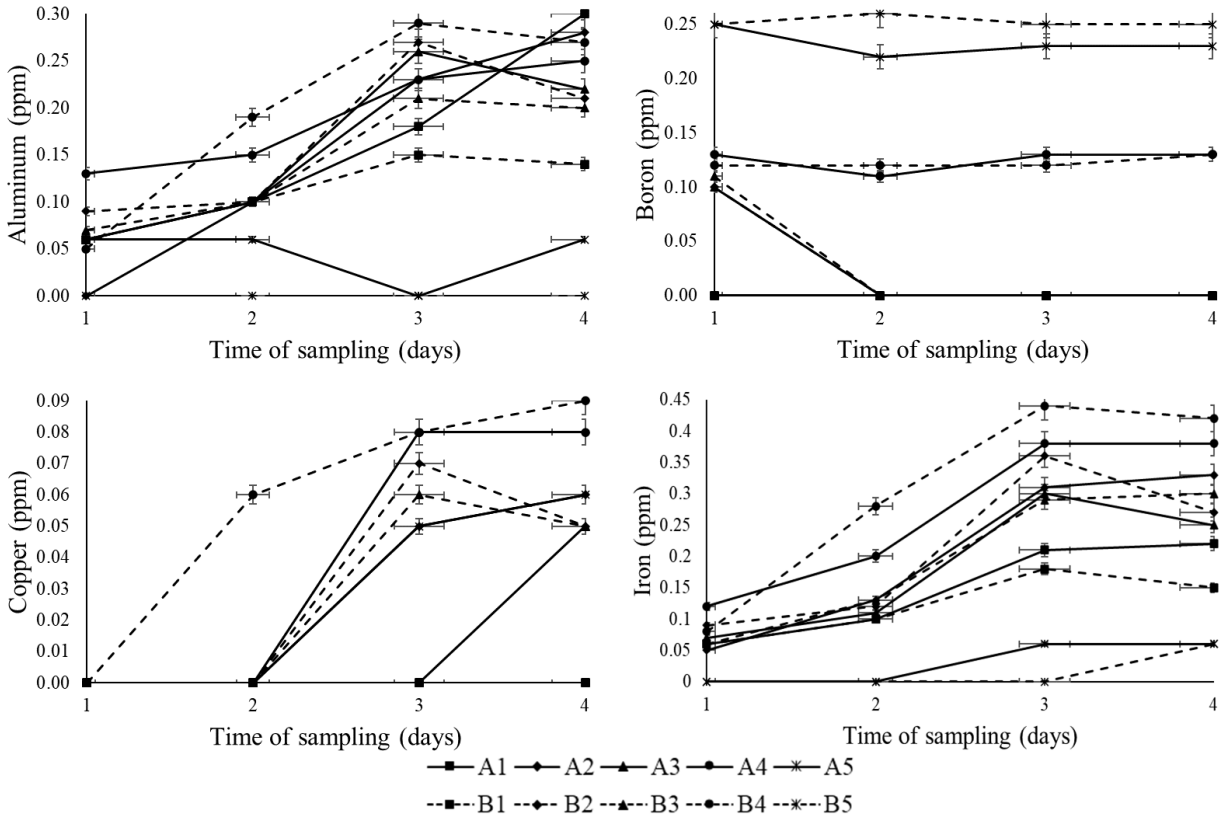
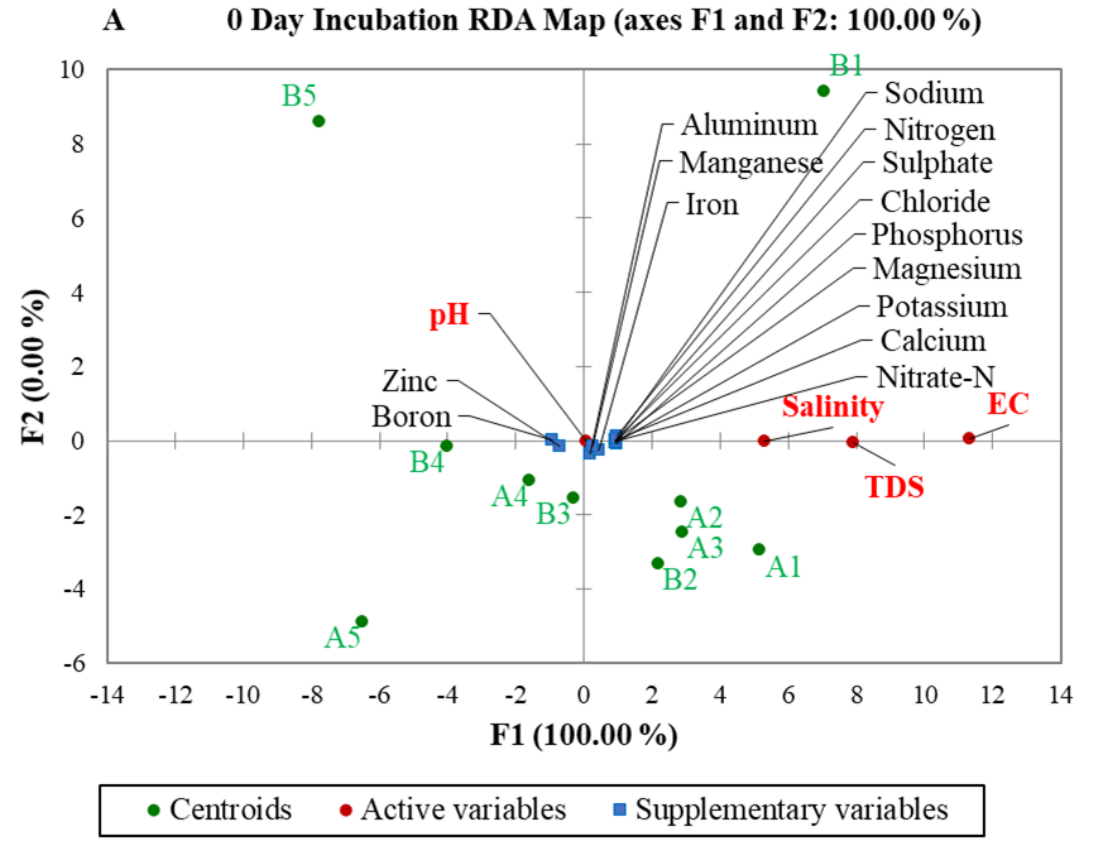
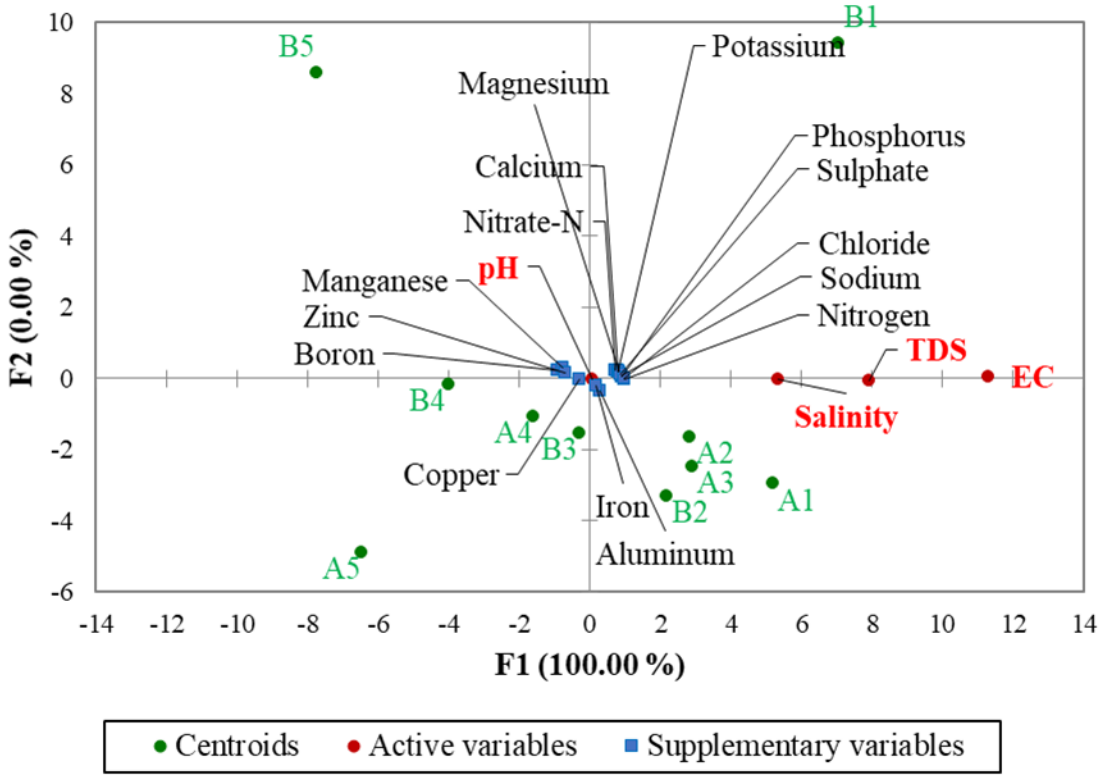


Figure 7. Changes in aluminum (Al), boron (B), copper (Cu) and iron (Fe) contents in different proportions of vermicast-sawdust mixed media with or without addition of *T. viride* during December 2018 to February 2019 incubation period. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.


A 0 Day Incubation RDA Map (axes F1 and F2: 100.00 %)
B1
B5
Sodium
Aluminum
Nitrogen
Manganese
Sulphate
Iron
Chloride
Phosphorus
Magnesium
pH
Potassium
Calcium
Zinc
Nitrate-N
Boron
Salinity
EC
B4
TDS
A4
B3
A2
A3
B2
A1
A5
F2 (0.00 %)
F1 (100.00 %)
Centroids
Active variables
Supplementary variables


B Days Incubation RDA Map (axes F1 and F2: 100.00 %)
B1
B5
Potassium
Magnesium
Phosphorus
Calcium
Sulphate
Nitrate-N
Chloride
pH
Sodium
Manganese
Nitrogen
Zinc
TDS
Boron
EC
B4
Salinity
A4
B3
A2
Copper
A3
A1
Iron
B2
Aluminum
A5
F2 (0.00 %)
F1 (100.00 %)
Centroids
Active variables
Supplementary variables

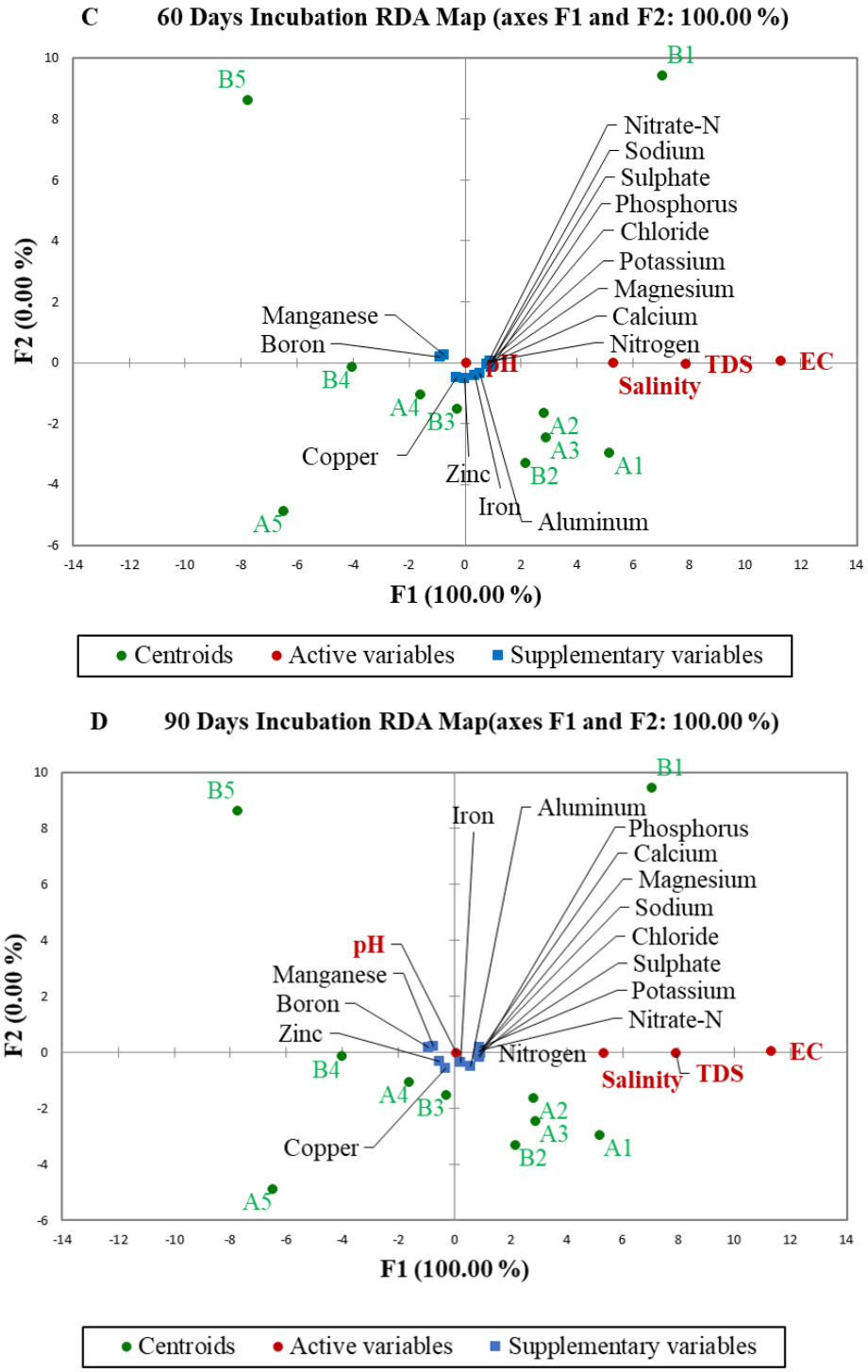


Figure 8 (A–D). Redundancy analysis (RDA) of the nutrient-release pattern and mineralization of the vermicast-*Trichoderma*-sawdust mixed media during December 2018 to February 2019 incubation period, 0 day (A), 30 days (B), 60 days (C), and 90 days (D). EC, electrical conductivity; TDS, total dissolved solids; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

The Figure 8 compared each mineralization sampling time on the electrical conductivity, total dissolved solids, salinity, and pH of sample released in water at 178.5 hrs. In 0 day incubation, A1, B1, A2, B2, and A3 had positive relationship with salinity, TDS, and EC. These five treatments also had positive relationship with most of minerals, except Zn and B had positive relationship with the rest treatments (Figure 8A). After 30 days incubation, the Mn and Cu changed and had opposite relationship with A1, A2, A3, and B2 (Figure 8B). The minerals distribution in 60 days was closed to the 0 day incubation, only Zn and B were had high correlation with B3, A4, B4, A5, and B5 (Figure 8A&C). Compared Figure 8D with Figure 8B&C, the distribution of minerals did not change too much. Overall, the A2, A3, B2, B3, A4, and B4 were closed to the central, which means the nutrient pattern of these treatments were mainly influenced by the minerals. Even from Figure 4-7, we concluded A1 and B1 had higher minerals than other treatments, Figure 8 showed the nutrient-release pattern of A2-A4 and B2-B4 were affected by minerals.

3.5 CONCLUSION

Total dissolved solids, electrical conductivity, and salinity in the solution of all the mixed growing medium treatments were progressively increased before reaching stable concentrations. Nutrient released fromA1 and B1 were the highest while A5 and B5 had the least and the others (i.e. A2, A3, A4, B2, B3 and B4) were intermediate. The interaction of Time *Treatment ($p<0.0001$) and the main effect of *T. viride* was significant ($p<0.0001$). Nutrients mineralization and availability were higher in the media treatments without *T. viride* (i.e. group A) compared to media with added *T. viride*. The highest EC combination was 34.5 hr with A1 and B1 and the least was 0.25 hr withA5 and B5). Moreover, the mixed media without *T. viride* had positively and significant effect on total

dissolved solids, electrical conductivity, and salinity. The N, Ca, K, Mg, Na, S and B contents of A1 and B1 were higher than the rest of the treatments. The RDA analysis showed the A5 and B5 had least relationship with nutrient-release pattern and mineralization in this experiment. A reduction in vermicast content of a mixed media leads to a significant reduction in mineral nutrients. This study did not report on microbial relationship with the different growing medium treatments. However, further studies are in progress to assess the growing media microbial dynamics and plants growth performance.

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3.7 SUPPLEMENTARY

Table S1. Least squares mean of total dissolved solids for Time*_Trichoderma viride_*Treatment interaction.

Effect	Time	*T. viride*	Treatment	Estimate	Estimate
Time**T. viride*Treatment	0	0	A1&B1	5.44	<.0001
Time**T. viride*Treatment	0	0	A2&B2	5.03	<.0001
Time**T. viride*Treatment	0	0	A3&B3	4.56	<.0001
Time**T. viride*Treatment	0	0	A4&B4	4.51	<.0001
Time**T. viride*Treatment	0	0	A5&B5	4.50	<.0001
Time**T. viride*Treatment	0	1	A1&B1	4.19	<.0001
Time**T. viride*Treatment	0	1	A2&B2	4.00	<.0001
Time**T. viride*Treatment	0	1	A3&B3	3.89	<.0001
Time**T. viride*Treatment	0	1	A4&B4	3.64	<.0001
Time**T. viride*Treatment	0	1	A5&B5	3.36	<.0001
Time**T. viride*Treatment	0.25	0	A1&B1	5.64	<.0001
Time**T. viride*Treatment	0.25	0	A2&B2	5.22	<.0001
Time**T. viride*Treatment	0.25	0	A3&B3	4.68	<.0001
Time**T. viride*Treatment	0.25	0	A4&B4	4.60	<.0001

Time*_T. viride_*Treatment	0.25	0	A5&B5	4.49	<.0001
Time*_T. viride_*Treatment	0.25	1	A1&B1	4.23	<.0001
Time*_T. viride_*Treatment	0.25	1	A2&B2	4.08	<.0001
Time*_T. viride_*Treatment	0.25	1	A3&B3	3.85	<.0001
Time*_T. viride_*Treatment	0.25	1	A4&B4	3.55	<.0001
Time*_T. viride_*Treatment	0.25	1	A5&B5	3.36	<.0001
Time*_T. viride_*Treatment	0.5	0	A1&B1	5.90	<.0001
Time*_T. viride_*Treatment	0.5	0	A2&B2	6.08	<.0001
Time*_T. viride_*Treatment	0.5	0	A3&B3	5.23	<.0001
Time*_T. viride_*Treatment	0.5	0	A4&B4	4.86	<.0001
Time*_T. viride_*Treatment	0.5	0	A5&B5	4.62	<.0001
Time*_T. viride_*Treatment	0.5	1	A1&B1	5.61	<.0001
Time*_T. viride_*Treatment	0.5	1	A2&B2	4.57	<.0001
Time*_T. viride_*Treatment	0.5	1	A3&B3	4.18	<.0001
Time*_T. viride_*Treatment	0.5	1	A4&B4	3.54	<.0001
Time*_T. viride_*Treatment	0.5	1	A5&B5	3.35	<.0001
Time*_T. viride_*Treatment	1	0	A1&B1	6.95	<.0001
Time*_T. viride_*Treatment	1	0	A2&B2	6.23	<.0001

Time*_T. viride_*Treatment	1	0	A3&B3	5.32	<.0001
Time*_T. viride_*Treatment	1	0	A4&B4	5.02	<.0001
Time*_T. viride_*Treatment	1	0	A5&B5	4.72	<.0001
Time*_T. viride_*Treatment	1	1	A1&B1	7.89	<.0001
Time*_T. viride_*Treatment	1	1	A2&B2	5.72	<.0001
Time*_T. viride_*Treatment	1	1	A3&B3	4.71	<.0001
Time*_T. viride_*Treatment	1	1	A4&B4	3.98	<.0001
Time*_T. viride_*Treatment	1	1	A5&B5	3.36	<.0001
Time*_T. viride_*Treatment	1.5	0	A1&B1	6.69	<.0001
Time*_T. viride_*Treatment	1.5	0	A2&B2	6.65	<.0001
Time*_T. viride_*Treatment	1.5	0	A3&B3	5.89	<.0001
Time*_T. viride_*Treatment	1.5	0	A4&B4	5.52	<.0001
Time*_T. viride_*Treatment	1.5	0	A5&B5	4.89	<.0001
Time*_T. viride_*Treatment	1.5	1	A1&B1	7.07	<.0001
Time*_T. viride_*Treatment	1.5	1	A2&B2	6.08	<.0001
Time*_T. viride_*Treatment	1.5	1	A3&B3	5.80	<.0001
Time*_T. viride_*Treatment	1.5	1	A4&B4	4.93	<.0001
Time*_T. viride_*Treatment	1.5	1	A5&B5	3.78	<.0001

Time*_T. viride_*Treatment	2	0	A1&B1	9.23	<.0001
Time*_T. viride_*Treatment	2	0	A2&B2	8.02	<.0001
Time*_T. viride_*Treatment	2	0	A3&B3	6.07	<.0001
Time*_T. viride_*Treatment	2	0	A4&B4	5.96	<.0001
Time*_T. viride_*Treatment	2	0	A5&B5	4.91	<.0001
Time*_T. viride_*Treatment	2	1	A1&B1	7.73	<.0001
Time*_T. viride_*Treatment	2	1	A2&B2	6.62	<.0001
Time*_T. viride_*Treatment	2	1	A3&B3	6.15	<.0001
Time*_T. viride_*Treatment	2	1	A4&B4	5.35	<.0001
Time*_T. viride_*Treatment	2	1	A5&B5	3.84	<.0001
Time*_T. viride_*Treatment	3	0	A1&B1	9.15	<.0001
Time*_T. viride_*Treatment	3	0	A2&B2	8.50	<.0001
Time*_T. viride_*Treatment	3	0	A3&B3	6.68	<.0001
Time*_T. viride_*Treatment	3	0	A4&B4	6.39	<.0001
Time*_T. viride_*Treatment	3	0	A5&B5	5.39	<.0001
Time*_T. viride_*Treatment	3	1	A1&B1	9.53	<.0001
Time*_T. viride_*Treatment	3	1	A2&B2	8.04	<.0001
Time*_T. viride_*Treatment	3	1	A3&B3	7.53	<.0001

Time*_T. viride_*Treatment	3	1	A4&B4	4.09	<.0001
Time*_T. viride_*Treatment	3	1	A5&B5	6.09	<.0001
Time*_T. viride_*Treatment	4	0	A1&B1	9.10	<.0001
Time*_T. viride_*Treatment	4	0	A2&B2	8.32	<.0001
Time*_T. viride_*Treatment	4	0	A3&B3	6.98	<.0001
Time*_T. viride_*Treatment	4	0	A4&B4	6.54	<.0001
Time*_T. viride_*Treatment	4	0	A5&B5	5.53	<.0001
Time*_T. viride_*Treatment	4	1	A1&B1	9.90	<.0001
Time*_T. viride_*Treatment	4	1	A2&B2	8.10	<.0001
Time*_T. viride_*Treatment	4	1	A3&B3	7.22	<.0001
Time*_T. viride_*Treatment	4	1	A4&B4	6.25	<.0001
Time*_T. viride_*Treatment	4	1	A5&B5	4.34	<.0001
Time*_T. viride_*Treatment	5	0	A1&B1	9.49	<.0001
Time*_T. viride_*Treatment	5	0	A2&B2	8.57	<.0001
Time*_T. viride_*Treatment	5	0	A3&B3	7.38	<.0001
Time*_T. viride_*Treatment	5	0	A4&B4	6.78	<.0001
Time*_T. viride_*Treatment	5	0	A5&B5	5.66	<.0001
Time*_T. viride_*Treatment	5	1	A1&B1	8.91	<.0001

Time*_T. viride_*Treatment	5	1	A2&B2	7.37	<.0001
Time*_T. viride_*Treatment	5	1	A3&B3	6.98	<.0001
Time*_T. viride_*Treatment	5	1	A4&B4	5.90	<.0001
Time*_T. viride_*Treatment	5	1	A5&B5	4.44	<.0001
Time*_T. viride_*Treatment	8	0	A1&B1	9.71	<.0001
Time*_T. viride_*Treatment	8	0	A2&B2	8.73	<.0001
Time*_T. viride_*Treatment	8	0	A3&B3	7.90	<.0001
Time*_T. viride_*Treatment	8	0	A4&B4	7.00	<.0001
Time*_T. viride_*Treatment	8	0	A5&B5	5.79	<.0001
Time*_T. viride_*Treatment	8	1	A1&B1	9.07	<.0001
Time*_T. viride_*Treatment	8	1	A2&B2	7.41	<.0001
Time*_T. viride_*Treatment	8	1	A3&B3	6.88	<.0001
Time*_T. viride_*Treatment	8	1	A4&B4	5.85	<.0001
Time*_T. viride_*Treatment	8	1	A5&B5	4.52	<.0001
Time*_T. viride_*Treatment	13.5	0	A1&B1	9.48	<.0001
Time*_T. viride_*Treatment	13.5	0	A2&B2	8.60	<.0001
Time*_T. viride_*Treatment	13.5	0	A3&B3	7.92	<.0001
Time*_T. viride_*Treatment	13.5	0	A4&B4	6.89	<.0001

Time*_T. viride_*Treatment	13.5	0	A5&B5	5.97	<.0001
Time*_T. viride_*Treatment	13.5	1	A1&B1	9.37	<.0001
Time*_T. viride_*Treatment	13.5	1	A2&B2	7.71	<.0001
Time*_T. viride_*Treatment	13.5	1	A3&B3	7.13	<.0001
Time*_T. viride_*Treatment	13.5	1	A4&B4	6.00	<.0001
Time*_T. viride_*Treatment	13.5	1	A5&B5	4.62	<.0001
Time*_T. viride_*Treatment	22.5	0	A1&B1	9.39	<.0001
Time*_T. viride_*Treatment	22.5	0	A2&B2	8.40	<.0001
Time*_T. viride_*Treatment	22.5	0	A3&B3	8.18	<.0001
Time*_T. viride_*Treatment	22.5	0	A4&B4	7.09	<.0001
Time*_T. viride_*Treatment	22.5	0	A5&B5	5.90	<.0001
Time*_T. viride_*Treatment	22.5	1	A1&B1	9.78	<.0001
Time*_T. viride_*Treatment	22.5	1	A2&B2	8.12	<.0001
Time*_T. viride_*Treatment	22.5	1	A3&B3	7.40	<.0001
Time*_T. viride_*Treatment	22.5	1	A4&B4	6.16	<.0001
Time*_T. viride_*Treatment	22.5	1	A5&B5	4.92	<.0001
Time*_T. viride_*Treatment	34.5	0	A1&B1	9.57	<.0001
Time*_T. viride_*Treatment	34.5	0	A2&B2	8.68	<.0001

Time*_T. viride_*Treatment	34.5	0	A3&B3	8.40	<.0001
Time*_T. viride_*Treatment	34.5	0	A4&B4	7.18	<.0001
Time*_T. viride_*Treatment	34.5	0	A5&B5	5.83	<.0001
Time*_T. viride_*Treatment	34.5	1	A1&B1	10.18	<.0001
Time*_T. viride_*Treatment	34.5	1	A2&B2	8.42	<.0001
Time*_T. viride_*Treatment	34.5	1	A3&B3	7.65	<.0001
Time*_T. viride_*Treatment	34.5	1	A4&B4	6.33	<.0001
Time*_T. viride_*Treatment	34.5	1	A5&B5	4.88	<.0001

T. viride levels, 0 means without it; 1 means with it. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

Table S2. Least squares mean of electrical conductivity for Time*Treatment interaction.

Effect	Time	Treatment	Estimate	Estimate
Time*Treatment	0	A1&B1	34.73	<.0001
Time*Treatment	0	A2&B2	29.61	<.0001
Time*Treatment	0	A3&B3	25.79	<.0001
Time*Treatment	0	A4&B4	25.48	<.0001
Time*Treatment	0	A5&B5	22.91	<.0001
Time*Treatment	0.25	A1&B1	35.69	<.0001
Time*Treatment	0.25	A2&B2	31.83	<.0001
Time*Treatment	0.25	A3&B3	26.54	<.0001
Time*Treatment	0.25	A4&B4	23.95	<.0001
Time*Treatment	0.25	A5&B5	22.67	<.0001
Time*Treatment	0.5	A1&B1	48.33	<.0001
Time*Treatment	0.5	A2&B2	41.85	<.0001
Time*Treatment	0.5	A3&B3	32.44	<.0001
Time*Treatment	0.5	A4&B4	26.05	<.0001
Time*Treatment	0.5	A5&B5	23.31	<.0001
Time*Treatment	1	A1&B1	80.58	<.0001

Time*Treatment	1	A2&B2	51.90	<.0001
Time*Treatment	1	A3&B3	36.55	<.0001
Time*Treatment	1	A4&B4	29.61	<.0001
Time*Treatment	1	A5&B5	24.31	<.0001
Time*Treatment	1.5	A1&B1	83.24	<.0001
Time*Treatment	1.5	A2&B2	56.49	<.0001
Time*Treatment	1.5	A3&B3	48.76	<.0001
Time*Treatment	1.5	A4&B4	39.23	<.0001
Time*Treatment	1.5	A5&B5	27.53	<.0001
Time*Treatment	2	A1&B1	104.35	<.0001
Time*Treatment	2	A2&B2	78.18	<.0001
Time*Treatment	2	A3&B3	53.74	<.0001
Time*Treatment	2	A4&B4	45.96	<.0001
Time*Treatment	2	A5&B5	27.91	<.0001
Time*Treatment	3	A1&B1	127.95	<.0001
Time*Treatment	3	A2&B2	98.86	<.0001
Time*Treatment	3	A3&B3	69.29	<.0001
Time*Treatment	3	A4&B4	41.51	<.0001

Time*Treatment	3	A5&B5	47.21	<.0001
Time*Treatment	4	A1&B1	121.70	<.0001
Time*Treatment	4	A2&B2	96.81	<.0001
Time*Treatment	4	A3&B3	72.36	<.0001
Time*Treatment	4	A4&B4	59.24	<.0001
Time*Treatment	4	A5&B5	35.66	<.0001
Time*Treatment	5	A1&B1	122.18	<.0001
Time*Treatment	5	A2&B2	91.99	<.0001
Time*Treatment	5	A3&B3	74.25	<.0001
Time*Treatment	5	A4&B4	58.25	<.0001
Time*Treatment	5	A5&B5	37.19	<.0001
Time*Treatment	8	A1&B1	126.89	<.0001
Time*Treatment	8	A2&B2	94.09	<.0001
Time*Treatment	8	A3&B3	78.75	<.0001
Time*Treatment	8	A4&B4	59.70	<.0001
Time*Treatment	8	A5&B5	38.48	<.0001
Time*Treatment	13.5	A1&B1	124.15	<.0001
Time*Treatment	13.5	A2&B2	92.43	<.0001

Time*Treatment	13.5	A3&B3	79.50	<.0001
Time*Treatment	13.5	A4&B4	58.46	<.0001
Time*Treatment	13.5	A5&B5	40.46	<.0001
Time*Treatment	22.5	A1&B1	136.31	<.0001
Time*Treatment	22.5	A2&B2	98.30	<.0001
Time*Treatment	22.5	A3&B3	87.83	<.0001
Time*Treatment	22.5	A4&B4	61.88	<.0001
Time*Treatment	22.5	A5&B5	41.26	<.0001
Time*Treatment	34.5	A1&B1	141.14	<.0001
Time*Treatment	34.5	A2&B2	104.72	<.0001
Time*Treatment	34.5	A3&B3	93.60	<.0001
Time*Treatment	34.5	A4&B4	65.74	<.0001
Time*Treatment	34.5	A5&B5	41.46	<.0001

T. viride levels, 0 means without it; 1 means with it. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

Table S3. Least squares mean of salinity for Time*_Trichoderma viride_ interaction.

Effect	Time	*T. viride*	Estimate	Estimate
Time**T. viride*	0	0	15.46	<.0001
Time**T. viride*	0	1	9.63	<.0001
Time**T. viride*	0.25	0	16.33	<.0001
Time**T. viride*	0.25	1	9.71	<.0001
Time**T. viride*	0.5	0	19.38	<.0001
Time**T. viride*	0.5	1	12.39	<.0001
Time**T. viride*	1	0	21.98	<.0001
Time**T. viride*	1	1	19.51	<.0001
Time**T. viride*	1.5	0	26.18	<.0001
Time**T. viride*	1.5	1	21.29	<.0001
Time**T. viride*	2	0	33.12	<.0001
Time**T. viride*	2	1	24.72	<.0001
Time**T. viride*	3	0	36.45	<.0001
Time**T. viride*	3	1	35.54	<.0001
Time**T. viride*	4	0	36.76	<.0001
Time**T. viride*	4	1	37.22	<.0001

Time*_T. viride_	5	0	39.73	<.0001
Time*_T. viride_	5	1	31.87	<.0001
Time*_T. viride_	8	0	42.35	<.0001
Time*_T. viride_	8	1	32.17	<.0001
Time*_T. viride_	13.5	0	41.51	<.0001
Time*_T. viride_	13.5	1	32.17	<.0001
Time*_T. viride_	22.5	0	41.22	<.0001
Time*_T. viride_	22.5	1	39.55	<.0001
Time*_T. viride_	34.5	0	43.40	<.0001
Time*_T. viride_	34.5	1	40.04	<.0001

T. viride levels, 0 means without it; 1 means with it.

Table S4. Least squares mean of salinity for Time*Treatment interaction.

Effect	Time	Treatment	Estimate	Estimate
Time*Treatment	0	A1&B1	15.65	<.0001
Time*Treatment	0	A2&B2	13.61	<.0001
Time*Treatment	0	A3&B3	11.90	<.0001
Time*Treatment	0	A4&B4	11.03	<.0001
Time*Treatment	0	A5&B5	10.53	<.0001
Time*Treatment	0.25	A1&B1	16.60	<.0001
Time*Treatment	0.25	A2&B2	14.49	<.0001
Time*Treatment	0.25	A3&B3	12.21	<.0001
Time*Treatment	0.25	A4&B4	11.38	<.0001
Time*Treatment	0.25	A5&B5	10.41	<.0001
Time*Treatment	0.5	A1&B1	22.36	<.0001
Time*Treatment	0.5	A2&B2	19.41	<.0001
Time*Treatment	0.5	A3&B3	14.99	<.0001
Time*Treatment	0.5	A4&B4	11.93	<.0001
Time*Treatment	0.5	A5&B5	10.73	<.0001
Time*Treatment	1	A1&B1	37.69	<.0001

Time*Treatment	1	A2&B2	24.25	<.0001
Time*Treatment	1	A3&B3	16.90	<.0001
Time*Treatment	1	A4&B4	13.71	<.0001
Time*Treatment	1	A5&B5	11.18	<.0001
Time*Treatment	1.5	A1&B1	38.15	<.0001
Time*Treatment	1.5	A2&B2	26.91	<.0001
Time*Treatment	1.5	A3&B3	22.94	<.0001
Time*Treatment	1.5	A4&B4	18.14	<.0001
Time*Treatment	1.5	A5&B5	12.53	<.0001
Time*Treatment	2	A1&B1	49.23	<.0001
Time*Treatment	2	A2&B2	35.95	<.0001
Time*Treatment	2	A3&B3	25.35	<.0001
Time*Treatment	2	A4&B4	21.28	<.0001
Time*Treatment	2	A5&B5	12.79	<.0001
Time*Treatment	3	A1&B1	60.03	<.0001
Time*Treatment	3	A2&B2	46.21	<.0001
Time*Treatment	3	A3&B3	32.35	<.0001
Time*Treatment	3	A4&B4	19.31	<.0001

Time*Treatment	3	A5&B5	22.05	<.0001
Time*Treatment	4	A1&B1	61.95	<.0001
Time*Treatment	4	A2&B2	45.23	<.0001
Time*Treatment	4	A3&B3	33.94	<.0001
Time*Treatment	4	A4&B4	27.34	<.0001
Time*Treatment	4	A5&B5	16.50	<.0001
Time*Treatment	5	A1&B1	57.45	<.0001
Time*Treatment	5	A2&B2	42.83	<.0001
Time*Treatment	5	A3&B3	34.45	<.0001
Time*Treatment	5	A4&B4	27.05	<.0001
Time*Treatment	5	A5&B5	17.21	<.0001
Time*Treatment	8	A1&B1	59.55	<.0001
Time*Treatment	8	A2&B2	44.11	<.0001
Time*Treatment	8	A3&B3	36.89	<.0001
Time*Treatment	8	A4&B4	27.83	<.0001
Time*Treatment	8	A5&B5	17.91	<.0001
Time*Treatment	13.5	A1&B1	58.16	<.0001
Time*Treatment	13.5	A2&B2	43.29	<.0001

Time*Treatment	13.5	A3&B3	37.08	<.0001
Time*Treatment	13.5	A4&B4	27.10	<.0001
Time*Treatment	13.5	A5&B5	18.58	<.0001
Time*Treatment	22.5	A1&B1	62.28	<.0001
Time*Treatment	22.5	A2&B2	45.90	<.0001
Time*Treatment	22.5	A3&B3	45.94	<.0001
Time*Treatment	22.5	A4&B4	28.86	<.0001
Time*Treatment	22.5	A5&B5	18.94	<.0001
Time*Treatment	34.5	A1&B1	66.51	<.0001
Time*Treatment	34.5	A2&B2	48.94	<.0001
Time*Treatment	34.5	A3&B3	43.55	<.0001
Time*Treatment	34.5	A4&B4	30.78	<.0001
Time*Treatment	34.5	A5&B5	18.81	<.0001

A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

Table S57. Least squares mean of salinity for *Trichoderma viride**Treatment interaction.

Effect	*T. viride*	Treatment	Estimate	Estimate
*T. viride**Treatment	0	A1&B1	47.31	<.0001
*T. viride**Treatment	0	A2&B2	38.55	<.0001
*T. viride**Treatment	0	A3&B3	30.15	<.0001
*T. viride**Treatment	0	A4&B4	24.79	<.0001
*T. viride**Treatment	0	A5&B5	18.37	<.0001
*T. viride**Treatment	1	A1&B1	45.86	<.0001
*T. viride**Treatment	1	A2&B2	30.85	<.0001
*T. viride**Treatment	1	A3&B3	26.54	<.0001
*T. viride**Treatment	1	A4&B4	17.63	<.0001
*T. viride**Treatment	1	A5&B5	12.11	<.0001

T. viride levels, 0 means without it; 1 means with it. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

Table S6. Least squares mean of pH for Time*_Trichoderma viride_*Treatment interaction.

Effect	Time	*T. viride*	Treatment	Estimate	Estimate
Time**T. viride*Treatment	0	0	A1&B1	7.25	<.0001
Time**T. viride*Treatment	0	0	A2&B2	7.21	<.0001
Time**T. viride*Treatment	0	0	A3&B3	7.26	<.0001
Time**T. viride*Treatment	0	0	A4&B4	7.21	<.0001
Time**T. viride*Treatment	0	0	A5&B5	7.20	<.0001
Time**T. viride*Treatment	0	1	A1&B1	6.81	<.0001
Time**T. viride*Treatment	0	1	A2&B2	6.82	<.0001
Time**T. viride*Treatment	0	1	A3&B3	6.79	<.0001
Time**T. viride*Treatment	0	1	A4&B4	6.77	<.0001
Time**T. viride*Treatment	0	1	A5&B5	6.74	<.0001
Time**T. viride*Treatment	0.25	0	A1&B1	7.19	<.0001
Time**T. viride*Treatment	0.25	0	A2&B2	7.21	<.0001
Time**T. viride*Treatment	0.25	0	A3&B3	7.17	<.0001
Time**T. viride*Treatment	0.25	0	A4&B4	7.26	<.0001
Time**T. viride*Treatment	0.25	0	A5&B5	7.19	<.0001
Time**T. viride*Treatment	0.25	1	A1&B1	6.92	<.0001
Time**T. viride*Treatment	0.25	1	A2&B2	6.88	<.0001
Time**T. viride*Treatment	0.25	1	A3&B3	6.91	<.0001
Time**T. viride*Treatment	0.25	1	A4&B4	6.90	<.0001
Time**T. viride*Treatment	0.25	1	A5&B5	6.85	<.0001

Time*_T. viride_*Treatment	0.5	0	A1&B1	7.26	<.0001
Time*_T. viride_*Treatment	0.5	0	A2&B2	7.23	<.0001
Time*_T. viride_*Treatment	0.5	0	A3&B3	7.20	<.0001
Time*_T. viride_*Treatment	0.5	0	A4&B4	7.19	<.0001
Time*_T. viride_*Treatment	0.5	0	A5&B5	7.19	<.0001
Time*_T. viride_*Treatment	0.5	1	A1&B1	7.00	<.0001
Time*_T. viride_*Treatment	0.5	1	A2&B2	6.98	<.0001
Time*_T. viride_*Treatment	0.5	1	A3&B3	7.00	<.0001
Time*_T. viride_*Treatment	0.5	1	A4&B4	6.95	<.0001
Time*_T. viride_*Treatment	0.5	1	A5&B5	6.94	<.0001
Time*_T. viride_*Treatment	1	0	A1&B1	7.24	<.0001
Time*_T. viride_*Treatment	1	0	A2&B2	7.23	<.0001
Time*_T. viride_*Treatment	1	0	A3&B3	7.24	<.0001
Time*_T. viride_*Treatment	1	0	A4&B4	7.17	<.0001
Time*_T. viride_*Treatment	1	0	A5&B5	7.20	<.0001
Time*_T. viride_*Treatment	1	1	A1&B1	7.06	<.0001
Time*_T. viride_*Treatment	1	1	A2&B2	7.02	<.0001
Time*_T. viride_*Treatment	1	1	A3&B3	6.98	<.0001
Time*_T. viride_*Treatment	1	1	A4&B4	7.00	<.0001
Time*_T. viride_*Treatment	1	1	A5&B5	6.90	<.0001
Time*_T. viride_*Treatment	1.5	0	A1&B1	7.20	<.0001
Time*_T. viride_*Treatment	1.5	0	A2&B2	7.22	<.0001

Time*_T. viride_*Treatment	1.5	0	A3&B3	7.26	<.0001
Time*_T. viride_*Treatment	1.5	0	A4&B4	7.20	<.0001
Time*_T. viride_*Treatment	1.5	0	A5&B5	7.19	<.0001
Time*_T. viride_*Treatment	1.5	1	A1&B1	7.01	<.0001
Time*_T. viride_*Treatment	1.5	1	A2&B2	6.95	<.0001
Time*_T. viride_*Treatment	1.5	1	A3&B3	6.97	<.0001
Time*_T. viride_*Treatment	1.5	1	A4&B4	6.96	<.0001
Time*_T. viride_*Treatment	1.5	1	A5&B5	6.83	<.0001
Time*_T. viride_*Treatment	2	0	A1&B1	7.23	<.0001
Time*_T. viride_*Treatment	2	0	A2&B2	7.27	<.0001
Time*_T. viride_*Treatment	2	0	A3&B3	7.20	<.0001
Time*_T. viride_*Treatment	2	0	A4&B4	7.16	<.0001
Time*_T. viride_*Treatment	2	0	A5&B5	7.01	<.0001
Time*_T. viride_*Treatment	2	1	A1&B1	7.02	<.0001
Time*_T. viride_*Treatment	2	1	A2&B2	6.98	<.0001
Time*_T. viride_*Treatment	2	1	A3&B3	6.98	<.0001
Time*_T. viride_*Treatment	2	1	A4&B4	6.90	<.0001
Time*_T. viride_*Treatment	2	1	A5&B5	6.80	<.0001
Time*_T. viride_*Treatment	3	0	A1&B1	7.34	<.0001
Time*_T. viride_*Treatment	3	0	A2&B2	7.32	<.0001
Time*_T. viride_*Treatment	3	0	A3&B3	7.30	<.0001
Time*_T. viride_*Treatment	3	0	A4&B4	7.28	<.0001

Time*_T. viride_*Treatment	3	0	A5&B5	7.04	<.0001
Time*_T. viride_*Treatment	3	1	A1&B1	7.07	<.0001
Time*_T. viride_*Treatment	3	1	A2&B2	7.08	<.0001
Time*_T. viride_*Treatment	3	1	A3&B3	7.05	<.0001
Time*_T. viride_*Treatment	3	1	A4&B4	6.82	<.0001
Time*_T. viride_*Treatment	3	1	A5&B5	7.00	<.0001
Time*_T. viride_*Treatment	4	0	A1&B1	7.38	<.0001
Time*_T. viride_*Treatment	4	0	A2&B2	7.39	<.0001
Time*_T. viride_*Treatment	4	0	A3&B3	7.36	<.0001
Time*_T. viride_*Treatment	4	0	A4&B4	7.29	<.0001
Time*_T. viride_*Treatment	4	0	A5&B5	7.07	<.0001
Time*_T. viride_*Treatment	4	1	A1&B1	7.15	<.0001
Time*_T. viride_*Treatment	4	1	A2&B2	7.12	<.0001
Time*_T. viride_*Treatment	4	1	A3&B3	7.05	<.0001
Time*_T. viride_*Treatment	4	1	A4&B4	7.02	<.0001
Time*_T. viride_*Treatment	4	1	A5&B5	6.78	<.0001
Time*_T. viride_*Treatment	5	0	A1&B1	7.34	<.0001
Time*_T. viride_*Treatment	5	0	A2&B2	7.29	<.0001
Time*_T. viride_*Treatment	5	0	A3&B3	7.31	<.0001
Time*_T. viride_*Treatment	5	0	A4&B4	7.28	<.0001
Time*_T. viride_*Treatment	5	0	A5&B5	7.16	<.0001
Time*_T. viride_*Treatment	5	1	A1&B1	7.16	<.0001

Time*_T. viride_*Treatment	5	1	A2&B2	7.14	<.0001
Time*_T. viride_*Treatment	5	1	A3&B3	7.10	<.0001
Time*_T. viride_*Treatment	5	1	A4&B4	7.02	<.0001
Time*_T. viride_*Treatment	5	1	A5&B5	6.82	<.0001
Time*_T. viride_*Treatment	8	0	A1&B1	7.38	<.0001
Time*_T. viride_*Treatment	8	0	A2&B2	7.37	<.0001
Time*_T. viride_*Treatment	8	0	A3&B3	7.33	<.0001
Time*_T. viride_*Treatment	8	0	A4&B4	7.26	<.0001
Time*_T. viride_*Treatment	8	0	A5&B5	6.94	<.0001
Time*_T. viride_*Treatment	8	1	A1&B1	7.24	<.0001
Time*_T. viride_*Treatment	8	1	A2&B2	7.20	<.0001
Time*_T. viride_*Treatment	8	1	A3&B3	7.15	<.0001
Time*_T. viride_*Treatment	8	1	A4&B4	7.09	<.0001
Time*_T. viride_*Treatment	8	1	A5&B5	6.79	<.0001
Time*_T. viride_*Treatment	13.5	0	A1&B1	7.44	<.0001
Time*_T. viride_*Treatment	13.5	0	A2&B2	7.43	<.0001
Time*_T. viride_*Treatment	13.5	0	A3&B3	7.33	<.0001
Time*_T. viride_*Treatment	13.5	0	A4&B4	7.25	<.0001
Time*_T. viride_*Treatment	13.5	0	A5&B5	6.88	<.0001
Time*_T. viride_*Treatment	13.5	1	A1&B1	7.24	<.0001
Time*_T. viride_*Treatment	13.5	1	A2&B2	7.20	<.0001
Time*_T. viride_*Treatment	13.5	1	A3&B3	7.15	<.0001

Time*_T. viride_*Treatment	13.5	1	A4&B4	7.09	<.0001
Time*_T. viride_*Treatment	13.5	1	A5&B5	6.79	<.0001
Time*_T. viride_*Treatment	22.5	0	A1&B1	7.51	<.0001
Time*_T. viride_*Treatment	22.5	0	A2&B2	7.46	<.0001
Time*_T. viride_*Treatment	22.5	0	A3&B3	7.42	<.0001
Time*_T. viride_*Treatment	22.5	0	A4&B4	7.34	<.0001
Time*_T. viride_*Treatment	22.5	0	A5&B5	6.94	<.0001
Time*_T. viride_*Treatment	22.5	1	A1&B1	7.25	<.0001
Time*_T. viride_*Treatment	22.5	1	A2&B2	7.31	<.0001
Time*_T. viride_*Treatment	22.5	1	A3&B3	7.30	<.0001
Time*_T. viride_*Treatment	22.5	1	A4&B4	7.21	<.0001
Time*_T. viride_*Treatment	22.5	1	A5&B5	6.72	<.0001
Time*_T. viride_*Treatment	34.5	0	A1&B1	7.43	<.0001
Time*_T. viride_*Treatment	34.5	0	A2&B2	7.38	<.0001
Time*_T. viride_*Treatment	34.5	0	A3&B3	7.35	<.0001
Time*_T. viride_*Treatment	34.5	0	A4&B4	7.32	<.0001
Time*_T. viride_*Treatment	34.5	0	A5&B5	6.90	<.0001
Time*_T. viride_*Treatment	34.5	1	A1&B1	7.10	<.0001
Time*_T. viride_*Treatment	34.5	1	A2&B2	7.02	<.0001
Time*_T. viride_*Treatment	34.5	1	A3&B3	7.16	<.0001
Time*_T. viride_*Treatment	34.5	1	A4&B4	7.14	<.0001
Time*_T. viride_*Treatment	34.5	1	A5&B5	6.62	<.0001

T. viride levels, 0 means without it; 1 means with it. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40%

sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

CHAPTER 4 MICROBIAL ACTIVITIES OF VERMICAST-TRICHODERMA-SAWDUST MIXED MEDIA

4.0 ABSTRACT

Vermicast-sawdust plant growing media are low-cost, rich in beneficial microbes and essential nutrients. However, very little is known concerning how sawdust-vermicast media combinations modulate the active microbial community, and nutrient mineralization. As such this study was conducted to determine whether vermicast-*Trichoderma*-sawdust mixed media favorably influences nutrient mineralization and the active microbial composition in vermicast-sawdust based mixed media. The mixed proportion of the vermicast-sawdust comprised of no *Trichoderma* (A), *Trichoderma* (B) containing 10^5 spores/g *T. viride*; and five levels of mixed media treatments (weight by weight) were delineated as: (A1) 80% vermicast+20% sawdust; (A2) 60% vermicast+40% sawdust; (A3) 40% vermicast+60% sawdust; (A4) 20% vermicast+80% sawdust; and (A5) sawdust (control). We observed that the addition of *T. viride* did not significantly ($p>0.05$) changed the active microbial community composition in any of the mixed media. We found six main microbial groups in the media following analysis of the microbial membrane phospholipid fatty acids (PLFA). These include: G+, G-, fungi, protozoa, eukaryotes and Archea. Treatments A2 and B2 showed higher pH values and amounts of Protozoa, F/B ratio and Fe. On the other hand, treatments B1 and B3 contained higher amounts of Na and PG. A moderate positive correlation ($0.3<r<0.7$) was observed between microbial groups and mineral nutrients in the mixed media. The results of phospholipid fatty acid (PLFA) determination showed that the mixed media with A2, A3, B2 and B3 had the most diverse active microbial community composition and the highest overall mineral nutrients content compared to the other media. Temporal reductions in microbial

community composition were observed in the incubated mixed growing media. After 30 days' incubation was significantly higher than the others for the sum of bacteria and fungi. For the sum of bacteria, samples within 30 days' incubation were higher. Thus, the vermicast-sawdust ratio of 40:60 and 60:40 (w/w) without incubation can potentially improve plant growth and productivity. Further study on DNA of the mixed media is in progress to assess the specific microbial population and their functionality on plant growth. Sequence-based approaches (i.e., DNA analysis) to study microbiomes in vermicast-sawdust based media can expose the associations between microbial taxa and a myriad of factors to improve crop production.

4.1 INTRODUCTION

Natural growing medium amendments such as compost and vermicast are universally acknowledged to improve growing medium quality and increase plant productivity. Vermicasts provide plants with numerous growth factors, including nutrients, amino acids. humins, fulvic or humic acids (Iheshiulo et al., 2017; Lazcano & Domínguez, 2011). Some studies indicated that under controlled environmental production systems, the performance of these natural growing media amendments is better than synthetic chemical fertilizers (Lazcano & Domínguez, 2011). Generally, the production of vermicast requires simple technology, is low cost, easily available, and is environmentally sustainable. Furthermore, vermicast increase growing medium quality by enhancing beneficial microbial activities or composition, as well as reducing risk of environmental pollution (Duong et al., 2012). Vermicast alone can be toxic to plants due to the concentration of mineral nutrients and other compounds (Abbey and Appah, 2017). However, vermicast mixed with other substrates, such as peat moss or coconut fiber was found to be efficacious and had positive

impact on plants performance. Sawdust, a waste product of the timber and wood processing industry is another option that can be used as a growing medium substrate for greenhouse plants (Sawan & Eissa, 1995), but not widely researched although it is low cost and more importantly, locally available, as well as have good water-holding capacity. Previous studies showed that application of sawdust-vermicompost extract (1:10, v/v; 1000 ppm) to the foliage of *Syngonium* plants increased mineral nutrients uptake, particularly N (Khomami et al., 2019).

Free-living fungi such as *Trichoderma viride* are also recognized to confer beneficial association with organic and inorganic natural substances (i.e., humic acids, protein hydrolysates, seaweed extracts, and silicon) (Fiorentino et al., 2018). *T. viride* are ubiquitous in different soils and root ecosystems. They have been reported to protect plants from pathogens by activating immunity mediated genes through the canonical defence signals jasmonic acid and salicylic acid pathways (Shoresh et al., 2010). Furthermore, soil microbes can form important associations or relationships with natural medium amendments including vermicast from which they can derive their nutrients for metabolism. Typically, these microbes mineralize or help to recycle nutrients during their metabolic activities in the growing media. This metabolic action can improve the media quality and nutrients availability for plant uptake, growth and productivity (Lichtfouse, 2010; He et al., 2013). Intact membranes of microbes contain phospholipids, which are characterized by acyl chains or fatty acids that can be associated to specific microbial taxa (Gómez-Brandón and Domínguez, 2010). Phospholipids are sufficiently complex to provide biomarkers for viable microbial biomass, community composition, nutritional and physiological status. A combination of molecular and biochemical screening can be used to determine active microbial community structure (Ying et al., 2013). Phospholipids fatty acids (PLFAs) are rapidly synthesized during microbial growth and are quickly degraded upon microbial death. They represent a 'fingerprint' of

the viable or active microbial community and do not function as storage compounds (Yao et al., 2000; Evershed et al., 2006; Gómez-brandón & Domínguez, 2010). Therefore, microbial PLFA provides qualitative and quantitative information about the structure of the microbial community, and it also indicates the presence and abundance of the main groups of microorganisms. PLFA reflect the current living community because those phospholipids are found in all living cell membranes, but not in storage molecules (Hirsch et al., 2010). Typically, PLFA can be used as biomarkers to assess the active microbial community composition such as gram-positive (G+) and gram-negative (G-) bacteria, actinomycetes, Archaea, fungi and protozoa living in the root rhizosphere (He et al., 2009; He et al., 2007; White et al., 1996). Hence, PLFA profiling is an efficient way to evaluate the active microbial community in the mixed media samples and can be used to represent and compare the biological components of growing media. Taking these into consideration, we hypothesized that vermicast-*Trichoderma*-sawdust (i.e. 40% sawdust + 60% vermicast formulation) mixed media used under controlled environmental conditions could enhance the growing media performance by improving the active microbial community composition and mineral nutrients content. Therefore, the object of the present study was to determine nutrient mineralization and active microbial composition and their relationship in incubated vermicast-*Trichoderma*-sawdust mixed media.

4.2 MATERIALS AND METHODS

4.2.1 Study Site and Sampling

A 3-month laboratory experiment on nutrient mineralization in incubated sawdust-vermicast mixed media was conducted in the Compost and Bio-stimulant Laboratory, Department of Plant, Food

and Environmental Science, Faculty of Agriculture, Dalhousie University, Canada. Samples of the incubated media were analyzed for active microbial composition at the Boreal Ecosystem and Agricultural Research Facility, Department of Environmental Science, Grenfell Campus, Memorial University of Newfoundland and Labrador, Canada.

Vermicast produced by earthworm castings excreted by Red wiggler worms (*Eisenia fetida*) was obtained from Pagonis Live Bait, ON, Canada; and heat-treated maple tree sawdust was obtained from Thermal Wood Canada, NB, Canada and used for the experiments. This experiment was arranged in a 2×5 factorial with four replications. The two factors were two levels of *Trichoderma viride* as follows: without (A) or with (B) 10^5 spores/g *T. viride*; and five levels of mixed media treatments (weight by weight) were delineated as: (A1) 80% vermicast + 20% sawdust; (A2) 60% vermicast + 40% sawdust; (A3) 40% vermicast + 60% sawdust; (A4) 20% vermicast + 80% sawdust; and (A5) sawdust alone (control). The corresponding mixed media treatments with added *T. viride* were labelled B1 – B5. Each treatment was mixed with 5 ml *T. viride* and 400 ml deionized water to make 10^5 spores/g by Fisherbrand Finnpipette II (https://www.pipette.com/21377822-Fisherbrand-Finnpipette-II-200-1000-uL). Every 1 kg sawdust-vermicast mixed media was partially decomposed by sufficiently mixed with tap water to improve its physicochemical properties before adding 2.5 L of distilled water. The individual mixed media were then incubated at room temperature for 90 days. A total of 160 mixed media samples were collected at the beginning followed by every 30 days during the incubation period. Thus, sampling was done at four different times. Therefore, the mixed media were coded as A1-1 to A5-4 and B1-1 to B5-4, where for example, A1-1 means 80% vermicast + 20% sawdust at the first sampling time. Each 100-g medium treatment sample were kept in a plastic bag and stored in a -20 ℃ freezer (Whirlpool, Mississauga, ON, CA) until analyzed.

4.2.2 Nutrient Mineralization

The media samples were sent to Nova Scotia Department of Agriculture Laboratory Services, Truro, Canada (http://www.gov.ns.ca/agri/qe/labserv/) for analyses of nitrate-N, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), chloride (Cl), sulphate (SO_4) and aluminum (Al) using the AOAC-968.08 inductively coupled plasma (ICP) mass spectrometer method (AOAC, 2003).

4.2.3 Lipid Extraction

The method of Zaeem et al. (2019) with slight modification was adopted for the extraction of PLFAs from the media samples. The total soil microbial fatty acids were extracted from 4-g ground mixed medium samples with 10 mL of 2:1 chloroform:methanol (v/v). The sample mixture was sonicated (Q700 Sonicator, Fisherbrand™ UK.) for 5 min at 50 amplitude followed by 5 sec of pulse on time, and then 10 sec of pulse off time. During sonication, the samples were kept in an ice bath to cool. After 24 hr incubation at room temperature, the supernatant was filtered with Whatman 42 filter paper (Sigma Aldrich, ON. Canada). The filtered samples were dried under a gentle stream of N in a pre-weighed sample vial. Extracted total lipids were resuspended in 2 ml chloroform followed by fractionation using a Visiprep™ SPE Vacuum Manifold and Discovery® DSC-Si SPE columns (50 µm, 70 Å, 100 mg/1 mL) (Sigma-Aldrich, Wilmington, DE, USA). Aliquots of 2.0 mL chloroform and 1.0 mL acetone were used to remove unwanted, undesired materials from the columns. Then 1.5 mL samples were added to the column followed by 2.0 mL chloroform, 2.0 mL acetone, and then 2.0 mL methanol were used to fractionate lipids into neutral

lipids, glycolipids, and phospholipids, respectively. The solution was slowly passed into the extraction tube under vacuum (approximately, 30 kPa). The phospholipid fractions were dried under a gentle stream of nitrogen and then resuspending in 500 µL of methyl tert-butyl ether (MTBE). 100 µL of solvent was used for GC-MS analysis, and the rest 400 µL of solvent was saved for later LC-MS analysis. The 100 µL of MTBE was derivatized using 50 µL trimethyl sulfonium hydroxide (TMSH) (Batista et al., 2001). The mixture in the vial was vortexed and incubated for 30 min at room temperature. After incubation, 50 µL samples were mixed with 10 µL of the internal standard methyl nonadecanoate (C18:0 Alkane/MTBE @ 1600 µg/mL) GC vials and the samples analyzed via GC-FID and GC-MS (Batista et al., 2001).

4.2.4 GC-FID/MS Analysis of Microbial PLFAs

Gas chromatography with flame ionization detection (GC-FID) or mass spectrometry (GC-MS) analysis method was adapted from Gómez-Brandón (2010) and Zaeem et al (2019). The methylated PLFAs were identified through retention times comparison and mass spectra obtained from commercial standards (i.e. Supelco 37 Component FAME Mix and Bacterial Acid Methyl Ester (BAME) CP mix) obtained from Sigma-Aldrich, Bellefonte, PA, USA) (Lazcano et al., 2013). BAME mix was prepared at five different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) diluted with n-hexane to obtain a standard curve. The standard curve was used to quantify individual PLFAs. A total of 25 BAME were identified (Table 2), which was used as biomarkers to assess the different active microbial groups living in the media (active microbial community composition) at the time of sampling. The PLFAs (Table S1) were used as biomarkers to determine the presence and abundance of specific microbial groups (Zelles, 1999, Zaeem et al 2019). The PLFAs considered to be predominantly of bacterial origin were further classified as G+ bacterial PLFAs

and G- bacterial PLFAs (Lazcano et al., 2013).

GC-FID was used for quantification while GC-MS was used for PLFA identification. GC-MS analysis was conducted on a Thermo Scientific Trace-1300 GC coupled to a Thermo Scientific TSQ 8000 Triple Quadrupole mass spectrometer (MS). GC-FID analysis was conducted on a Thermo Scientific Trace-1300 gas chromatography coupled to a flame ionization detector. Methylated fatty acids were separated with a BPX 70 high-resolution column (10 m × 0.1 mm × 0.2 μm; Life Science, Peterborough, Ontario, Canada) using helium (He) as the carrier gas at a flow rate of 15 mL/min. One (1 μL) of each sample was injected 1:15 in split mode using a Tri-plus autosampler. The oven temperature was programmed as follows: the initial oven temperature of 50°C was held for 0.75 min; then programmed to increase at 40°C min^{-1} to 175°C; increased at 6°C min^{-1} to 210°C; increased at 15°C min^{-1} to 250°C where it was held for 2 min. Total analysis time was 13.8 mins.

4.2.5 LC-MS Analysis of Intact Microbial Lipids

The intact microbial lipidome was also used to assist in evaluating the active microbial community composition in the media. A modified Bligh and Dyer (1959) method was used to extract total lipids from each sample. The samples extracted for GC-MS analysis was partitioned and 300 uL saved for LC-MS analysis (See extraction above). Prior to LC-MS analysis, the lipid extracts were re-filtered by Whatman™ Mini-UniPrep G2 Hand Compressor and syringeless disposable filter vials (GE Healthcare Biosciences, Pittsburgh, PA, USA).

4.2.5.1 Analysis of the intact lipids using C30 reverse-phase liquid chromatography coupled to high-resolution accurate mass tandem mass spectrometry (C30-RPLC-HRAM-MS/MS)

An Accucore C30 column (150×2 mm I.D., particle size: 2.6 µm, pore diameter: 150 Å) obtained from ThermoFisher Scientific (ON, Canada) was used to separate the intact lipids according to our previously published method (Pham et al., 2019). Briefly, reconstituted lipids (10 µL) were injected onto the column using the following mobile phase system: solvent A (acetonitrile:water, 60:40, v/v) containing 10 mM ammonium formate and 0.1% formic acid; and solvent B (isopropanol:acetonitrile:water, 90:10:1, v/v/v) containing 10 mM ammonium formate and 0.1% formic acid. A C30-RPLC separation was carried out with a flow rate of 0.150 mL/min at 30°C column oven temperature, and 10 µL of the lipid extract suspended in chloroform: methanol (1:1 v/v) was injected onto the column. The following system gradient was used for separating the lipid classes and molecular species: 30% solvent B for 3 min; then solvent B increased to 43% over 5 min, then to 50% B in 2 min, then to 90% B over 5 min, then to 99% B over 17 min and finally kept at 30% B for 8 min. The column was re-equilibrated to starting conditions (70% solvent A) for 10min prior to each new injection.

4.2.5.2 High-resolution accurate mass tandem mass spectrometry analysis

A Q-Exactive Orbitrap mass spectrometer was used to analyze the intact microbial lipids, and X-Calibur software 4.0 (TermoScientifc, MO, USA) was used to operate the instrument. The Q-Exactive mass spectrometer was controlled under the following parameters: sheath gas, 40; auxiliary gas, 2; ion spray voltage, 3.2 kV; capillary temperature, 300°C; S-lens RF, and 30V (Supplementary Table S2). The instrument was externally calibrated to 1 ppm using both positive and negative electrospray ionization mass spectrometry (ESI/MS) tune solutions (ThermoScientifc,

MO, USA). Moreover, tune parameters were optimized in both negative- and positive-ion modes using a mixture of lipid standards (Avanti Polar Lipids, Alabama, USA). Precise lipid identification was accessed from LipidSearch 4.1.9 software (Thermo Fisher Scientific, 2016; Taguchi & Ishikawa, 2010; Pham et al., 2019) with manual confirmation using Xcalibur 4.0.

4.2.6 Data Processing and Statistical Analysis

Chromatographic peaks were integrated using Xcalibur Quan Browser in order to construct calibration curves. Redundancy analysis (RDA) was done to determine the association between the growing media nutrient and microbial community composition. Pearson correlation coefficient was performed to assess the strength of the relationship. Analysis of variance (ANOVA) was done to determine whether treatments had any significant effects on microbial community composition and mineral nutrient content. Where treatment effects were significant, the means were separated using Fisher's least significant difference (LSD) test at α=0.05. A total of 40 samples were analyzed per experimental replicate. Statistical analysis was conducted using XLSTATS premium version (Addinsoft Inc, Paris, France). The comparison of treatments with and without *T. viride* was performed using group comparison analysis at α=0.05.

4.3 RESULTS AND DISCUSSION

A total of 26 PLFAs were identified and used as biomarkers to assess the presence of different active microbial groups in the media [A1-A5 and B1-B5] (Supplementary Table S1). The PLFA were used to diagnose the presence of the different groups of microbes and their rate of change in the media habitat as explained by Zelles (1999). The diversity of the active microbial community

is an important indicator of growing media quality and health status (Kong et al., 2011). A combination of PLFA markers previously reported in the literature was used to explain changes in the microbial community composition or structure in response to variations in the growing media composition. According to standard community structure method mentioned by Hedrick et al. (2005), we found six main microbial groups present as follows in the media: G+, G-, G+G-, fungi, protozoa and eukaryotes (Figure 1). Archea was also found in LC-MS analysis (Figure 3). All the treatments were combined into a single group to have an overview of the diversity in microbial population. Bacterial groups identified as G+, G- and G+G- were significant, and consistently higher in all the media treatments during the incubation time compared to fungi, eukaryotes and protozoan populations. G+ and G- contributed 18.98% and 35.53%, respectively, to the overall total microbial population observed on vermicast-sawdust media (Figure 1A). Compared to without adding *Trichoderma*, adding *Trichoderma* had lower ratio of fungi, protozoa, and eukaryote (Figure 1B). The most abundant microbes in the soil are bacteria (Reid, G. & Wong, P., 2005). In this project, the total of G+ and G- bacteria (sum of G+, G-, and G+G-) of vermicast-sawdust mixed media without (93.82%) and with (94.97%) adding *Trichoderma* made up the most of part of total microbial community (Figure 1).Forests and woodlot samples were observed to have higher relative abundance of G- bacteria in addition to monounsaturated fatty acids (Zelles, 1999; Bossio et al., 2005). Conversely, agricultural soil samples were reported to be indicative of higher levels of G+ bacteria in association with branched fatty acids (Haack et al., 1994). Therefore, the proportion of G+ bacteria (18.9%) relative to the overall observed total microbial population suggested that the content of both monounsaturated fatty acids and branched fatty acids might not be very high. According to Mathew et al. (2012), soil organic carbon positively correlates with PLFA biomarkers (r=0.98). A lower proportion of G+ bacterial population compared to G–

suggested a sufficiency of organic carbon in the media (Herman et al., 2012; Mathew et al., 2012; Pham et al., 2019). Consequently, our results indicated that the G+ bacterial population was higher than the G- bacterial population in the growing media irrespective of sawdust composition (Figure 1). The addition off sawdust to the growing media provided an excellent source of organic carbon to the media.

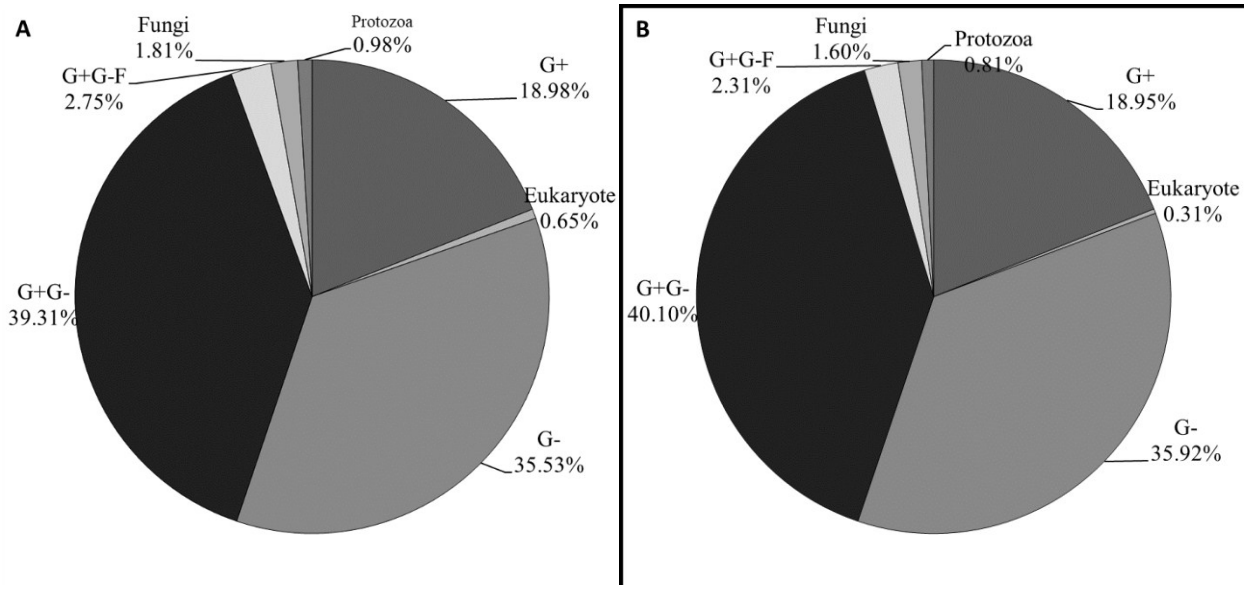
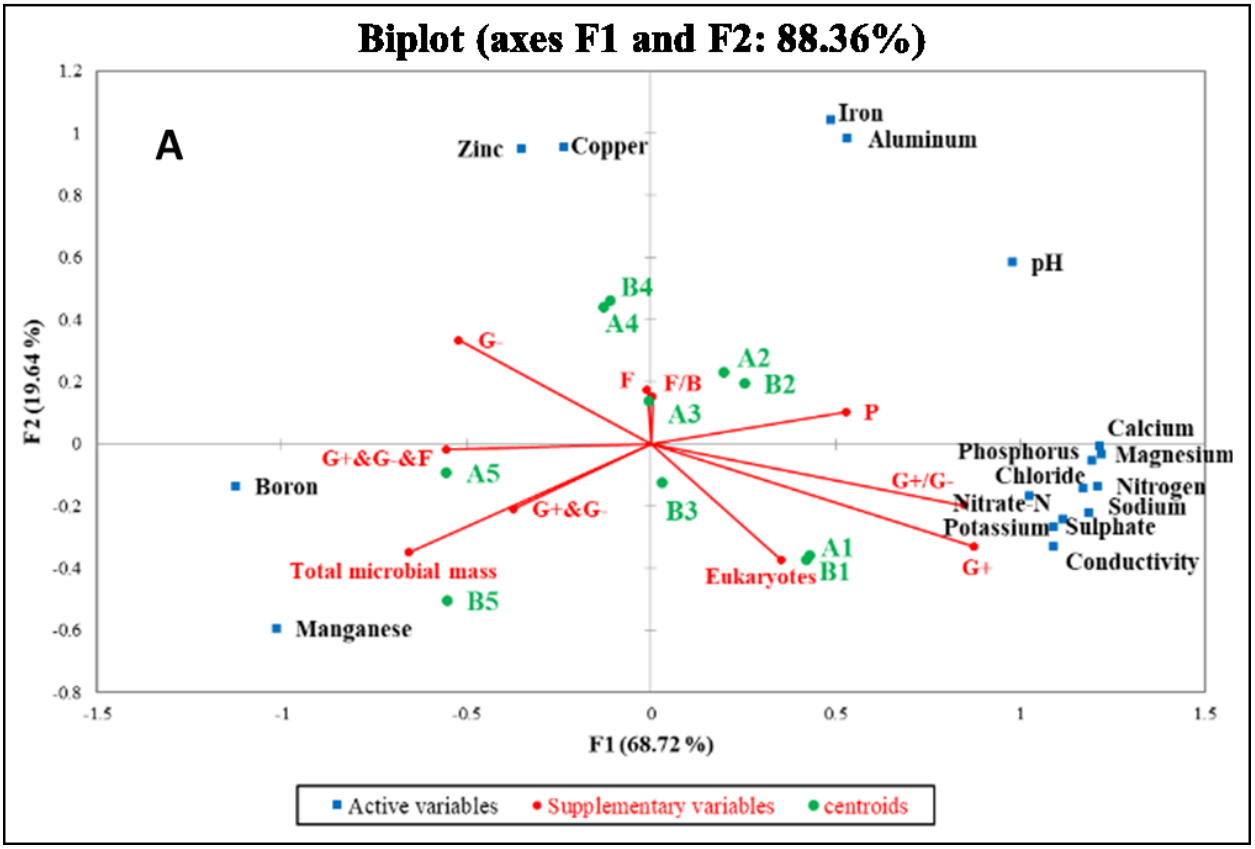


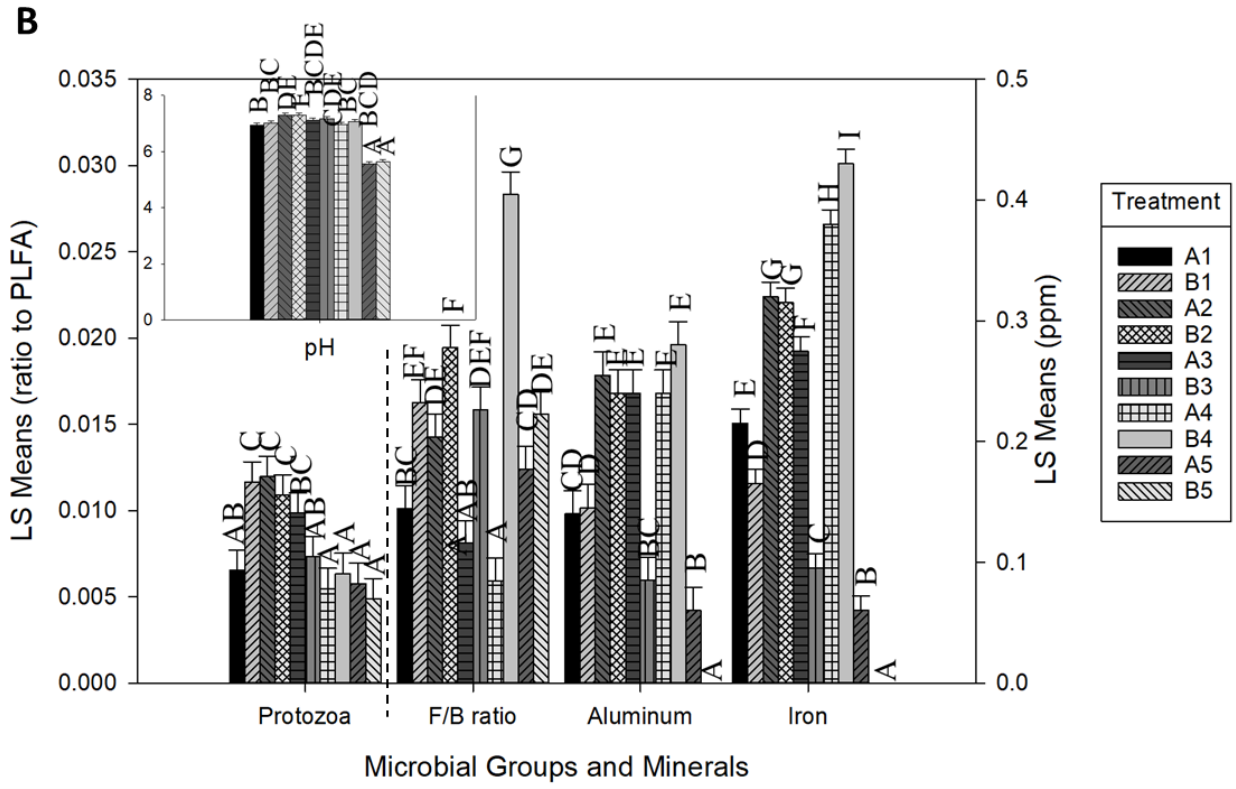
Figure 1. A pie chart showing average levels of six main microbial groups (G+, G-, G+G-, fungi, protozoa and eukaryotes) in percentage of vermicast-sawdust mixed media without (A) and with (B) adding Trichoderma during December 2018 to February 2019 incubation period. G+, gram-positive; G-, gram-negative; G+G-, microbes that cannot tell belonging to G+ or G-; G+G-F, microbes that cannot tell belonging to G+, G-, or F.

4.3.1 Comparison of Media Active Microbial Composition Following the Addition of *Trichoderma*

The media without *T. viride* (A) and with *T. viride* (B) were considered as two different treatment groups. A 2-sample t-test showed that eukaryotes, fungi and the ratio of fungi to bacteria differ significantly between the treatments A and B (Supplementary Table S3). *T. viride* showed little but

non-significant inhibitions (limitations) on the abundance of bacteria in the media. These results are in contrast with the results obtained by Leelavathi et al. (2014). In their work, the concentration of the bacterial species *Staphylococcus aureus* (G+), *Escherichia coli* (G-), and *Klebsiella* (G-) was reduced significantly by *T. viride* extract in a mixed medium comprising 0.2 g $MgSO_4$ ($7H_2O$), 0.9 g K_2HPO_4, 0.15 g KCl, 1.0 g NH_4NO_3, 3.0 g D+ glucose anhydrous, 0.15 g rose Bengal and 20g agar. The difference in the results can be ascribed to the differences in growing media composition, which suggested that *Trichoderma's* effect on bacterial composition is dependent on media components and the *Trichoderma* species (Leelavathi et al., 2014). In Leelavathi's studies, the strain of *Trichoderma* showed various degrees of inhibition of various plant pathogens. In our study, the G+ was slightly inhibited by the addition of *Trichoderma* (Supplementary Table S3) although it was not significant. Many findings also suggested that the development and biochemistry of plants were strongly affected by *Trichoderma* strains (Mach et al., 1993). Another confounding factor in our study was that we evaluated the effects of *T. viride*. on the bacterial classes or groups, while Leelavathi's studies did their evaluation at the species level. This could be the reason for the no statistically significant effect of the added *T. viride*.


Biplot (axes F1 and F2: 88.36%)
A
Zinc
Copper
Iron
Aluminum
pH
B4
A4
G-
A2
B2
F
F/B
A3
P
Calcium
Phosphorus
Magnesium
G+&G-&F
A5
Boron
Chloride
G+/G-
Nitrogen
Nitrate-N
Sodium
G+&G-
B3
Potassium
Sulphate
A1
Total microbial mass
Eukaryotes
B1
G+
Conductivity
B5
Manganese
F2 (19.64 %)
F1 (68.72 %)
Active variables
Supplementary variables
centroids


B
LS Means (ratio to PLFA)
LS Means (ppm)
pH
Protozoa
F/B ratio
Aluminum
Iron
Microbial Groups and Minerals
Treatment
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5

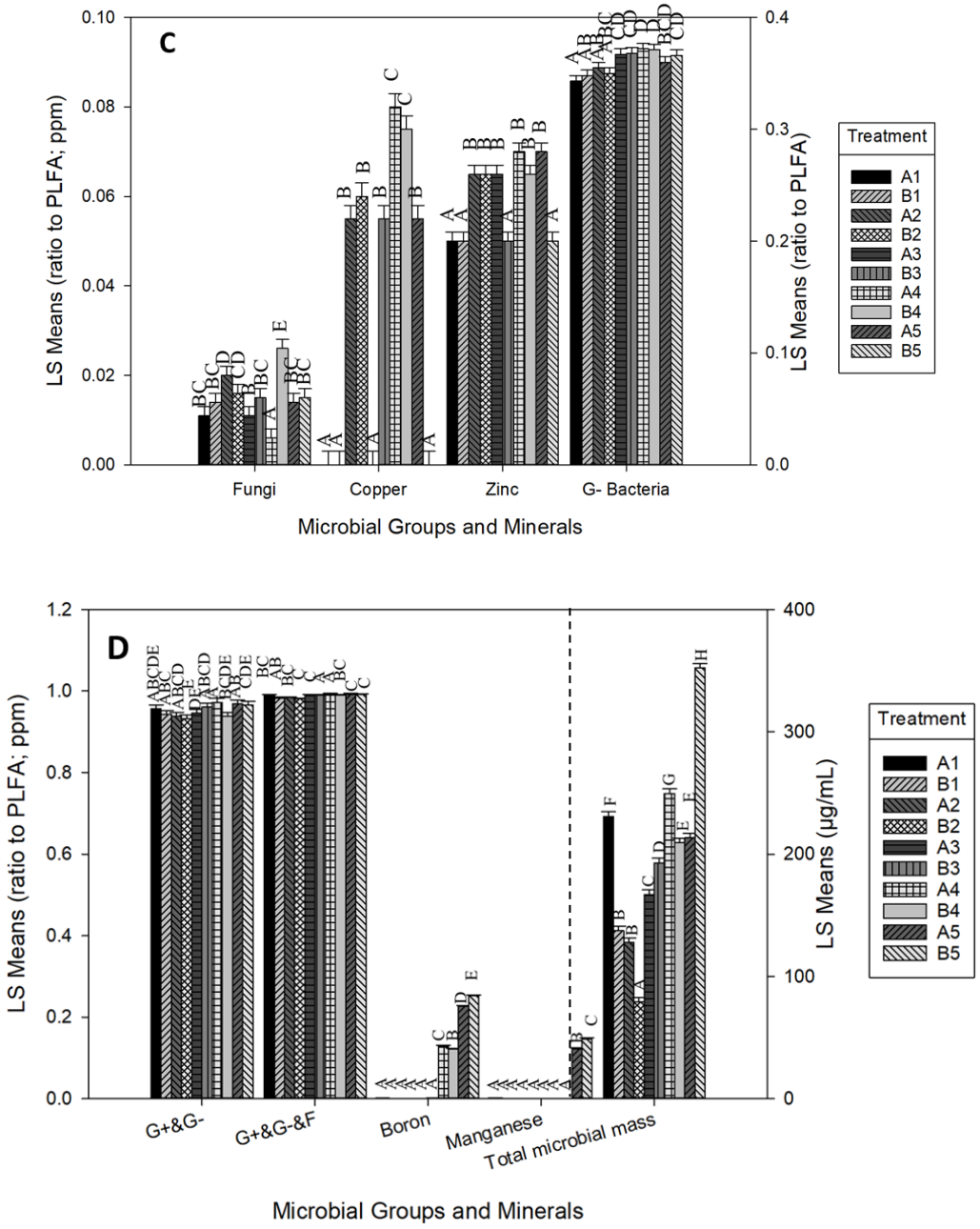


Figure 2. (A–D). The relationship between the active soil microbial community (PLFA), nutrient mineralization, and media amendments during December 2018 to February 2019 incubation period. A principal component analysis (PCA) biplot (A) showing the groupings of the nutrients, and treatments with active microbial composition. One-way ANOVA shows microbial groups and nutrient mineralization following principal component analysis of components segregated in (B) quadrants 1 (Q1), (C) Q2, and (D) Q3. Means in the same row accompanied by different superscripts are significantly different at LSD $\alpha = 0.05$, $n = 4$ per experimental replicate. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

4.3.2 Relationship Between Mineral Nutrients and Active Microbial Community

The microbial composition of the growing media is known to mineralize organic matter and other sources of plant nutrients in the media, so it is important to confirm their availability for plant growth and development (He et al., 2013). The PLFA are effective taxonomic markers because different groups of microorganisms synthesize a variety of PLFA through various biochemical pathways (David et al., 1998). In this section, the relationship between available mineral nutrients and active microbial community composition in the growing media during the incubation period was assessed through principal component analysis (PCA) and redundancy analysis (RDA). PCA was used to assess the relationships between the active microbial community composition and the mineral nutrients in the different media combinations (Figure 2a). The exact content of mineralized nutrients in all the media treatments (A1-A5 and B1-B5) are shown in the Supplementary Table S4. Output from the PCA analysis indicated that the growing media amendments were segregated into distinct quadrants of the biplot based on the composition of the microbial groups and the mineral nutrients. This segregation accounted for 88.4% of the total variability observed in the data. Aluminum (Al) content was significantly higher ($p<0.05$) in treatments A2, A3, A4, B2 and B5 than the other treatments (Figure 2a & b). Treatments A2, A3, A4, B2 and B4 had a significantly higher content of iron (Fe) compared to the other treatments (Figure 2a & b). Overall, treatments A2 and B2 showed higher pH, protozoa, F/B ratio, and Fe. Moreover, protozoa had a moderate linear relationship with Al and pH (Figure 2a & b).

Correlations between the various nutrients and microbial groups were assessed to confirm their relationships. Copper (Cu) was significantly higher ($p<0.0001$) in the treatments A4 and B4 (Table 1). Copper and zinc (Zn) did not show a strong correlation with fungi and G- bacteria (Figure 2c). Total bacterial (G+&G-), as well as the total bacterial and fungi (G+&G-&F), Boron (B),

Manganese (Mn), and total microbial mass were located in Quadrant 3 (Figure 2a). The dataset also showed that treatments A5 and B5 had higher B and Mn contents (Figure 2d). A moderate but significant correlation was observed between total microbial mass and B ($r=0.586$, $p<0.0001$), between G+G- and B ($r=0.394$; $p=0.012$), as well as G+&G-&F and B ($r=0.526$, $p<0.05$) (Table 1). Similarly, Mn was moderately correlated with total microbial mass ($r=0.636$, $p<0.0001$) but slightly with G+G- ($r=0.350$, $p=0.027$), and G+&G-&F ($r=0.367$, $p=0.020$).

Table 1. Pearson's Correlation coefficients for microbial populations and the minerals that were found to be significant in different quadrants of the RDA plot.

Quadrant	Variables	Aluminum	pH	Variables	Iron
1	Protozoa	0.35 **(0.028)**	0.50 **(0.001)**	F/B ratio	0.17 **(0.029)**
			Copper		
2	G-		0.309 **(0.052)**		
			Boron		Manganese
3	G+&G-		0.39 **(0.012)**		0.35 **(0.027)**
	G+&G-&F		0.53 **(< 0.05)**		0.37 **(0.020)**
	Total microbial mass		0.59 **(< 0.0001)**		0.64 **(< 0.0001)**
		Eukaryotes	G+/G-		G+
4	Phosphorus		**0.79 (< 0.0001)**		**0.87 (< 0.0001)**
	Chloride	0.45 **(0.004)**	**0.91 (< 0.0001)**		**0.91 (< 0.0001)**
	Sodium	0.39 **(0.013)**	**0.87 (< 0.0001)**		**0.93 (< 0.0001)**
	Nitrogen	0.40 **(0.011)**	**0.89 (< 0.0001)**		**0.90 (< 0.0001)**
	Potassium	0.40 **(0.011)**	**0.76 (< 0.0001)**		**0.89 (< 0.0001)**
	Conductivity	0.44 **(0.004)**	**0.84 (< 0.0001)**		**0.91 (< 0.0001)**

Sulphate	0.55 **(< 0.05)**	**0.88 (< 0.0001)**	**0.88 (< 0.0001)**
Nitrate-N		0.66 **(< 0.0001)**	**0.72 (< 0.0001)**
Magnesium	0.44 **(0.005)**	**0.89 (< 0.0001)**	**0.90 (< 0.0001)**
Calcium	0.41 **(0.008)**	**0.86 (< 0.0001)**	**0.87 (< 0.0001)**

Value are correlation values (p-value), with a significance level alpha=0.05. The other components were not shown if they were not significantly correlated.

High correlation of nitrogen content with G+ (r=0.90) and G+/G- (r=0.89) (Table 1), which might be due to N fixers in the media. Same, we found the high correlation of SO_4^{2-} and G+ (r=0.88) and G+/G- (r=0.88). From Reid and Wong fact sheet (2005), *Thiobacillus* bacteria can covert sulfides into sulfates. These bacteria exist in the media might lead the high correlation between sulphate and bacteria. G+ had high correlation with P, Cl, Na, K, NO_3^-, Mg, and Ca, those nutrients are essential for later plant growth. G+ had bad been proved that it could increase more than other taxonomic microbial groups on plant growth because of its greater ability on root exudates utilizing (Li et al., 2007). Even F/B ratio had significantly (p=0.027) weak correlation with Fe, F/B ratio was closed to A2, B2, A3, and B3 treatments on the biplot (Figure 2A). In Li et al. (2017) experiment, they found the plant growth significantly shifted the F/B ratio.

Treatments A1 and B1 were in quadrant 4 and had higher contents of minerals among the group (Table 2). All quadrant 4 minerals had significantly ($p<0.001$) stronger linear relationship with G+ bacteria and G+/G- ratio. However, the eukaryotes had a moderate correlation with chlorine (Cl^-), sodium (Na), nitrogen (N), potassium (K), conductivity, sulphur (S), magnesium (Mg) and calcium (Ca).The significantly ($p<0.0001$) high P correlation with G+ and G+/G- in the mixed medium was found in Table 1.

The highest electric conductivity was observed in treatments A1, B1, A2 and B2 (Table 2), and

suggested that these media amendment had the higher concentration of dissolved ions in solution. These ions might contain mineral nutrients and other dissolved chemicals. This would be considered to help plant growth. Aeorobic G+ cocci were not significant between group A and B (Supplementary Table S3). This was evident based on the ratio of i-C15 to a-C15 which is characteristic for aerobic G+ cocci (Sasek et al., 2012). Therefore, the media did not contain micro niche of aerobic G+ cocci.

Table 2. One-way ANOVA showed microbial groups and nutrient mineralization segregated in quadrant 4 of PCA biplot.

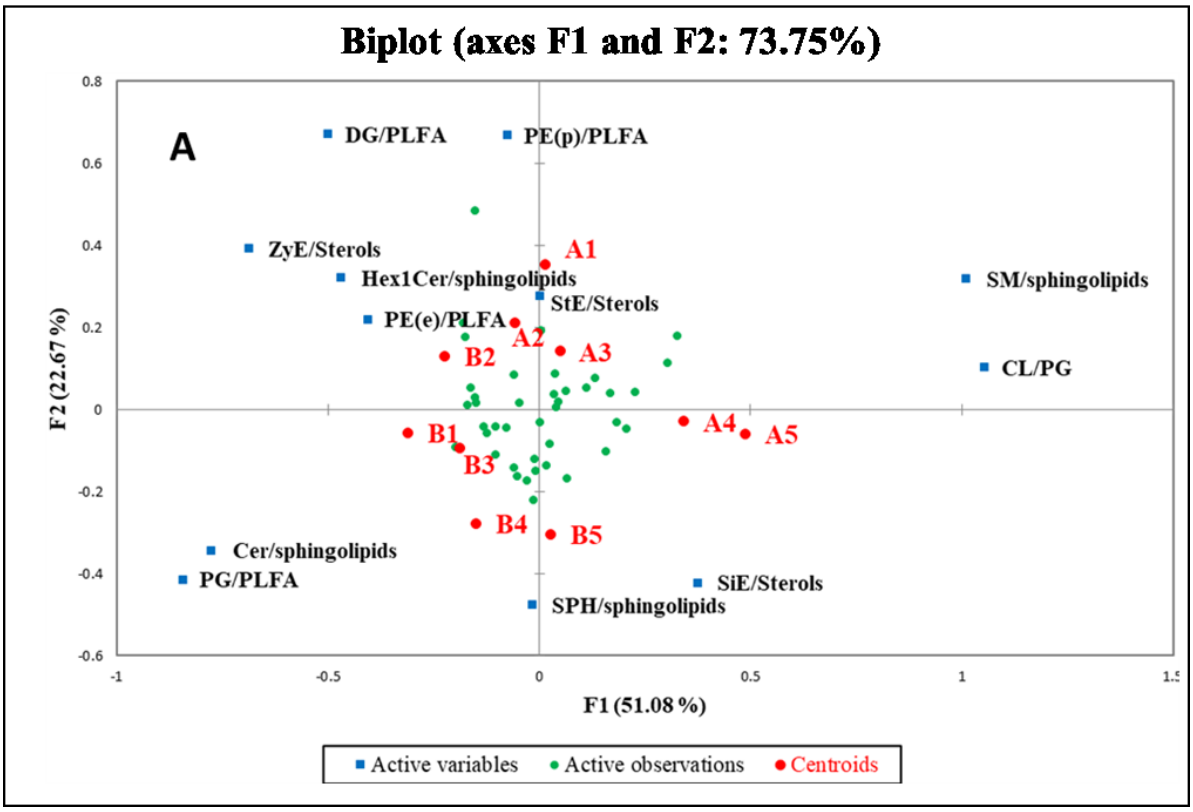
	A1	B1	A2	B2	A3	B3	A4	B4	A5	B5
E	0.003 ±0.00^{b}	0.006 ±0.00^{c}	0.001 ±0.00^{a}	0.006 ±0.00^{c}	0.000± 0.00^{a}	0.004 ±0.00^{b}	0.001 ±0.00^{a}	0.003± 0.00^{b}	0.001 ±0.00^{a}	0.004 ±0.00^{b}
G+ /G-	0.56± 0.01^{d}	0.71± 0.01^{f}	0.50± 0.01^{c}	0.66± 0.01^{e}	0.46±0. 01^{c}	0.49± 0.01^{c}	0.37± 0.01^{b}	0.39±0 .01^{b}	0.38± 0.01^{b}	0.33± 0.01^{a}
G+	0.27± 0.01^{e}	0.26± 0.01^{e}	0.19± 0.01^{c}	0.23± 0.01^{d}	0.17±0. 01^{b}	0.18± 0.01^{bc}	0.14± 0.01^{a}	0.14±0 .01^{a}	0.14± 0.01^{a}	0.12± 0.01^{a}
PO $_4^{2-}$	21.14 ±0.43^{g}	18.35 ±0.43^{f}	17.68 ±0.43^{f}	16.39 ±0.43^{e}	13.10± 0.4``3^{c}	14.68 ±0.43^{d}	11.25 ±0.43^{b}	12.12± 0.43^{bc}	6.36± 0.43^{a}	6.55± 0.43^{a}
Cl^-	477.0 ±1.23^{f}	477.0 ±1.23^{f}	286.0 ±1.23^{e}	477.0 ±1.23^{f}	286.0± 1.23^{e}	286.0 ±1.23^{e}	191.0 ±1.23^{d}	114.3± 1.23^{c}	29.0± 1.23^{b}	19.0± 1.23^{a}
Na	234.3 ±6.84^{e}	214.8 ±6.84^{e}	156.9 ±6.84^{d}	171.9 ±6.84^{d}	97.6±6. 84^{c}	109.2 ±6.84^{c}	61.8± 6.84^{b}	69.1±6 .84^{b}	12.1± 6.84^{a}	11.8± 6.84^{a}
N	0.90±	0.96±	0.76±	0.77±	0.53±0.	0.62±	0.41±	0.41±0	0.19±	0.16±

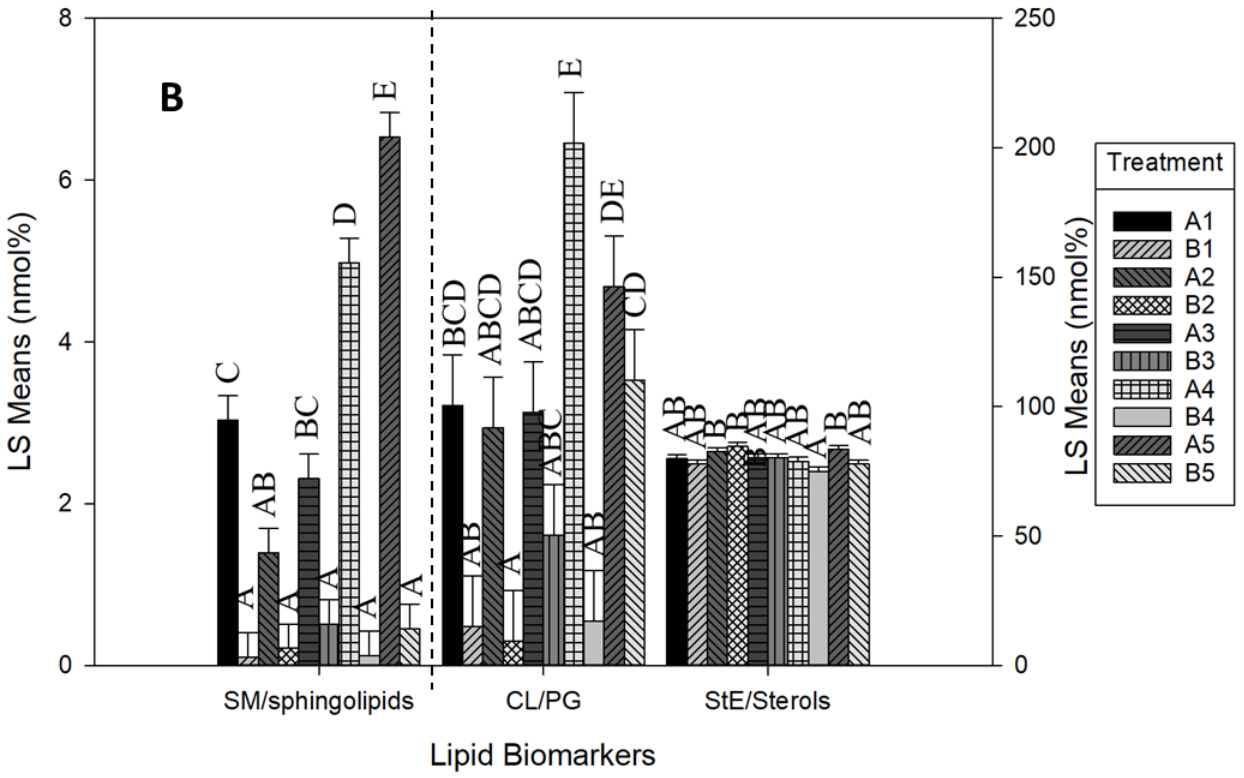
	0.01^{f}	0.01^{g}	0.01^{e}	0.01^{e}	01^{c}	0.01^{d}	0.01^{b}	.01^{b}	0.01^{a}	0.01^{a}
K	407.0 ±4.38^{g}	322.7 ±4.38^{f}	244.1 ±4.38^{d}	291.7 ±4.38^{e}	198.0± 4.38^{b}	216.9 ±4.38^{c}	216.8 ±4.38^{c}	213.7± 4.38^{c}	154.9 ±4.38^{a}	153.5 ±4.38^{a}
EC	3.37± 0.21^{e}	3.46± 0.21^{e}	2.08± 0.2^{cd}	2.46± 0.2^{d}	1.43±0. 2^{b}	1.62± 0.21^{bc}	1.12± 0.21^{ab}	1.06±0 .21^{ab}	0.58± 0.21^{a}	0.54± 0.2^{a}
SO_4^{2-}	231.0 ±4.13^{e}	326.8 ±4.13^{f}	137.2 ±4.13^{c}	190.0 ±4.13^{d}	119.0± 4.13^{b}	140.2 ±4.13^{c}	122.5 ±4.13^{b}	119.0± 4.13^{b}	30.4± 4.13^{a}	39.8± 4.13^{a}
NO_3^{2-}	2.60± 0.15^{fg}	2.23± 0.15^{f}	2.88± 0.15^{g}	1.57± 0.15^{e}	1.12±0. 15^{cd}	1.25± 0.15^{de}	0.77± 0.15^{abc}	0.86±0 .15^{bcd}	0.36± 0.15^{a}	0.48± 0.15^{ab}
Mg	55.74 ±0.35^{g}	57.30 ±0.35^{h}	41.77 ±0.35^{e}	52.03 ±0.35^{f}	30.42± 0.35^{c}	34.70 ±0.35^{d}	25.13 ±0.35^{b}	25.71± 0.35^{b}	3.46± 0.35^{a}	4.22± 0.35^{a}
Ca	82.08 ±0.25^{i}	92.35 ±0.25^{j}	67.89 ±0.25 ^{g}	70.73 ±0.25^{h}	49.62± 0.25^{d}	59.10 ±0.25^{f}	47.57 ±0.25^{c}	50.76± 0.25^{e}	16.18 ±0.25^{b}	15.28 ±0.25 ^{a}

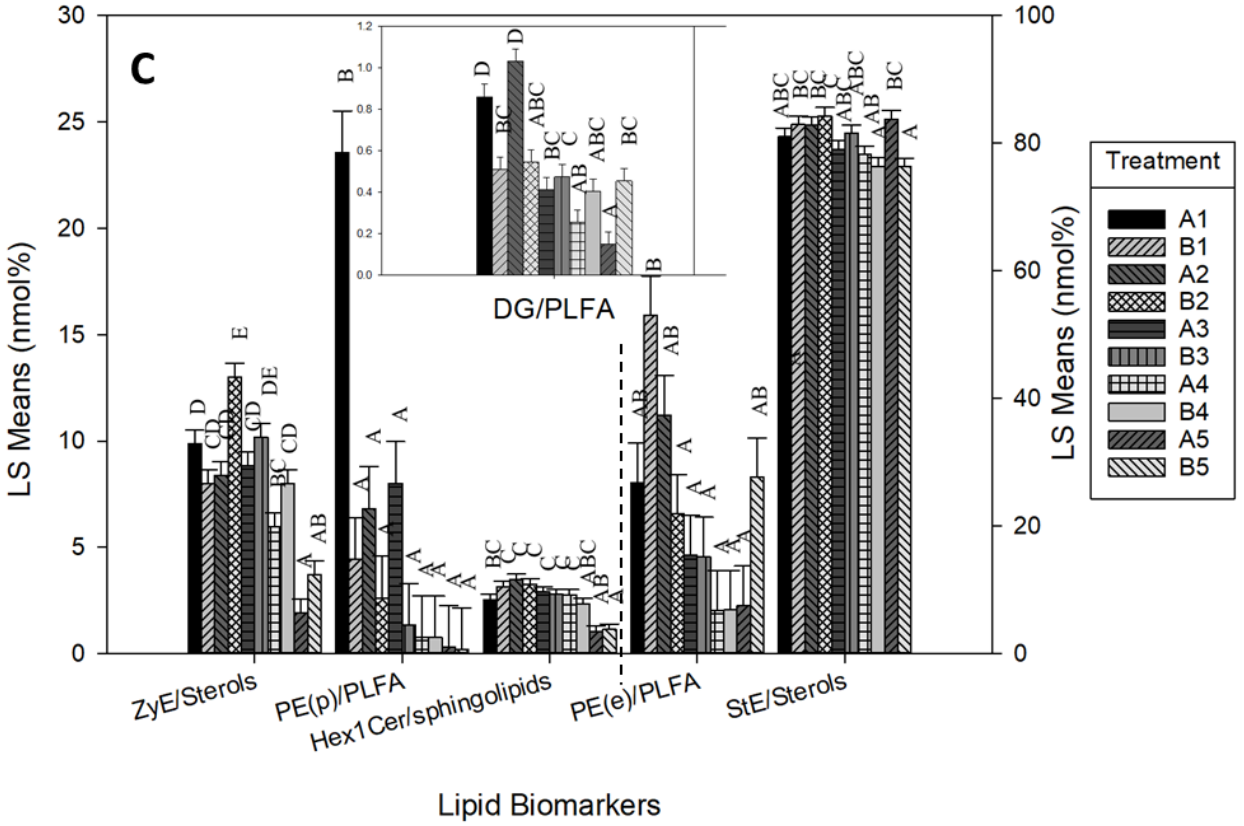
Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (at LSD $\alpha = 0.05$, n = 4 per experimental replicate). G+, gram-positive; G-, gram-negative; EC, electronic conductivity; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*. The unit of microbial groups is ratio to total microbial mass, and the unit of total microbial mass is µg/mL. Units of all minerals were ppm, except N was %, and electronic conductivity was nmol.

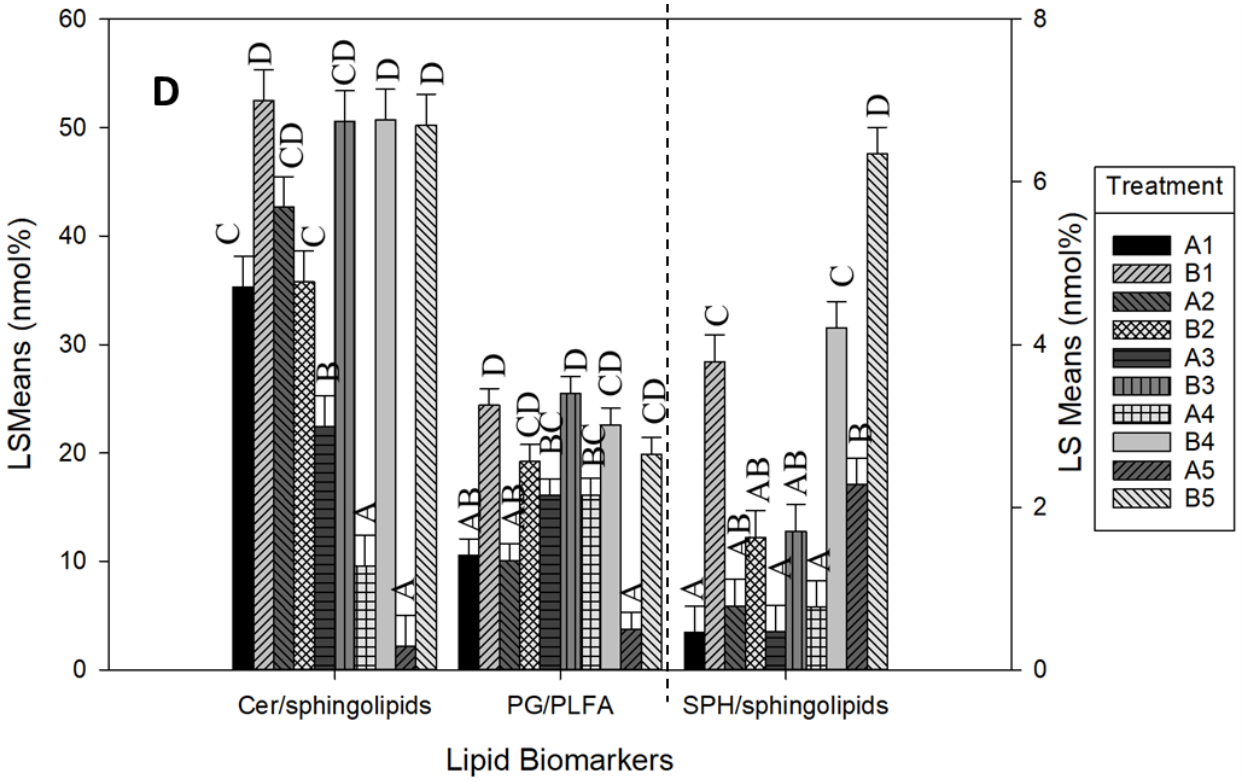
In the present study, the lipids were extracted by LC-MS (David et al., 1998, Pham et al 2019). Plasmalogens were found in phosphatidylcholine (PC) or phosphatidylethanolamine (PE), indicating the presence of archaea in the media. (Supplementary Table S1). Figure 3a showed the segregation of the media inoculated with *Trichoderma* based on RDA analysis. This segregation

accounted for approximately 73.8% of the total variability presented in the data due to the treatments. All treatments with and without *Trichoderma* were separated into two parts.




B
LS Means (nmol%)
LS Means (nmol%)
Treatment
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5
SM/sphingolipids
CL/PG
StE/Sterols
Lipid Biomarkers


C
DG/PLFA
LS Means (nmol%)
LS Means (nmol%)
Treatment
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5
ZyE/Sterols
PE(p)/PLFA
Hex1Cer/sphingolipids
PE(e)/PLFA
StE/Sterols
Lipid Biomarkers


D
LSMeans (nmol%)
LS Means (nmol%)
Cer/sphingolipids
PG/PLFA
SPH/sphingolipids
Lipid Biomarkers
Treatment
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5

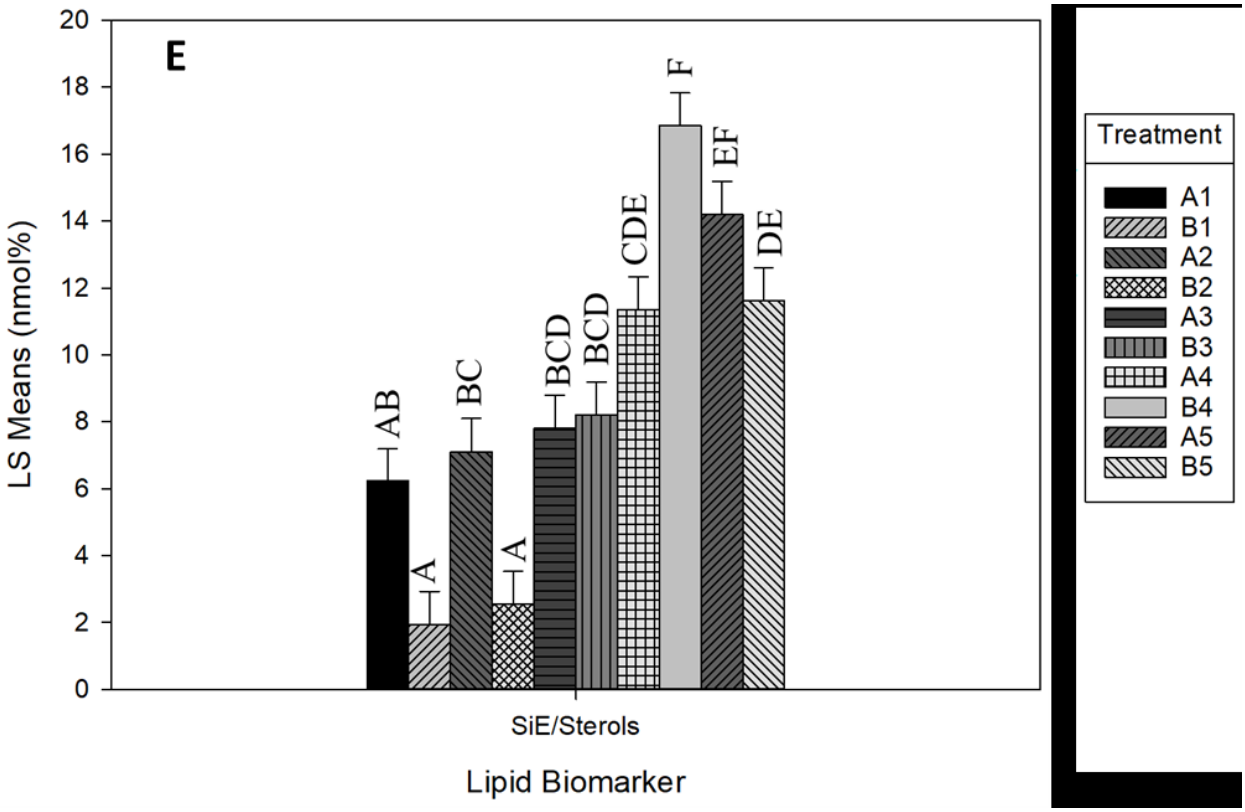

E
LS Means (nmol%)
SiE/Sterols
Lipid Biomarker
Treatment
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5

Figure 3. (A–E). Variation in Biomarkers used to assess microniche or physiological status of the vermicast-Trichoderma-sawdust mixed media during December 2018 to February 2019 incubation period. The observed lipids and media amendments were done under control environmental (lab) conditions. (**A**) Redundancy analysis (RDA) biplot showing relationships between observed lipid biomarkers and media amendments. One-way ANOVA showing lipids segregated in different quadrants (Q) of the biplot following redundancy analysis as followed: (**B**) quadrants 1 (Q1), (**C**) Q2, (**D**) Q3, and (**E**) Q4. Means in the same row accompanied by different superscripts are significantly different at LSD $\alpha = 0.05$, $n = 4$ per experimental replicate. CL, cardiolipin; Cer, Ceramide; DG, diglyceride; Hex1Cer, monohexosylceramide; PE(e), plasmanyl phosphatidylethanolamine; PE(p), plasmalogen phosphatidylethanolamine; PG, phosphatidylglycerol; SPH, sphinganine; SM, sphingomyelin; SiE, phytosterol; StE, beta sitosterols; ZyE, zymosterols; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

We observed that zymosterols (ZyE) and beta sitosterols (StE) decreased with an increase in the proportion of sawdust (Figure 3c). Overall, Group B showed higher ZyE and StE than Group A. These sterols are used to detect yeasts, protozoans, fungi and microalgae in the media (Monreal & Schnitzer, 2013; Volkman, 2003). Sphinganine (SPH) was also observed to be higher in Group B than in Group A. This means that Group B based media had higher redox potential (Figure 3a). Ether lipids as represented by plasmalogen phosphatidylethanolamine [PE (p)] and plasmanyl phosphatidylethanolamine [PE (e)] indicating the presence of archaea in the mix media (Sasek et al., 2012). Both PE (p)/PLFA and PE (e)/PLFA had a higher ratio in Group B, which means Group B contained more Clostridia and methanogenic Archae than Group A (Figure 3c). Furthermore, microbial membrane phospholipids can be used to demonstrate the physiological or nutritional status of the media or soil (Green & Scow, 2000; Nickels et al., 1979). For example, the ratio of cardiolipin (CL) to phosphatidylglycerol (PG) is suggestive of microniche with suboptimal growth conditions in the media (Sasek et al., 2012; Guckert et al., 1986). As such, the high (greater than 1) CL to PG ratio observed in A4, A5 and B5 indicate these media mixes appears to have elevated microniches with suboptimal growth conditions (Figure 3b). Group B had higher amount of sphingolipids and PG than Group A (Figure 3d). Highuptake of growing medium Na in roots can

be a source of endogenous Na that forms adduct with PG (Johanson & Cheeseman, 1983). In this study, we found that treatments B1 and B3 contained higher amounts of Na and PG compared to all other treatments (Figures 1a and 2a). Lipids can be used as indicators of microniche or physiological status of the soil and can enhance the quality of the media leading to enhancement or reduction of the ability to promote crop growth. Therefore, in this section, we concluded that microbes appear in growing media can influence the nutrient status, especial majority content is G+, which had high correlation with necessary nutrients for plants, such as P, Cl, Na, K, NO_3^-, Mg, and Ca.

4.3.3 Temporal Changes in Microbial Community Composition

All the 10 treatments (A1-A5 and B1-B5) were sampled every 30 days to evaluate the incubation effect on media active microbial compositions as presented in Table 3. For the 80% vermicast-20% sawdust (A1) treatment, one-way ANOVA test suggested that after 30 days G+ ($p=0.004$), as well as the sum of G+, G-, and fungi ($p=0.042$) were significantly higher than those media sampled before 30 days. The bacterial content of A1 to A4, B2 and B5 increased after 30 days incubation (Table 3). In Lanza et al. (2016) study, the incubation of different media, soil, pyrolysis char, and hydrothermal carbonisation char, generated an increased in the abundance of all microbial taxa. In contrast, the content of A5, B1, B3 and B4 decreased after 30 days incubation consistent with the findings reported by Chen et al. (2016). Furthermore, we observed the content of the bacterial group was significantly ($p=0.009$) higher in the growing media incubated for less than 30 days. Overall, we found that the third sampling was significantly higher than the others for the sum of bacteria and fungi. For the sum of bacteria, the first two samplings were higher. However, for the

same combination of treatments with *T. viride*, none of the microbial groups showed any difference. For the A2 and A3 treatments, four samples had significant differences on G+ ($p=0.002$) G+&G- ($p=0.01$), G- ($p=0.005$), G+&G-&F ($p=0.014$). So, the treatments A2 and A3 were similar to each other in terms of microbial groups. The fourth sampling was significantly higher than the other sampling times for the G+ group. After 60 days' incubation, the amount of all the bacteria groups was lower than before. The total bacteria and fungi percentages were higher in the first sampling time than the fourth sampling time.

Table 3. Active microbial community composition in each incubated mixed growing media.

	Mean (%)						
Treatment	G+	E	G+G-	G+, G-, F	G-	F	P
A1-1	19±0.9	0.4±0.06	42±0.8	2±0.3	35±0.6	1±0.2	0.7±0.05
A1-2	18±1.1	0.3±0.15	44±1.3	1±0.2	36±0.3	1±0.2	0.4±0.13
A1-3	24±2.2	1.2±0.86	33±4.0	5±1.5	34±1.5	2±1.3	1.0±0.24
A1-4	29±2.6	0.3±0.02	30±3.3	4±0.7	35±0.4	1±0.2	0.7±0.09
A2-1	24±3.4	0.2±0.19	34±4.3	4±0.9	34±0.8	3±0.7	1.3±0.22
A2-2	18±1.4	0.0±0.00	40±3.1	2±0.4	38±0.4	2±0.3	1.1±0.78
A2-3	20±1.9	0.3±0.12	39±2.4	3±0.6	36±0.4	1±0.3	0.9±0.16
A2-4	33±1.6	0.6±0.20	24±1.6	5±0.2	34±0.6	2±0.3	1.5±0.02
A3-1	19±0.9	0.0±0.00	39±1.1	3±0.2	34±0.5	4±0.3	0.7±0.25
A3-2	15±0.9	0.2±0.17	45±1.1	1±0.2	38±0.4	1±0.2	0.3±0.18
A3-3	16±0.5	0.2±0.13	45±0.6	1±0.2	36±0.2	1±0.1	1.0±0.19
A3-4	26±2.8	0.0±0.00	29±4.4	4±0.9	36±0.4	3±0.9	1.4±0.22
A4-1	13±0.5	0.5±0.05	48±0.6	0±0.1	37±0.2	1±0.2	0.5±0.07
A4-2	13±0.5	0.3±0.04	48±0.7	1±0.2	37±0.1	0±0.2	0.5±0.07
A4-3	17±1.9	0.1±0.09	42±2.4	2±0.4	37±0.5	2±0.2	1.2±0.43

A4-4	15±0.4	0.1±0.08	45±0.6	1±0.1	37±0.1	1±0.1	0.7±0.09
A5-1	14±1.0	0.1±0.05	47±1.0	1±0.2	36±0.5	2±0.3	0.5±0.11
A5-2	18±3.1	1.4±1.30	39±6.5	4±2.5	35±2.4	3±2.0	0.4±0.14
A5-3	14±0.5	0.2±0.08	46±1.2	1±0.3	37±0.4	2±0.6	0.8±0.14
A5-4	13±0.3	0.1±0.08	48±0.4	1±0.1	37±0.1	1±0.2	0.7±0.06
B1-1	21±2.5	0.0±0.00	37±2.7	3±0.6	36±0.9	2±0.3	1.5±0.29
B1-2	30±3.5	6.7±5.58	27±2.8	4±1.6	28±4.2	3±0.9	0.6±0.32
B1-3	24±2.0	0.6±0.08	34±2.6	4±0.6	35±0.5	1±0.3	1.1±0.11
B1-4	25±0.7	0.7±0.04	33±0.6	4±0.2	35±0.4	1±0.1	1.2±0.11
B2-1	29±2.1	0.3±0.29	25±0.9	6±0.3	33±1.9	5±0.3	2.2±0.42
B2-2	23±5.2	0.8±0.46	35±6.5	3±0.9	35±1.3	2±0.6	1.6±0.74
B2-3	21±1.7	0.2±0.15	38±1.9	3±0.5	36±0.5	1±0.2	1.2±0.18
B2-4	24±1.0	0.5±0.16	32±1.3	4±0.3	36±0.4	2±0.4	1.2±0.19
B3-1	12±0.5	0.0±0.00	49±0.7	1±0.1	37±0.2	1±0.2	0.5±0.17
B3-2	16±1.7	0.2±0.12	41±3.9	2±0.7	37±0.6	2±0.5	1.3±0.66
B3-3	18±2.1	0.4±0.12	41±3.1	2±0.8	36±0.4	1±0.3	1.0±0.09
B3-4	19±1.0	0.4±0.17	39±1.6	3±0.4	36±0.4	2±0.3	1.1±0.26
B4-1	14±2.8	0.0±0.00	44±3.3	1±0.4	37±0.4	2±1.0	0.5±0.23

B4-2	14±0.6	0.7±0.41	43±2.0	4±1.6	35±1.4	3±1.1	0.6±0.06
B4-3	14±0.8	0.4±0.13	44±1.3	2±0.4	37±0.6	2±0.4	0.7±0.08
B4-4	16±1.1	0.2±0.10	42±2.4	2±0.5	37±0.4	2±0.5	1.2±0.46
B5-1	22±2.6	0.4±0.04	42±2.0	1±0.5	33±1.1	1±0.1	0.6±0.05
B5-2	12±0.5	0.5±0.12	47±1.3	2±0.6	36±0.4	2±0.4	0.5±0.09
B5-3	12±0.3	0.2±0.08	48±0.5	1±0.2	37±0.2	1±0.2	0.5±0.03
B5-4	13±0.6	0.1±0.06	47±1.1	1±0.3	37±0.4	2±0.4	0.6±0.16

Values are means ± standard errors. G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryotes. A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*. Treatment-1, first time sampling of treatment; Treatment-2, second time sampling of treatment; Treatment-3, third time sampling of treatment; Treatment-4, forth time sampling of treatment. Units are ug/mL.

For the B2 treatment, the first sampling media had a significantly higher content of fungi ($p<0.001$) and the sum of bacteria and fungi ($p=0.006$). For the B3 treatment, only the G+ group was significantly lower at the first sampling ($p=0.025$). In the 20% vermicast+80% sawdust (A4) treatment, the sum of bacteria and fungi was higher after 60 days (Table 3). There was a significant ($p<0.05$) increase in the inoculated bacterial count (G+G-) in the mixed media after incubation and reached its maximum level between 45 and 60 days consistent with previous reports in the literature (Kaushik et al., 2008). Moreover, for the sawdust alone treatment (A5), none of the microbial groups varied throughout the incubation period. For the B5 treatment, even the first sample media had significantly higher amounts of G+ and eukaryotes, and the media sampled at the three subsequent times had more G- and sum of bacteria (G+G-). For the fourth sampling time, the

mineral nutrients were significantly different for Al ($p<0.001$), Fe ($p<0.001$), Mn ($p=0.012$), and Zn ($p<0.001$) across all treatments. Between 60 and 90 days after incubation, there was a decline in the bacterial count (G+G-). This is consistent with the work of Kaushik et al. (2008), who reported that aged vermicompost decreased the active microbial population between 60- and 70-days incubation. The decreased microbial activity in the aged vermicast perhaps occur due to diminution in the available carbon in the media substrate, lower microbial population, and nutrient status (Tiwari et al., 1993; Parle, 1963). We found N had high correlation with G+G- bacteria, so the reduction in total bacteria population appears to be associated with the decreased N content (Table 1). With the passage of time, the growing media composition changes and the substrate becomes less suitable for micro-organism as an energy substrate thus decreasing the activity of nitrogen fixing bacteria (Kaushik et al., 2008). We noted that previous reports in the literature suggest that 45–60 days incubation at 28℃ is appropriate to induce beneficial physical and chemical properties of vermicompost (Alikhani et al., 2017), which differed from our findings. Temporal changes in microbial community composition in the vermicast-sawdust mixed media suggested that incubation for 0-30 days gives higher microbial community composition, which means no incubation for these media combination showed superior microbial compositions and community structure.

Sequence-based approaches to study microbiomes, such as V6-V8 16S rRNA gene (16S), V4 18S rRNA gene (18S), fungal ITS2 region (ITS) sequencing and metagenomics, are uncovering associations between microbial taxa and a myriad of factors (Comeau et al., 2017). The PLFA data confirmed the presence of active microbial populations in the mixed media, but the exact species were not determined. Therefore, further study on DNA tests of the mixed media is in progress to assess the specific microbial populations and their functionality on plant growth.

4.4 CONCLUSIONS

The results obtained from the present study demonstrate that the addition of *Trichoderma* was not significantly beneficial in enhancing the microbial activities compared to growing media without *Trichoderma viride*. The mineral nutrients released in the mixed media had a high correlation ($r>0.7$) with the active microbial groups present. We found six main microbial groups present in the sawdust-vermicast based media as follows: G+, G-, G+G-, fungi, protozoa, archaea and eukaryotes. G+, G- as well as the total of G+ and G- bacteria contributed 18.9%, 35.7% and 39.8%, respectively, to the overall total microbial population observed in the media. Treatments A2 and B2 (60% vermicast-40% sawdust, without and with *T. viride*) showed higher pH, Protozoa, F/B ratio, and Fe. We found that treatments B1 and B3 (80% vermicast-20% sawdust and 40% vermicast-60%sawdust, both with *T. viride*) contained higher amounts of Na and PG than the other treatments. The mineralized nutrients (e.g. chloride, sodium, nitrogen, potassium, sulphate, magnesium, and calcium) in the mixed growing media is associated with increased active microbial community composition (eukaryotes, G+, and the ratio of G+/G-). Additionally, the vermicast-sawdust in the ratio of 40:60 and 60:40 had superior effects on the microbial composition under the conditions of the present study. After 30 days (i.e. sampling times 3 and 4), the sum of bacteria and fungi ($p=0.042$) were significantly higher than those media sampled before 30 days (i.e. sampling times 1 and 2). On the contrary, the content of the bacterial group was significantly ($p=0.009$) higher in the growing media incubated for less than 30 days. For the sum of bacteria, the first two samplings time were higher. The decreased microbial activity in the aged mixed media appears to be related to diminution in the available carbon in the substrate and lower microbial population and nutrient status. Overall, no incubation of the sawdust vermicast based media appears to be more beneficial in supporting a superior active microbial composition. For this

project, the ratio of 40:60 and 60:40 vermicast-sawdust without incubation would be suggested as superior in terms of improved active microbial composition, health status and nutrient mineralization for plant growth.

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4.6 Supplementary

Table S1. Phospholipid fatty acids biomarkers used to characterize the active microbial community structure.

Shorthand notation	Name	Microbial group	References
C11:0	Methyl undecanoate	G+	(Alenius et al., 2009)
C12:0	Methyl dodecanoate	E	(Cavigelli et al., 1995; Zhang et al., 2017)
C13:0	Methyl tridecanoate	G+, G-	(Amir et al., 2008; Alenius et al., 2009)
(2-OH) C10:0	Methyl 2-hydroxydecanoate	G-	(Lasater et al., 2017)
C14:0	Methyl tetradenote	G+	(Sheng et al., 2012)
i-C15:0	Methyl 13-mmethylteradenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
a-C15:0	Methyl 12-methylteradenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
C15:0	Methyl pentadecanoate	G+	(Huygens et al., 2011;

			Papatheodorou et al., 2012)
i-C16:0	Methyl 14-methyl pentadecanoate	G+	(Wang et al., 2016; Zhang et al., 2016)
(2-OH) C12:0	Methyl 2-hydroxydodecanoate	G-	(Lasater et al., 2017)
C16:0	Methyl Hexadecenoate	G+, G-	(Kujur and Patel, 2014; Wu et al., 2015)
C16:1n7	Methyl cis-9-Hexadecenoate	G+, G-	(Brockett et al., 2012; Wang et al., 2016)
C17:0; C16:1n5	Methyl 15-Methylhexadecanote; Methyl 11(Z)-hexadecenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
C17:0	Methyl Heptadecanoate	G+	(Huygens et al., 2011; Papatheodorou et al., 2012)
(3-OH) C12:0	Methyl 3-hydroxydodecanoate	G-	(Spring et al., 2000; Kaur et al., 2005)
Δ17:0	Methyl cis-9.10-Methylenehexadecanote	G-	(Wang et al., 2016; Zhang et al., 2016)
(2-OH) C14:0	Methyl 2-Hydroxytetradecanoate	G-	(White and Rice, 2009)

C18:0	Methyl Octadecanoate	G+, G-	(Brockett et al., 2012; Wu et al., 2015)
C18:1n9 t	Methyl octadecenoate(trans-9)	G-	(Moreno et al., 2017)
C18:1n9 c	Methyl octadecenoate(cis-9)	G+, G-, F	(Brockett et al., 2012; Zhang et al., 2016)
C18:2n6	Methyl octadecadienoate (all cis-9,12)	F	(Joergensen and Potthoff 2005; eukaryote White et al., 2009; Zhang et al., 2016)
C19:0	Methyl nonadecanoate	G+	(Gharaibeh and Voorhees, 1996)
(3-OH) C14:0	Methyl 3-Hydroxytetradecanoate	G-	(Papatheodorou et al., 2012)
∆C19:0	Methyl methyleneoctadecanoate (all cis-9,10)	G-	(Wang et al., 2016)
C20:0	Methyl eicosenoate	P	(Schindlbacher et al., 2011)
(2-OH) C16:0	Methyl 2-hydroxyhexadecanoate	G-	(Sheng et al., 2012)

G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryotes.

Table S2. The parameters used for Q-Exactive mass spectrometer.

General	
Runtime	0-40 min
Polarity	Positive/ Negative
Default charge	1
Full MS	
Resolution	70,000
AGC target	1e5
Maximum IT	100 ms
Scan range	200 to 2000 m/z
dd-MS^2/ dd-SIM	
Resolution	35,000
AGC target	2e4
Maximum IT	100 ms
Loop count	20
Isolation window	1.0 m/z
(N)CE/ stepped nce	30, 35
dd Setting	
Minimum AGC target	5.00e2

Table S3. A t-test for each microbial group in Group A and B.

Microbial Group	A	B	P-Value
G+	0.18±0.05	0.19±0.06	0.583
E	0.001±0.001	0.005±0.002	<0.001
G+G-	0.96±0.02	0.95±0.02	0.212
G-	0.36±0.01	0.36±0.01	0.754
G+, G-, F	0.99±0.004	0.99±0.006	0.094
F	0.01±0.006	0.02±0.005	0.012
P	0.01±0.004	0.01±0.003	0.713
G+/G-	0.45±0.08	0.52±0.15	0.120
F/B	0.01±0.004	0.02±0.005	<0.001
T	197.7±10	194.1±21	0.881
i /a C15	0.79±0.03	0.83±0.02	0.175

Unit is the ratio of each microbial group to total microbial population. G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryote; T, Total microbial mass; A, treatment without Trichoderma; B, treatment with *Trichoderma viride*.

Table S4. The changes of aluminum, boron, copper, iron, manganese, and zinc contents as time going by among 10 sample treatments.

Treatments	Sampling time points	Aluminum (ppm)	Boron (ppm)	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)
A1	1	0.06	ND	ND	0.06	0.17	0.03
	2	0.1	ND	ND	0.1	ND	0.03
	3	0.18	ND	ND	0.21	ND	0.05
	4	0.3	ND	ND	0.22	ND	0.05
A2	1	ND	ND	ND	0.05	0.29	0.03
	2	0.1	ND	ND	0.13	ND	0.04
	3	0.23	ND	0.05	0.31	ND	0.06
	4	0.28	ND	0.06	0.33	ND	0.07
A3	1	0.06	0.1	ND	0.07	0.24	0.03
	2	0.1	ND	ND	0.11	ND	0.03
	3	0.26	ND	ND	0.3	ND	0.07
	4	0.22	ND	0.05	0.25	ND	0.06
A4	1	0.13	0.13	ND	0.12	0.16	0.05
	2	0.15	0.11	ND	0.2	ND	0.04
	3	0.23	0.13	0.08	0.38	ND	0.07
	4	0.25	0.13	0.08	0.38	ND	0.07
A5	1	0.06	0.25	ND	ND	0.2	0.07
	2	0.06	0.22	ND	ND	0.11	0.05
	3	ND	0.23	0.05	0.06	0.12	0.06

	4	0.06	0.23	0.06	0.06	0.14	0.07
B1	1	0.06	ND	ND	0.06	0.21	0.03
	2	0.1	ND	ND	0.1	ND	0.04
	3	0.15	ND	ND	0.18	ND	0.05
	4	0.14	ND	ND	0.15	ND	0.05
B2	1	0.09	0.1	ND	0.09	0.3	0.04
	2	0.1	ND	ND	0.12	ND	0.05
	3	0.27	ND	0.07	0.36	ND	0.07
	4	0.21	ND	0.05	0.27	ND	0.06
B3	1	0.07	0.11	ND	0.06	0.28	0.04
	2	0.1	ND	ND	0.13	ND	0.04
	3	0.21	ND	0.06	0.29	ND	0.05
	4	0.2	ND	0.05	0.3	ND	0.05
B4	1	0.05	0.12	ND	0.08	0.16	0.03
	2	0.19	0.12	0.06	0.28	ND	0.05
	3	0.29	0.12	0.08	0.44	ND	0.06
	4	0.27	0.13	0.09	0.42	ND	0.07
B5	1	ND	0.25	ND	ND	0.21	0.05
	2	ND	0.26	ND	ND	0.15	0.05
	3	ND	0.25	ND	ND	0.14	0.05
	4	ND	0.25	ND	0.06	0.15	0.06

ND, data was not detected; A1, 80% vermicast+20% sawdust; A2, 60% vermicast+40% sawdust; A3, 40% vermicast+60% sawdust; A4, 20% vermicast+80% sawdust; and A5, sawdust alone (control). The corresponding treatments B1-B5 contained *T. viride*.

CHAPTER 5 ACTIVE MICROBIAL COMPOSITION AND MICROGREENS PLANT GROWTH RESPONSE TO VERMICAST-SAWDUST MIXED MEDIA

5.0 ABSTRACT

Microgreens are tender young vegetables grown for their high nutritional values and functional properties for human health benefit, but largely understudied. An experiment was performed to compare the effects of two combinations of vermicast-sawdust mixed growing media (T1: 60% vermicast+40% sawdust and T2: 40% vermicast+60% sawdust), and a control Promix-BX on active microbial composition and the growth of three different microgreen plant species; namely, kale (*Brassica oleraceae var.* sabellica), Swiss chard(*Beta vulgaris* subsp. Maritima), and Pak choi (*Brassica rapa* subsp. chinensis). Comparatively total calcium, magnesium, sodium and sulphate contents in T1 were higher than in T2 by 60%, 65%, 59% and 53% respectively. Similar observations were made on total nitrogen ($p<0.001$), total potassium ($p=0.004$) and electric conductivity ($p=0.004$). Overall, mineral nutrients content had significantly ($p<0.05$) highly positive correlation ($r<0.7$) with active microbial composition in the mixed media. Combined data for phospholipid fatty acids and mineral nutrients with plant growth showed that T1 significantly ($P<0.05$) enhanced the physiological profile of kale, Swiss chard and Pak choi plants as compared to T2 plants. Furthermore, T1 was confirmed to be better than T2 in a combined (treatments vs plant species) plant growth indicators versus mineral nutrients and active microbial group analyses. In conclusion, T1 mixed medium was the most efficacious for microgreen production.

5.1 INTRODUCTION

The global population is expected to increase by approximately 26% from 7.7 billion in mid-2019 to 9.7 billion by 2050 (UN DESA, 2019). The expected increase in global population will require sustainable effort to meet food demand. However, food and nutrition security are faced with grave challenges such as global climate change, increased soil degradation and pressure on farmlands. To meet the ever-increasing global food demand, it is universally acknowledged that an important area for investigation is growing media substrates and natural amendments. These investigations can help improve and maintain soil quality, soil health and increase plant productivity per unit area.

Microgreens, frequently called vegetable confetti, are a novel class crops, defined as immature greens harvested without roots from the tender seedlings of vegetables, herbs, grains, and wild species (Xiao et al., 2012; Kyriacou et al., 2019). Some literature exhibited that microgreens contain higher amounts of phytonutrients (ascorbic acid, b-carotene, a-tocopherol and phylloquinone), vitamins, minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo), and lower nitrate content than their mature leaf counterparts (Xiao et al., 2012; Pinto et al., 2015; Kyriacou et al. 2016). The test plants in this experiment were kale, Swiss chard, and Pak choi as they are widely grown for microgreens and known to be rich in vitamins A, C, and K, essential lipids, carotenoids and mineral nutrients (Di Noia, 2014; Pham et al., 2018).

Vermicast, compost and humates are not only used as organic amendments to growing media but also, as supplements of synthetic chemical fertilizers. There are many advantages to these natural

amendments, which include low technology or cost input, enhanced beneficial microbial activities, richness and diversity in nutrients and environmental friendliness (Duong et al., 2012; Abbey et al., 2018). According to a report by Ramnarain et al. (2018), for pac choi (*Brassica rapa* var. chinensis) growth, almost all soil quality parameters including mineral nutrients and soil structure were increased following the application of vermicompost in a greenhouse. In a previous study, the application of vermicast had a positive effect on the growth and yield of Swiss chard (Smith et al., 2001). In a comparative controlled environment/soilless study, it was reported that plants treated with dry vermicast recorded increased levels of plant tissue macro-and micro-nutrients more than the potassium humate and the volcanic minerals amendments (Abbey et al., 2018). It was also suggested that dry vermicast could be a potential target natural media amendment for the biofortifying of kale plants. The amended media substrate for these studies was peat moss.

Farmers operating in controlled environment production systems usually use natural soilless substrates. Such natural substrates are preferred because of their low cost, biodegradability and overall good properties to support plant growth (Raviv et al., 2002; Caron et al., 2015). A typical example is forestry and sawmill residue such as sawdust and bark which according to Bradley (2007), are the most available source of biomass in Canada and many other countries. According to Depardieu et al. (2016), sawdust-based growing medium can potentially be used as substitutes to coconut fiber (coir) for soilless culture for strawberry production in Canada. However, despite the good porosity and high saturated hydraulic conductivities, sawdust has low water retention capacity (Depardieu et al., 2016). Additionally, sawdust may have negative effects on plant growth due to salt and toxic compound accumulations, such as tannins, resins, and turpentine (Dorais et

al., 2006). There is also no specific research on the effects of sawdust as growing medium substrates on plants grown and harvested as microgreens. the limited information on the proper use of sawdust as growing medium substrates does not encourage its use for plant production in the horticulture sector.

Based on previous literatures, we postulated that the addition of vermicast to heat-treated sawdust can detoxify the sawdust, increase growing medium microbial activities and nutrition availability and ultimately, and improve plant growth. A recent work by Lin et al. (unpublished) showed that nutrients mineralization and active microbial composition were increased in growing media comprising 40% vermicast+60% heat-treated sawdust or 60% vermicast+40% heat-treated sawdust. Based on this recent study, we hypothesized that vermicast and heat-treated sawdust blend would enhance active microbial activity and the growth and yield of kale, Swiss chard and pak choi microgreens. Therefore, the objective of the present study was to compare the efficacy of two different mixed proportions of vermicast-sawdust media to produce microgreens under greenhouse conditions.

5.2 MATERIALS AND METHODS

A greenhouse experiment was carried out in the Department of Plant, Food and Environmental Science, Faculty of Agriculture, Dalhousie University, Canada from October 2019 to February 2020.Vermicast (i.e. earthworm castings excreted by Red wiggler worms, *Eisenia fetida*) was obtained from Pagonis Live Bait (ON, Canada); and thermally treated maple tree sawdust was

purchased from Thermal Wood Canada (NB, Canada). The seeds of Green Curled kalewere purchased from Halifax Seeds (NS, Canada). Seeds of Pak choi and Swiss chard seeds were purchased from West Coast Seeds (Ottawa, ON, Canada) for the study. The Promix-BX potting medium with added *Mycorrhizae* fungi (Premier Horticulture Inc., PA, United States) was also purchased from Halifax Seeds.

5.2.1 Growing Media Composition

The growing media treatments comprised of mixed proportions of vermicast and sawdust (w/w) as follows 60% vermicast+40% sawdust (T1), 40% vermicast+60% sawdust (T2) and Promix-BX alone (control); and three species of leafy vegetable plants as follows: kale, pak choi and Swiss chard. The individual growing medium samples (200 g) were analyzed at 30-day intervals for nitrate-N (NO_3^--N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), chloride (Cl^-), sulphate (SO_4^{2-}), aluminum (Al) and nitrogen (N). The samples were sent to Nova Scotia Department of Agriculture Laboratory Services, Truro, NS, Canada for analyses using the AOAC-968.08 inductively coupled plasma (ICP) spectrometer method (AOAC, 2003).The active microbial community composition was also assessed at 30-day intervals using microbial phospholipid fatty acids (PLFA) according to the modified method of Gómez-Brandón et al. (2010). Gas chromatography analyzed the fatty acids as methyl esters with flame-ionization detection/ mass spectrometry (GC-FID/MS), and the active microbial community structure in the individual media was determined by PLFA platform.

5.2.3 Raising and Transplanting of Seedlings

Seeds of kale, pak choi, and swiss chard were germinated in a 36 cell-tray containing Promix-BX,

with regular watering (every two-day). The trays were placed on a shelving with 120 V and 80 WLED lighting.28 days post germination, the seedlings were transplanted into 15.24 cm-diameter plastic pots containing 1 kg of the individual mixed medium treatment. The heat-threated sawdust was dry, so 1.5 L of distilled water was added to every 1-kg vermicast-sawdust mixed medium and stirred with a wooden ladle. The pots were arranged on a galvanized-steel bench in the greenhouse. Each growing medium treatment was made up of a total of 45 pots (i.e.3x3x5 factorial experiment; 3 plant species, 3 treatments, and5 replications per treatment). The potted plants were rearranged randomly on the benches to offset any unpredictable environmental effects. Plant growing temperatures in the greenhouse were set at 24°C and 16°C in the day and night, respectively. The humidity was set at 75%. The supplementary lighting was supplied by high-pressure sodium lamps (600 W HS2000) and NAH600.579 ballasts (P.L. Light Systems, ON, Canada).

5.2.4 Plant Growth Analysis

Plant height was measured from the tip of the leaf to the collar of the stem. Green leaf numbers were counted at 35 days after transplanting (DAT). Stem diameter was measured at the middle portion of the stem by Mastercraft digital calliper (Canadian Tire, NS, Canada). Leaf area was measured using LI-3100 C leaf area meter (LI-COR Biosciences, NE, USA). The leaf fresh weights were recorded at the final harvest using HR60 analytical balance (Data Weighing Systems, Inc., IL, USA). The leaves were dried at 65℃ for 48 hr using a mechanical convection oven (Cole-Parmer Instrumental Co., Vernon Hills, IL). Leaf moisture (LM) content was estimated as:

$$LM\ (\%) = 100 \times \left[\frac{M_f - M_d}{M_d}\right]$$

Where M_f and M_d are the fresh mass and the dry mass of the plant tissue, respectively.

5.2.5 Leaf Chlorophyll Indices

Leaf greenness as an estimator of leaf chlorophyll content was determined using a SPAD 502 Chlorophyll meter (Konica Minolta, Inc., IL, USA). Anthocyanin content index (ACI) of the leaves was determined using Anthocyanin content meter (Opti-Sciences Inc., NH, USA). Chlorophyll fluorescence indices are commonly usedto test plant photosynthesis efficiency and ecophysiological performance, which was determined using a portable OS30p+ Chlorophyll fluorometer (Opti-Sciences, Inc., NH, USA) before harvesting (Maxwell & Johnson, 2000). The indices included maximum quantum efficiency of photosystem II photochemistry (F_v/F_m) calculated as:

$$F_v/F_m = (F_m - F_0)/F_m$$

$$F_v/F_O = (F_M - F_O)/F_O$$

Where $F_0 = F_{50\mu s}$, the fluorescence at 50 μs; F_m, maximal fluorescence intensity; Fv,variable fluorescence indices.

5.2.6 Experimental Design and Statistical Analysis

The two factors were three levels of different growing media and three plant species, with 5 replications per treatment. Analyzed of variance (ANOVA) was performed using Minitab version 18.3 (Minitab Inc., PA, USA) to determine whether the treatments had any significant effects on plant growth. Means were separated by Fisher's least significant difference at α = 0.05when the

ANOVA indicated that significant differences exist between treatments at $P \leq 0.05$. Principal component analysis was also performed to determine the association between the growing media mineral nutrients and microbial community composition using the XLSTATS premium version (Addinsoft Inc, PAR, France).

5.3 RESULTS AND DISCUSSION

In this part, we did the analysis of vermicast media as a standard to compare the mineral nutrients in the three media we interested in. Physical factors of growing media and biological processes either separately or in together can drive decomposition, although the innate characteristics (i.e., functional traits) of crop residues are important in controlling their decomposition rate in media (Trinsoutrot et al., 2000, Jensen et al., 2005, Aulen et al., 2012). In the nutrient mineralization experiment, the contents of Ca, Mg, P and Mn in the Promix-BX and the vermicast were higher than T1 and T2 but the contents of K, Na in the T1 and T2 treatments were higher in the latter than the former (Table 1A). The content of Ca and N were highest in the organic amendments (i.e., seafood waste compost, municipal solid waste compost, and vermicompost), while Mg and P were the least. Comparatively, the vermicompost had the highest Ca content but the least P and K contents (Abbey et al., 2018). Comparatively, Ca, K, Mg, P, Na, S, and Mn in T1 were higher than in T2 by 60%, 24%, 65%, 23%, 59%, 53% and 21%, respectively.

Table 1A. Nutrients contents of the separate substrates and mixed media.

Treatment	Ca	K	Mg	P	Na	S	Mn

	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
T1	137.99	330.19	99.34	22.49	177.78	266.02	0.29
T2	86.09	267.13	60.12	18.36	112.19	173.72	0.24
Promix-BX	1692.80	199.60	179.60	101.60	31.20	95.20	10.00
Vermicast	4718.40	83.60	485.60	37.60	28.80	182.00	12.00

T1, 60% vermicast + 40% sawdust; T2, 40% vermicast + 60% sawdust.

The contents of Al, B, Cu, Fe, and Zn in the Promix-BX and vermicast were higher than mixed media treatments T1 and T2 (Table 1B). The pH of T1 and T2 were higher than the Promix-BX and vermicast. The remaining mineral nutrients i.e. Al, B, Cu, Zn and Fe were higher in T2 than in T1.

Table 1B. Nutrients contents of the separate substrates and mixed media.

Treatment	Al (mg/l)	B (mg/l)	Cu (mg/l)	Fe (mg/l)	Zn (mg/l)	pH
T1	0.00	0.00	0.00	0.05	0.03	6.64
T2	0.06	0.10	0.00	0.07	0.03	6.71
Promix-BX	34.00	0.00	1.74	60.00	3.30	5.97
vermicast	21.00	1.94	1.04	220.00	3.78	5.89

T1, 60% vermicast + 40% sawdust; T2, 40% vermicast + 60% sawdust.

The content of N, EC and NO_3^-N of T1 was increased by 52%, 41% and 73%, respectively compared to T2. Based on Martínez-Suller et al. (2008) report, there was a high positive correlation between EC and nutrients concentration. EC was particularly found to be the best estimator for N and K. Because we are interested the T1 and T2 treatments, the data of T1 and T2 were made in Table 2. In this study, the EC of T1 was higher than T2 in association with higher values of N and K (Tables 1A-B & 2). Comparing to vermicast only, there are only a little element (Al, B, Cu, Fe, and Zn) detected in the mixed media T1 and T2. This might be due to the sawdust adding to vermicast and limit the elements mineralized.

Table 2. Nitrogen, electric conductivity, nitrate-N and chloride content in the mixed media.

Treatment	N (%)	EC (mmhos)	NO_3^-N (mg/l)	Cl^- (mg/l)
T1	0.75	3.25	148.90	286
T2	0.53	2.30	86.22	286

T1, 60% vermicast + 40% sawdust; T2, 40% vermicast + 60% sawdust.

The active microbial community diversity can be used as an important indicator of growing media quality and health status (Kong et al., 2011). In this experiment, a total of 26 phospholipid fatty acids (PLFAs) were detected and used as biomarkers to define the presence of active microbial groups in the vermicast-sawdust mixed media i.e. T1 and T2 (Table 3).Six main microbial groups in GC-FID analysis was confirmed as G+, G-, G+G-, fungi, protozoa, and eukaryotes(Table 3). Total 37 PLFA's were identified as depicted in Table 3, and they are used as biomarkers to assess different active microbial groups in the growing media environment at the sampling time (Zaeem, 2018).

Table 3. Phospholipid fatty acids biomarkers used to characterize the active microbial community structure.

Shorthand notation	Name	Microbial group	References
C11:0	Methyl undecanoate	G+	(Alenius et al., 2009)
C12:0	Methyl dodecanoate	E	(Cavigelli et al., 1995; Zhang et al., 2017)
C13:0	Methyl tridecanoate	G+, G-	(Amir et al., 2008; Alenius et al., 2009)
(2-OH) C10:0	Methyl 2-hydroxydecanoate	G-	(Lasater et al., 2017)
C14:0	Methyl tetradenote	G+	(Sheng et al., 2012)
i-C15:0	Methyl 13-mmethylteradenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
a-C15:0	Methyl 12-methylteradenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
C15:0	Methyl pentadecanoate	G+	(Huygens et al., 2011; Papatheodorou et al., 2012)
i-C16:0	Methyl 14-methyl pentadecanoate	G+	(Wang et al., 2016; Zhang et al., 2016)
(2-OH) C12:0	Methyl 2-hydroxydodecanoate	G-	(Lasater et al., 2017)

C16:0	Methyl Hexadecenoate	G+, G-	(Kujur and Patel, 2014; Wu et al., 2015)
C16:1n7	Methyl cis-9-Hexadecenoate	G+, G-	(Brockett et al., 2012; Wang et al., 2016)
C17:0 **C16:1n5**	Methyl 15-Methylhexadecanote; Methyl 11(Z)-hexadecenoate	G+	(Wang et al., 2016; Zhang et al., 2016)
C17:0	Methyl Heptadecanoate	G+	(Huygens et al., 2011; Papatheodorou et al., 2012)
(3-OH) C12:0	Methyl 3-hydroxydodecanoate	G-	(Spring et al., 2000; Kaur et al., 2005)
Δ17:0	Methyl cis-9.10-Methylenehexadecanote	G-	(Wang et al., 2016; Zhang et al., 2016)
(2-OH) C14:0	Methyl 2-Hydroxytetradecanoate	G-	(White and Rice, 2009)
C18:0	Methyl Octadecanoate	G+, G-	(Brockett et al., 2012; Wu et al., 2015)
C18:1n9 t	Methyl octadecenoate(trans-9)	G-	(Moreno et al., 2017)
C18:1n9 c	Methyl octadecenoate(cis-9)	G+, G-, F	(Brockett et al., 2012; Zhang et al., 2016)

C18:2n6	Methyl octadecadienoate (all cis-9,12)	F	(Joergensen and Potthoff 2005; White et al., 2009; Zhang et al., 2016)
C19:0	Methyl nonadecanoate	G+	(Gharaibeh and Voorhees, 1996)
(3-OH) C14:0	Methyl 3-Hydroxytetradecanoate	G-	(Papatheodorou et al., 2012)
ΔC19:0	Methyl methyleneoctadecanoate (all cis-9,10)	G-	(Wang et al., 2016)
C20:0	Methyl eicosenoate	P	(Schindlbacher et al., 2011)
(2-OH) C16:0	Methyl 2-hydroxyhexadecanoate	G-	(Sheng et al., 2012)

G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryotes

Plant heights recorded for the three plant species i.e. kale, Swiss chard and pak choi were significantly ($P<0.05$) higher in the T1 treatment than T2 (Table 4). The suitable multiple mean comparison (MMC) method was used for those treatments, which showed significant effect (i.e., p-value is less than 0.05) for main effects and/or interaction effects. If the interaction effect was significant, then 1) MMC would be done on the interactions, 2) The significance of lower-order interactions would be ignored, and the main effects associated with the significant interaction effect

as well. In analysis, if there was a significant interaction, then the main effects would be ignored. The interaction effect of mixed media treatments and plant species on plant height was highly significant ($p= 0.007$). The height for plants grown in T1 might be due to the availability of adequate amounts of essential plant nutrients and other growth factors when the vermicast content of the medium was increased as compared to T2. The number of plant green leaves was higher for plants grown in the Promix-BX alone (control) medium as compared to T1 and T2. The number of leaves on T1 grown plants were more than five times higher than that for the T2 grown plants (Table 4). These again can be attributed to the higher nutrients content and improved growing medium properties following the application of higher amount of the vermicast.

Table 4. Effects of different mixed media on growth components of three different species of microgreen plants.

Treatment*Crops	Plant height	Number of green leaves	Fresh weight	Dry weight	Leaf area	Stem diameter
Control*pak choi	12.73b	7.2b	9.22b	1.19a	158.50a	6.43a
Control*Swiss chard	18.58a	11.4a	10.51a	0.92b	166.36a	5.90a
Control*kale	15.15b	8.2b	4.47c	0.80b	89.00b	4.04b
T1*kale	5.20c	4.2cd	0.54de	0.11c	13.12cde	1.84ef
T1*pak choi	3.65d	3.5de	0.71d	0.09c	15.13cd	2.49c
T1*Swiss chard	5.85c	4.9c	0.93d	0.09c	16.06c	2.39cd
T2*kale	2.33e	2.2f	0.07e	0.01c	1.58e	1.55f
T2*pak choi	2.06e	2.7ef	0.12e	0.02c	2.29e	2.01de
T2*Swiss chard	2.32e	1.75f	0.07e	0.01c	1.80de	1.45f

Means that do not share the same alphabetical letter in a column are significantly different.

Analysis of variance showed that the grown plants were non-significant (p=0.782) among mixed media T1, T2 and the control (Promix-BX). Similarly, all the treatments did not have significant (P>0.05) effect on leaf dry weight, but they were smaller than the control. However, Swiss chard

and pak choi leaf fresh weights, leaf area and stem diameter were significantly ($P<0.05$) increased by T1 than T2 (Table 4). Pak choi and Swiss chard plant stem diameter were increased by T1 and were significantly ($p<0.05$) higher than T2, and both T1 and T2 were lower than control.

The anthocyanin content index (ACI) of the Swiss chard plant leaves was higher than those of pak choi and kale. The ACI of plants grown in the T1 medium was higher than their counterparts in the T2 medium (Table 5). For the Fo value, only the Swiss chard showed that T1 was significantly higher than T2 (Table 5). Conversely, Fm value of kale and Swiss chard plants was significantly ($P<0.05$) different between T1 and T2.

Table 5. Mean Fm, Fv, Fo and ACI of kale, Swiss chard and pak choi plants as affected by combined medium treatment and plant species.

Treatment*Crops	F_M	Fv	F_O	ACI
Control*pak choi	933.90a	768.50a	165.40ab	8.14a
Control*Swiss chard	888.71abc	705.00ab	183.71ab	4.63bc
Control*kale	944.90a	758.40a	186.50a	6.78a
T1*kale	862.11ab	686.40a	175.71ab	3.68c
T1*pak choi	861.66ab	690.41a	171.24ab	4.09bc
T1*Swiss chard	839.00abc	659.00ab	180.00a	4.45b
T2*kale	740.37c	584.53b	155.84b	1.82de

T2*pak choi	761.20bc	595.20b	166.00ab	2.32de
T2*Swiss chard	477.39d	354.23c	123.15c	2.81d

Means that do not share the same alphabetical letter in a column are significantly different.

Chlorophyll fluorescence activity was used to assess photosynthetic activities in the microgreen plants (i.e. kale, pak choi and Swiss chard) grown in the different treatments. The ratio of Fv/Fo stand for the structural alterations in chlorophyll fluorescence and indicated the electron donation efficiency (Havaux & Lannoye, 1985).The Fv/Fm and Fv/Fo can be used to determine the maximum quantum yield of chlorophyll fluorescence photochemistry and the potential photosynthetic capacity, respectively (Abbey et al., 2018).We did not find the differences among interaction effect on plant and treatments, the comparison was done among treatments. It was found that the value of Fv, Fv/Fm and Fv/Fo of T1 was significantly higher than T2 on all the three plant species (Table 6). Lower values of T2 of physiological indices revealed the reduce chloroplasts photochemical activities and decreased photosynthesis (Garousi et al., 2015).

Table 6. Mean Fv/Fm and Fv/FO of plants (kale, pac chi, and Swiss chard) on vermicast-sawdust mixed medium treatments.

Crops	Fv/Fm	Fv/Fo
Control	0.804a	4.177a
T1	0.791a	3.903a
T2	0.766b	3.408b

Means that do not share the same alphabetical letter in a column are significantly different.

The different combinations of media treatments were compared by principal component analysis

(PCA) segmentation on plant growth parameters and media biomarkers (Figure 1). Biplots in Figure 1showedthe relationships between observed plant growth components, microbial groups, nutrients mineralization and growing media treatments. The growing media amendments were segregated into distinct quadrants of the biplot based on microbial group distributions and nutrient mineralization. This segregation was based on PCA analysis, where 89.6% of the variations in the dataset for plant growth components as affected by the media mineral nutrients and active microbial compositions were explained (Figure 1).

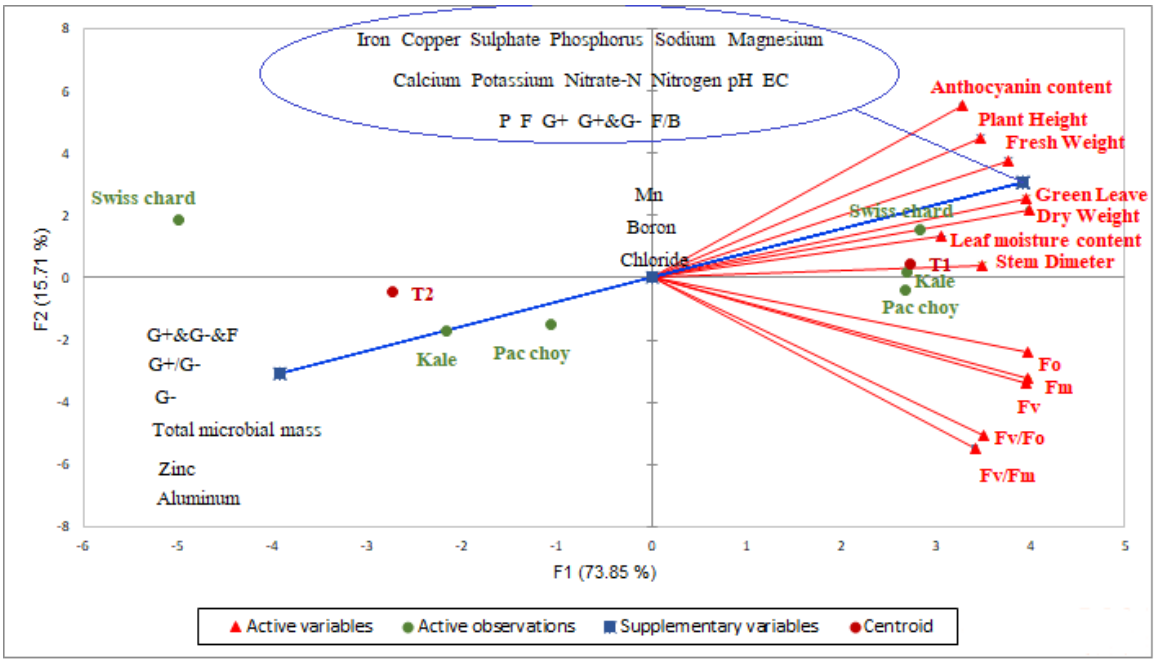

Figure 1. A biplot showing the relationship between active microbial composition and mineral nutrients of the mixed growing media. T1, 60% vermicast + 40% sawdust; T2, 40% vermicast + 60% sawdust. G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryotes. The right top is quadrant 1, and quadrants counted clockwise.

From quadrant 1 in Figure 1, the properties of T1 was majorly due to nutrients mineralization and active microbial structure. Plant stem diameter, fresh weight, dry weight, leaf moisture content,

plant height, number of green leaf, and anthocyanin content had strong correlations($r>0.7$) with G+ bacteria, eukaryotes, fungi, protozoa, G+/G-ratio, F/B ratio; and also N, electric conductivity, pH, NO_3^--N, Ca, K, Mg, P, Na, SO_4^{2-}, Cl^-, Al, copper, Fe and Zn (Table 7). The optimum growth pH of kale is around 5.5 to 6.5 (Lannotti, 2019). Bok choi requires a soil pH between 6.0 and 7.5 (Lannotti, 2019). The pH had a high correlation with plant growth parameters (Table 7). The optimum pH for Swiss chard plant growth is between 6.0 and 6.4 although it can tolerate a more neutral soil (Lannotti, 2018). This means the pH of the growing medium must be controlled to meet plant requirement. If the pH level is below 6, the ground limestone could be added to growing media; if the pH level is above 7.5, the sulfur could be a choice to adjust the amendment pH.

Table 7. Pearson's Correlation coefficients for plant growth components, microbial populations and mineral nutrients that was found significant in quadrants 1 of the PCA biplot.

Variables	Stem diameter	Fresh weight	Dry weight	Leaf moisture content	Plant height	Green leaf number	ACI
G+	0.73	0.94	0.98	0.78	0.90	0.90	0.92
F	0.73	0.94	0.98	0.78	0.90	0.90	0.92
P	0.73	0.94	0.98	0.78	0.90	0.90	0.92
G+/G-	-0.73	-0.94	-0.98	-0.78	-0.90	-0.90	-0.92
F/B ratio	0.73	0.94	0.98	0.78	0.90	0.90	0.92
N (%)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
EC (µS/cm)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
pH	0.73	0.94	0.98	0.78	0.90	0.90	0.92
NO_3^--N	0.73	0.94	0.98	0.78	0.90	0.90	0.92
Ca (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
K (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
Mg (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
P (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
Na (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
SO_4^{2-} (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
Cu (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92
Fe (mg/l)	0.73	0.94	0.98	0.78	0.90	0.90	0.92

Value are correlation values (p-value), with a significance level alpha=0.05. G+, gram-positive bacteria; G-, gram-negative bacteria; F, fungi; P, protozoa; E, Eukaryotes;NO_3^---N; Ca, calcium; P, potassium, Mg, magnesium; P, phosphorus; Na, sodium; SO_4^{2-}, sulphate; Cl^-, chloride; Al, aluminum; Cu, copper; Fe, iron; Zn, zinc. Protozoa, fungi and G+ bacteria present its ratio to total microbial mass.

5.4 CONCLUSION

The total calcium, magnesium, sodium and sulphate contents in T1 (60% vermicast+40% sawdust) were higher than in T2 (40% vermicast+60% sawdust) by 60%, 65%, 59% and 53% respectively. Similarly, the total nitrogen ($p<0.001$), total potassium ($p=0.004$) and electric conductivity ($p=0.004$) in T1 were significantly higher than in T2. The mineral nutrients content had significantly ($p<0.05$) highly positive correlation ($r<0.7$) with active microbial composition in the mixed media. Merged data for phospholipid fatty acids and mineral nutrients with plant growth showed that T1 significantly ($P<0.05$) enhanced the plant morphological and physiological profile of kale, Swiss chard and pak choi plants as compared to T2 plants. In addition, in a combined (treatment X plant species) plant growth indicators versus mineral nutrients and active microbial group analyses, T1 has been shown to be superior to T2.In summary, the T1 mixed medium was the most effective combination for producing microgreens (kale, pac choi, and Swiss chard in this experiment). Further research should investigate edible quality components of the microgreens produced.

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CHAPTER 6 CONCLUSION

Conventionally, organic amendments were used as the sources of plant nutrients. As the well understanding of the roles of organic amendments on plant growth and soil fertility, farmers use organic amendments instead of synthetic chemical fertilizers and pesticides to reduce environmental pollution, production cost, as well as to restore or reclaim degraded soils. The organic amendment used in this project was the mixed media, vermicast-sawdust. Many agricultural problems could be avoided by vermicast application, such as soil structural degradation, erosion, nutrient loss, nutrient toxicity and salinity attributable to its physicochemical nature, microbial richness and properties. Due to these functions on plant growth, vermicast is treated as one of the most promising organic amendments in agriculture and horticulture. Sawdust is widely used as a growing medium substrate in greenhouse production because it is low cost and local available technology, as well as its good water-holding capacity.

In this study, the total dissolved solids, electrical conductivity and salinity in the solution of all the mixed growing medium treatments were progressively increased before reaching stable concentrations. Nutrient released from A1 and B1 were the highest while A5 and B5 had the least and the others (i.e. A2, A3, A4, B2, B3 and B4) were intermediate. The interaction of Time *Treatment ($p<0.0001$) and the main effect of *T. viride* was significant ($p<0.0001$). Nutrients mineralization and availability were higher in the media treatments without *T. viride* (i.e. group A) compared to media with added *T. viride*. The highest EC combination was 34.5 hr with A1 and B1 and the least was 0.25 hr with A5 and B5). Moreover, the mixed media without *T. viride* had positive and significant effect on total dissolved solids, electrical conductivity and salinity. The N, Ca, K, Mg, Na, S and B contents of A1 and B1 were higher than the rest of the treatments. A

reduction in vermicast content of a mixed media leads to a significant reduction in mineral nutrients. This study did not report on microbial relationship with the different growing medium treatments. However, further studies are in progress to assess the growing media microbial dynamics and plants growth performance.

The results obtained from the present study demonstrate that the addition of *Trichoderma* was not significantly beneficial in enhancing the microbial activities compared to growing media without *Trichoderma viride*. The mineral nutrients released in the mixed media had a high correlation ($r>0.7$) with the active microbial groups present. We found six main microbial groups present in the saw vermicast based media as follows: G+, G-, G+G-, fungi, protozoa, archaea and eukaryotes. G+, G- as well as the total of G+ and G- bacteria contributed 18.9%, 35.7% and 39.8%, respectively, to the overall total microbial population observed in the media. Treatments A2 and B2 (60% vermicast-40% sawdust, without and with *T. viride*) showed higher pH, Protozoa, F/B ratio, and Fe. We found that treatments B1 and B3 (80% vermicast-20% sawdust and 40% vermicast-60%sawdust, both with *T. viride*) contained higher amounts of Na and PG than the other treatments. The mineralized nutrients (eg chloride, sodium, nitrogen, potassium, sulphate, magnesium, and calcium) in the mixed growing media is associated with increased active microbial community composition (eukaryotes, G+, and the ratio of G+/G-). Additionally, the vermicast-sawdust in the ratio of 40:60 and 60:40 had superior effects on the microbial composition under the conditions of the present study. After 30 days (i.e. sampling times 3 and 4), the sum of bacteria and fungi ($p=0.042$) were significantly higher than those media sampled before 30 days (i.e. sampling times 1 and 2). On the contrary, the content of the bacterial group was significantly ($p=0.009$) higher in the growing media incubated for less than 30 days. For the sum of bacteria, the first two samplings time were higher. The decreased microbial activity in the aged mixed media appears to be related

to diminution in the available carbon in the substrate and lower microbial population and nutrient status. Overall, no incubation of the sawdust vermicast based media appears to be more beneficial in supporting a superior active microbial composition. For this project, the ratio of 40:60 and 60:40 vermicast-sawdust without incubation would be suggested as superior in terms of improved active microbial composition, health status and nutrient mineralization for plant growth.

The total calcium, magnesium, sodium and sulphate contents in T1 (60% vermicast+40% sawdust) were higher than in T2 (40% vermicast+60% sawdust) by 60%, 65%, 59% and 53% respectively. Similarly, the total nitrogen ($p<0.001$), total potassium ($p=0.004$) and electric conductivity ($p=0.004$) in T1 were significantly higher than in T2. The mineral nutrients content had significantly ($p<0.05$) highly positive correlation ($r<0.7$) with active microbial composition in the mixed media. Merged data for phospholipid fatty acids and mineral nutrients with plant growth showed that T1 significantly ($P<0.05$) enhanced the plant morphological and physiological profile of kale, Swiss chard and pak choi plants as compared to T2 plants. In addition, in a combined (treatment X plant species) plant growth indicators versus mineral nutrients and active microbial group analyses, T1 has been shown to be superior to T2. In summary, the T1 mixed medium was the most effective combination for producing microgreens. Further research should investigate edible quality components of the microgreens produced.

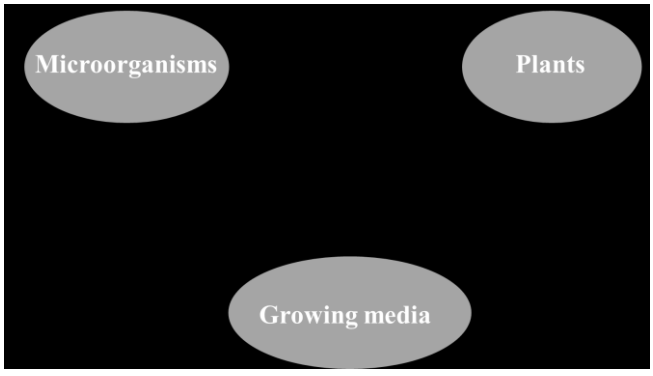

Figure 1. Interactions between plants, microbiota, and growing media (Jacoby et al., 2017).

Organic input could be one of the alternative methods of sustaining plant nutrition to lower inputs of mineral fertilizers and to supply plants with specific root-associated microbes (Jacoby et al., 2017; Foley et al., 2011). From Figure 1, we found that the plants gained nutrients from the growing media through activities of microorganisms that made the nutrients available. Microbes can depolymerize and mineralize the organic-bound nutrients, which was made available to the plants.

Taken together; our results demonstrated that 60% vermicast+40% sawdust could be a new growing media for kale, pac choi, and Swiss chard growth. The nutrient-release pattern experiment proved that growing media without incubation had more nutrient released than 30 days', 60 days', and 90 days' incubation. The nutrient mineralization experiment showed that increased proportion of vermicast increased mineral nutrients composition of the growing media. Adding extra *Trichoderma* on mixed media did not showed significant ($p<0.05$) effect regarding to the results of both nutrient-release pattern and nutrient mineralization experiments. The active microbial composition experiment showed that 60% vermicast+40% sawdust, 40% vermicast+60% sawdust, 60% vermicast+*Trichoderma*+40% sawdust, 60% vermicast+ *Trichoderma*+40% sawdust had higher connection with minerals. In addition, the data of plant growth showed that 60% vermicast+40% sawdust had better plant performance than 40% vermicast+60% sawdust. These studies have important value in the investigating new combination of growing media.

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