Quantifying the Role of Soil Microbial Activity in Cover Crops on Newly Established Fruit Trees in the Western Cape

by

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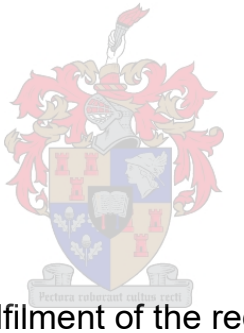

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

Limited research is available on the role of cover crops in newly established deciduous fruit orchards in South Africa. Thus, three field studies were conducted to evaluate the contribution of cover crops towards soil health/fertility. The effect of cover crop combinations on soil characteristics (mineral composition, soil moisture, soil compaction, water holding capacity, microbial activity and tree performance) and the contribution towards weed suppression and biomass production for mulching in a perennial orchard were quantified during two and three consecutive seasons in two locations. In addition, soil microbial activity and diversity were quantified with three locally available methods (Molecular fingerprinting, CO_2-burst test and gas chromatography). A two-year field study (Trial 1 and 2) was conducted in Stellenbosch, (33°56'51.27" S 18°52'19.29" E) and a three-year field study (Trial 3), in Elgin (34°09’16.83’ S, 19°02’28.01” E), Western Cape. Trial 1 (Multi species: forage rye, radish and white mustard) and trial 2 (Single specie: forage rye) comprised four treatments each: i) Control (natural vegetation), ii) Cover crop with no fertilizer application (MNFA; SNFA), iii) Cover crop with a single organic fertilizer application (MSFA; SSFA) and iv), Cover crop with a double organic fertilizer application (MDFA; SDFA). Trial 3 consisted of five annual winter cover crop treatments: i) Phacelia (PC) ii) Forage rye & vetch (FRVC) iii) Forage radish & white mustard (FRWMC), iv) Forage barley & peas (FBPC) v), Forage rye (FRC). Cover crops provided a significantly higher above- and below ground biomass compared to natural vegetation in trial 1 and 2, however, biomass differed amongst cover crop treatments. Biomass production differed significantly between cover crop treatments in trial 3. FRC performed the best, followed by FRVC and FRWMC. Crop species were the primary factor in plant performance and other factors contributed less towards biomass. However, weed suppression efficiency was affected by cover crop biomass and species. In trial 3, Forage rye, in combination or monoculture, performed the best in terms of consistency, biomass for mulching purposes, root length and weed suppression. Cover crops did not affect tree performance at this stage but should be monitored in future when tree roots will reach the work row. Cover crops directly contributed towards stimulation of the microbial community and conserved soil moisture compared to the control (natural vegetation) during spring. However, from our results it was evident that soil is an interlinking system and that it should be managed holistically in

terms of soil physical, chemical and biological components. A long-term study should be initiated to evaluate the effect of using cover crops to improve soil health (Soil physical, chemical and biological interrelationship) and how improving the soil biological component will contribute to an alternative system such as farming with nature, minimizing external inputs, building soil structure and providing nutrient dense food.

OPSOMMING

Daar is beperkte inligting beskikbaar oor die implimentering van dekgewasse in nuut gevestigde vrugte-boorde in Suid-Afrika. Drie veld studies is uitgevoer om die bydra van dekgewasse op grond-gesondheid en -vrugbaarheid te evalueer. Die effek van dekgewas kombinasies op grond einskappe (minerale-samestelling, grondvog, grond-kompaksie, waterhouvermoë, mikrobiese aktiwiteit) en boom prestasie en die bydra tot onkruid-onderdrukking en biomassa produksie van ‘n deklaag in ‘n meerjarige boord is gekwantifiseer gedurende twee en drie opeenvolgende seisoene, in twee verskillende geografiese gebiede. Additioneel is grond-mikrobe aktiwiteit en -diversiviteit gekwantifiseer met drie plaaslik beskikbare metodes (molekulêre vingerafdruk, ‘CO_2-burst’ en gas-chromatografie). ‘n Twee-jaar veld-proef (Proef 1 en 2) is uitgevoer in Stellenbosch (33°56'51.27"S 18°52'19.29"E) en ‘n drie-jaar veld proef (Proef 3), in Elgin (34°09’16.83”S, 19°02’28.01” E), Wes-kaap. Proef 1 (Multi-spesies: voerrog, radys en wit mosterd) en proef 2 (Enkel spesies: voerrog) het beide uit vier behandelinge bestaan: i) Kontrole (onkruid), ii) Dekgewas met geen kunsmis (MNFA; SNFA), iii) Dekgewas met ‘n enkele organiese kunsmis toediening (MSFA; SSFA) en iv), Dekgewas met ‘n dubbele organiese kunsmis toediening (MDFA; SDFA). Proef 3 het uit vyf eenjarige dekgewasse bestaan: i) Phacelia (PC), ii) Voerrog & wieke (FRVC), iii) Radys & wit mosterd (FRWMC), iv) Gars & ertjies (FBPC) en v), Voerrog (FRC). Dekgewasse het ‘n betekenisvolle hoër bo- en ondergrondse biomassa gelewer in vergelyking met die kontrole in proef 1 en 2, maar die biomassa het wel verskil tussen die dekgewas-behandelings. Biomassa produksie het betekenisvol verskil tussen die dekgewas-behandelings in proef 3. FRC het die beste gevaar, gevolg deur FRVC en FRWMC. Plant spesies was die primêre faktor wat plant-prestasie bepaal het en enige additionele faktore het minder bygedra tot biomassa produksie. Dekgewasse het ‘n positiewe effek op onkruid-onderdrukking getoon en kan as alternatief gebruik word. Die effektiwiteit van onkruid-onderdrukking is beïnvloed deur dekgewas-biomassa, asook die spesies. In proef 3, het voerrog, in kombinasie met ander dekgewasse of as monokultuur het die beste gevaar in terme van konsekwentheid, biomassa vir deklaag doeleindes, wortel-lengte en onkruid-onderdrukking. Dekgewasse het geen effek op boom prestasie gehad nie, maar dit moet gemonitor word oor tyd. Dekgewasse het ‘n direkte bydra gehad tot die

stimulasie van mikrobiese-populasies en grondvog beter bewaar as die kontrole gedurende die lente. Dit was duidelik uit ons resultate dat grond ‘n dinamiese sisteem is en dat dit holisties bestuur moet word in terme van grond fisiese, chemiese en biologiese komponente. ‘n Lang-termyn studie is nodig om die gebruik van dekgewasse om grond-gesondheid (grond fisiese, chemiese en biologiese verhouding) te verbeter te evalueer en te bepaal hoe verbetering van die biologiese komponent sal bydra tot ‘n alternatiewe sisteem soos natuur boerdery, minimalisering van toediening van eksterne produkte, die verbetering van grond-struktuur en voorsiening van voedingsdigte voedsel.

DEDICATION

First and foremost, I would like to dedicate my thesis to my Heavenly Father for giving me the strength and ability to start and complete my thesis.

Blessed is the man who trusts in the Lord, whose trust is the Lord. He is like a tree planted by water, that sends out its roots by the stream, and does not fear when heat comes, for its leaves remain green, and is not anxious in the year of drought, for it does not cease to bear fruit – Jeremia 17:7-8.

Secondly, I would like to dedicate my thesis to my mom and dad. As a thank you for their unconditional love, support and always being there for me.

Lastly, I would also like to dedicate my thesis to my older brother as a thank you for his love and support.

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GENERAL INTRODUCTION

Soil health consists of soil physical, chemical and biological components and it is important to consider the inter-relationship (Magdoff and van Es, 2009). When these three components are managed optimally, healthy soils enable high yields (Magdoff and van Es, 2009). However, mainstream agriculture and plant nutrition are based on soil chemistry as the only parameter as indicator of soil fertility and for the past few decades, the focus was on the chemical and physical aspects, neglecting the soil biology component. Of late, there was a shift towards the consideration of all three categories in order to protect long-term sustainability of soils, water and cropping systems (Woodyward and Kladivko, 2017).

According to FAO (2011): “Systems at risk are production systems where the land and water resources supporting agricultural production are constrained to a point where their capacity to meet current and future needs is seriously jeopardized”. These constraints may be intensified by unsustainable agriculture practices, pressure from the social and economic sectors and climate change. To ensure that the current food production systems will meet future needs and provide nutrient dense food, alternative approaches are necessary to meet challenges climate change, agricultural practices leading to soil degeneration and self-induced droughts and increasing fertilizer and pesticide costs. Cover crops are known for their benefits, i.e., improving soil health and rendering the soil more resilient to drought and other extreme environmental factors (Doran and Zeiss, 2000)

Our study quantified different roles of cover crops in newly established deciduous fruit orchards, in the Western Cape. We evaluated the effect of cover crops on soil microbial communities and soil physical, chemical and biological components in three sites. A two-year field study (Trial 1 and 2) was conducted in Stellenbosch, (33°56'51.27"S 18°52'19.29"E) and a three-year field study (Trial 3), in Elgin (34°09’16.83”S, 19°02’28.01”E), Western Cape. Trial 1 (Multi specie: forage rye, radish and white mustard) and trial 2 (Single specie: forage rye) comprised four treatments each: i) Control (natural vegetation), ii) Cover crop with no fertilizer application (MNFA; SNFA), iii) Cover crop with a single organic fertilizer application (MSFA; SSFA) and iv), Cover crop with a double organic fertilizer application (MDFA; SDFA). Trial 3 consisted of five annual winter cover crop treatments: i) Phacelia (PC)

ii) Forage rye & vetch (FRVC) iii) Forage radish & white mustard (FRWMC), iv) Forage barley & peas (FBPC) v), Forage rye (FRC).

Research aimed at quantifying the effect of cover crops on the soil environment (soil structure, soil compaction, soil moisture, water holding capacity, microbial activity and diversity and tree performance) was conducted in paper 1. The study was conducted in Stellenbosch and Elgin.

Our second paper evaluated three locally available soil health tests to quantify microbial activity and diversity in soil. The study was conducted in Elgin, soil samples were taken and analysed at the beginning of the season (March) and at the end of cover crop lifecycle (September).

Our third paper evaluated the contribution of different cover crop species (single or multi species) towards biomass production to apply as a mulch, as well as for suppression of weeds. The study was conducted in Stellenbosch and Elgin, excavation of cover crops and determination of weed suppression efficiency took place at the end of August/ beginning of September.

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Literature review: Quantifying the role of cover crops on soil biology, physics and chemistry as well as plant performance.

Introduction

The use of cover crops to improve soil health and yield dates back to ancient times and the use of green manure has been practiced for 3000 years according to Chinese manuscripts (Magdoff and van Es, 2009a). Cover crops are close-growing crops with various functions, but are grown primarily to protect soil and improve soil health. Soil health consists of three categories: soil physical, chemical and biological properties. Cover crops in the context of a cash cropping system are defined as any living ground cover crop planted between main cash crop cycles to provide ground cover during a fallow period and prevent soil from being bare or crops intercropped with main cash crops (Reeves, 1994; Hartwig and Ammon, 2002). In contrast, in a perennial orchard, a cover crop is any living ground cover of annuals, biennials or perennials and include grasses, legumes or various broadleaf plants grown on bare soil in the work row, in between tree rows (Hartwig and Ammom, 2002; Hammermeister, 2016). Cover cropping is used in regenerative agriculture to increase soil health, rendering the soil more resilient to drought and other extreme environmental factors (Doran and Zeiss, 2000). Incorporation of cover crops in a perennial orchard contributes to high specie richness and promotes a positive plant-soil feedback by promoting plant performance, beneficial microbial communities and reducing disease outbreaks (Keesing *et al*., 2010). Cover crops produce above as well as below ground biomass. Above ground biomass improves the photosynthetic capacity and rate of orchards, the total soil C content and reduces carbon dioxide (CO_2) emission. Below ground biomass improves soil structure and texture and creates a suitable environment for beneficial microbial communities. Jackson *et al.* (2017) reported that stabilisation of root-derived C was five times higher than above ground biomass in annual systems and suggested that this could be even higher in perennial-based ecosystems. Cover crops also protects main crops against pests (Steyn *et al*., 2014) by providing a habit for natural predators such as spiders, which in turn assist with a reduction in pest populations (Magdoff and van Es, 2009a).

Suitable Cover Crops for Perennial Orchard

Cover crops can be divided into two groups: legume and non-legume plants. Legumes consist of winter and summer annual, biennial and perennial legumes. Non-legumes consist mainly of grasses and broadleaf plants. There is a cover crop option that will fit every situation. The aim should be to choose the best recommended cover crop species for the region (Clark, 2012a). The selection of cover crops plays a critical role in the success of the main desired outcome. Factors such as climate, water availability, soil type, soil conditions and slope of the orchard should be considered, as well as when (winter or summer) the cover crop should be established, the soil preparation required for successful establishment and the termination of cover crops.

Legume Group

Legumes provide an economical source of N to the orchard through fixing atmospheric nitrogen (N_2) by forming symbioses with N-fixing bacteria such as *Rhizobium*, *Actinomycetes* and *Cyanobacteria*, which forms nodules on roots (Brady and Weil, 2008b; Willis, 2008; Hatfield and Sauer, 2011a). Rhizobia is one of the familiar strains (Willis, 2008). According to Pavek and Granatstein (2014), an economic analysis of legume N indicated that it is comparable with commercial N-sources and less expensive than organic sources. Nitrogen fixed from legumes does not cause acidification and salinization issues associated with commercial fertilizers such as ammonium (NH_4) or nitrate (NO_3) based fertilizers (Pavek and Granastein, 2016).

Forage Peas

Forage Peas (*Pisum sativum*) is an ideal crop to use in a cover cropping system. It is known for rapid establishment and ability to grow in well-drained soil types (Barenbrug, 2020) and short-term soil conditioning (Clark, 2012b). Forage peas can be grouped as summer or winter annuals. Forage Peas have taproot systems that forms nodules on the root surface. The plant can reach a height between 30-75 cm and produces reddish, purple or white flowers (Nda, 2021). In South Africa, the ideal sowing time ranges from end of April to mid-June, depending on the soil moisture and it grows well with most forage cereals like oats, triticale and barley. Forage Peas play an important role as a plough-down N source, providing high biomass production and suppression

of weeds (Clark, 2012b). Jahanzad *et al*. (2017) reported that peas, as a rotation crop, were effective in reducing N fertilization requirements for potatoes.

Medics

Medics (*Medicago* spp.), also known as black or burr medic, is a summer or winter annual, and includes 35 known species that vary widely in habit, maturity date and cold tolerance (Clark, 2012b). Medics are adapted to grow in dryland production areas and are a good source of N under low moisture conditions which improves when moisture levels are higher (Nivelle *et al*., 2016). It establishes quickly, suppresses weeds, is effective in erosion control, as well as increases OM and stimulates the microbial communities in soil (Nivelle *et al*., 2016; Lupwayi *et al*., 2018).

Hairy Vetch

Hairy vetch (*Vicia villosa*) is adapted to grow in cold climates, but can be used as a winter or summer annual legume. It grows well over a wide range of soil types, but prefers a sandy to loamy soil, which is well drained (Clark, 2012b; Undersander *et al*., 2020). Hairy vetch can be combined with small grains, field peas, bell peas, crimson clover and buckwheat in a cover crop mixture (Clark, 2012b). This plant grows between 90 and 120 cm in height and produces blue-violet flowers. Root architecture consists of a weak tap root system, capable of growing 60 to 90 cm deep into the soil, but most roots are concentrated in the top 20 cm of soil. Hairy vetch acts as a good N source (Clark, 2012b; Ates *et al*., 2013; Barenbrug, 2020), weed suppressor (Clark, 2012b; Barenbrug, 2020) and soil conditioner by improving topsoil tilth, creating a loose friable soil structure (Clark, 2012b). Hairy vetch also contributes to stimulating the microbial community by increasing fungal biomass and diversity (Kataoka *et al*., 2017), is an effective phosphorous (P) scavenger (Clark, 2012b) and able to increase the rate of P solubilizing fungi and soil phosphatase activity (Kataoka *et al*., 2017).

Cow Peas

Cowpeas (*Vigna unguiculate*) is a summer annual legume and indigenous to Africa. Also known as blackeye peas, they thrive under warm, moist conditions, but are able to withstand drought and low soil fertility (Clark, 2012b). Cowpeas can grow well over a wide range of soil types, but prefers sandy soil. They vary in growth forms and can

be erect, trailing, climbing or bushy with a strong taproot system with numerous lateral roots (Production guidelines for cowpeas, 2011). Cowpeas establish quickly to cover soil and smother weeds, they are involved in N-fixation and act as an integrated pest management (IPM) insectary crop that attracts beneficial insects (Clark, 2012b).

Alfalfa

Alfalfa (*Medicago sativa* L.) is an herbaceous perennial legume and not commonly used as an annual cover crop. It is an important forage crop, grown worldwide for animal feed, green manure and as land cover (Chandel *et al*., 2021). Alfalfa grows best on a deep permeable soil, with adequate soil moisture and prefers a soil pH above 6.2. With the establishment of Alfalfa, it is necessary to inoculate seeds if not grown regularly. This specie has a strong taproot system with lateral roots that has the potential to produce 5 to 25 stems per crown and reach a height of 40 to 60 cm. Alfalfa can be used to reduce compaction and erosion, improve soil infiltration and permeability, and assist with weed suppression and N-fixation (Latrach *et al*., 2014; Barenbrug, 2020). Alfalfa is a host to slugs and can serve as a trap crop.

Clover

Clover (*Trifolium*) may be divided into an annual or perennial legume. Annual clover consists of: i) Arrowleaf clover (*T. vesiculsum*), ii) Balansa clover (*T. michelianum*), iii) Crimson clover (*T. incarnatum*), iv) Persian clover (*T. resupinatum*) and v) Subterranean clover (*T. subterranean*). Perennial clover consists of: i) White clover (*T. repens*), ii) Red clover (*T. pretense*) and iii) Strawberry clover (*T. fragiferum*). Clover prefers a moderate acid-alkaline soil pH (Barenbrug, 2020). Crimson and Subterranean clover are commonly used as annual perennial cover crops (Clark, 2012c). Crimson clover establishes quickly and possesses a taproot system, provides N early spring and prevents erosion as well as provide a good inter-row ground cover (Clark, 2012c). Clover does not grow well in high pH soils or poorly drained soils (Magdoff and van Es, 2009a) and is able to scavenge and fixate N (Clark, 2012c). Clover grows well with small grains, grasses and other clovers such as sub-clover and red clover (Clark, 2012c). Subterranean clover has a shallow root system, but is drought tolerant (Barenbrug, 2020). It assists with loosening the soil, fixate N (Clark, 2012c), is an effective weed suppressor and reduces soil erosion. It provides a good soil cover in the orchard, however, it is perceived as a potential weed (Clark, 2012c;

Barenbrug, 2020). White clover is commonly used as a perennial cover crop and possesses a small shallow root system, making it sensitive to dry conditions (Magdoff and van Es, 2009a; Barenbrug, 2020). It is useful as a living mulch in an orchard because it is short and can tolerate shading better than many other legumes (Magdoff and van Es, 2009a). It can also fixate N, tolerate traffic and attracts beneficial insects (Clark, 2012c).

Non-legume Group

Cover crops in the non-legume group include cereals, forage grasses and broadleaf species. Generally, these crops are useful for scavenging nutrients, conditioning soil, reducing erosion, suppressing weeds and producing organic material (OM) as a mulch (Clark, 2015). Brassicas for orchards include forage radish, mustard and rapeseed. Commonly used grass cover crops include the annual cereals forage rye, wheat, barley and oats, as well as annual or perennial forage grasses such as ryegrass and warm season grasses such as sorghum-sudan grass. Phacelia and buckwheat attract natural predators and beneficial insects.

Forage Radish

Radish (*Raphanus sativus*) is a fall/winter cover crop and forms part of the *Brassica* family. According to Magdoff and van Es (2009a), these cover crops maintain soil quality and fertility, as well as cropland productivity. It is recommended to combine radish with a grass such as forage rye, to promote *mycorrhizae* formation, because *Brassica* family don't develop a symbiotic association with *mycorrhizal* fungi (Magdoff and van Es, 2009a; Phillips, 2017). Radish prefers sandy to loamy soil and if soil moisture is adequate at planting, germination and establishment of seedling is promoted. Radish can reach a height of 60 cm and has a taproot system with potential to grow to a depth of 3 m (Jacobs, 2012). The thick, fleshy upper part of the taproot grows 30 to 50 cm and 5 – 7cm in diameter (Weil *et al*., 2009). The root architecture of radish enables the plant to eleviate soil compaction and penetrate plough pans better than crops such as rye that are usually used as cover crops (William and Weil, 2004). Williams and Weil (2004) reported that radish can be a no-till alternative to mechanical ripping. Radish is a good nutrient scavenger (Magdoff and van Es, 2009a; Jahanzad *et al*., 2017), weed suppressor (Weil *et al*., 2009; Barenbrug, 2020) and act

as a bio-fumigator by producing a chemical known as glucosinolate, which helps to suppress soil pests, especially root pathogens and plant parasitic nematodes (Ngouaiio and Mutch, 2004; Magdoff and van Es, 2009a). Hemayati *et al*. (2017) reported that sugar beet cyst nematode (*Heterodera schachtii*) was significantly reduced after the incorporation of oilseed radish and white mustard into the planting program.

Mustard

Mustard (*Brassicaceae*) is an annual cover crop, usually planted in autumn and grows throughout the winter, i.e., white mustard (*Sinapus alba or Brassica hirta*), brown mustard (*Brassica juncea*) and black mustard (*Brassica nigra L*.). Mustard establishes quickly, grows well in autumn. It has potential for a high biomass production, as well as ability to scavenge nutrients and act as a biofumigant, suppressing soil borne diseases and nematodes (St. John *et al*., 2017). As a biofumigant, mustard must be mowed before seed set and incorporated into soil, because fumigant chemicals are produced when plant cells are ruptured (St. John *et al*., 2017). It is important to mow mustard before seed ripeness, otherwise the plant can become weedy. White mustard is commonly used as a cover crop and is characterized as a plant that grows about 25-38 cm tall (Bone *et al*., 2009). This variety has a strong taproot with the ability of growing up to 70 cm deep and has fibrous roots near the soil surface (De Baets *et al*., 2011; St. John *et al*., 2017). White mustard provides good ground cover, suppresses weeds and its taproot enables deep growth and scavenging for nutrients (Barenbrug, 2020).

Rapeseed

Rapeseed (*Brassica napus*) or canola is an autumn/winter crop that grows well under relatively cool, moist conditions (Magdoff and van Es, 2009a). Rapeseed has a taproot system with lateral roots, and rooting depth may vary from 90 to 190 cm and height between 75 and 175 cm. Rapeseed functions as biofumigants, suppressors of soil pests, especially root pathogens and plant parasitic nematodes (Magdoff and van Es, 2009a), weed suppressors, and their root systems act as a biological ploughing system (Barenbrug, 2020).

Phacelia

Phacelia (*Phacelia tanacetifolia*), blue tansy or purple tansy is an annual broadleaf plant, with light-blue flowers native to the southwest region of the USA and Mexico (St. John *et al*., 2017). This crop is grown as a bee forage, because of the high quality nectar and pollen, and is rated among the top 20 honey producing plants worldwide (Stanek *et al*., 2019). According to Nielsen *et al*. (2018), it differs taxonomically from most cover crops and is thus easy to intermix with different cover crops without the fear of allopathic reactions. Phacelia is fairly drought tolerant (Kilian, 2016), robust to different sowing periods and has a long flowering period, starting at about eight weeks after sowing, attracting birds and bees (Nielsen *et al*., 2018). It prefers a loam to clay type of soil with a pH range of 6.0-7.5, and is more tolerant to cold and drought than buckwheat (Barenbrug, 2020). Phacelia has an erect growth habit, height varies from 30 to 90 cm (Smither-Kopperl, 2018) and it has a small shallow taproot system with numerous fibrous lateral roots forming an intensive network (De Baets *et al*., 2011). However, Kilian (2016) reported that roots can grow 25 to 76 cm. Aside from attracting natural predators and beneficial insects, phacelia provides ground cover and suppress weeds (Barenbrug, 2020), stimulates microbial life (Patkowska and Konopinski, 2013), improve soil structure, reduces soil erosion, is a good nutrient scavenger, absorbs excess calcium (Ca) and NO_3^- (Barenbrug, 2020). Patkowska and Konopinski (2013), determined the effect of cover crops (oats, common vetch and phacelia) on soil microbial populations under the cultivation of root vegetables and found that it promoted the development of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.).

Buckwheat

Buckwheat (*Fagopyrum esculentum*) is an annual broadleaf specie used as a summer cover crop. It establishes easily and quickly (Clark, 2012a) and can thrive in a wide variety of soils, especially poor soils (Magdoff and van Es, 2009a). Buckwheat forms a dense fibrous root system with a deep taproot and has an upright growth habit reaching a height of 60 to 150 cm (Valenzuela and Smith, 2002). Buckwheat suppresses summer annual and perennial weeds and condition the soil by improving soil aggregation and soil tilth (Björkman *et al*., 2008; Clark, 2012a). When buckwheat starts to flower, it attracts beneficial insects such as bees, predatory wasps and lady

beetles. Buckwheat is a nutrient scavenger, especially phosphate (P), releasing it to the main crop when it decomposes (Clark, 2012a).

Forage Rye

Forage rye (*Secale cereal*) is a cool season annual cereal grain and outperforms all other cover crops on infertile, sandy or acidic soil (Clark, 2012a). Forage rye has an upright growth habit with a height of 90 to 180 cm (Casey, 2012). It has a quick growing, fibrous root system (Clark, 2012a), can serve as windbreaks for low growing crops and reduce the impact of rain droplets on soil during the winter (Clark, 2012a), as well as attract beneficial insects such as lady beetles (Bugg *et al*., 1990). Forage rye establishes quickly, provides a large amount of OM, reduces soil erosion and acts as a nutrient scavenger, especially N, as well as increases the concentration of exchangeable potassium (K^+) near the soil surface (Clark, 2012a). Forage rye suppresses light sensitive annual weeds such as lambsquarters, redroot, chickweed, foxtail and dandelions through allopathy (Clark, 2012a).

Forage Barley

Forage Barley (*Hordeum vulgare*) is a cool season annual cereal grain recommended to combine with annual legumes, ryegrass or other small grains to improve soil tilth and nutrient cycling in perennial orchards (Clark, 2012a). Barley possesses a fibrous root system and is known to prevent erosion, suppresses weeds, scavenge nutrients and adding OM to the soil (Clark, 2012a).

Forage Oats

Forage oats (*Avena sativa*) is a cool season annual cereal recommended to combine with other small grains and legumes such as clover, pea and hairy vetch (Clark, 2012a). Oats can reach a height of 1.3 m and is characterized by a fibrous root system. Oats can prevent erosion, suppress weeds, scavenge nutrients, produce biomass and act as a fall legume nurse crop (Clark, 2012a).

All the cover crops discussed are, in theory, suitable for deciduous ochards, but the characteristics were described in detail, as this will influence the aim and success of

the selection of the individual crops for site specific conditions and can partly explain the lack of results that may be experienced under certain conditions.

Cover Crops and their Role in a Perennial Orchard

Erosion Control

Due to human activity, the process of soil erosion is accelerated, resulting in degeneration of soil and the loss of one or more soil functions (Jones *et al*., 2008). A perennial orchard is susceptible to wind and water erosion when there is no vegetative ground cover or plant residues in the work row (Hartwig and Ammon, 2002). Erosion has an onsite effect as well as an offsite effect and leads to degradation in soil fertility, loss of soil C and negatively influences soil water infiltration and holding capacity ability (Van Pelt and Zobeck, 2007). Under conventional farming practices, work rows of perennial cropping systems for nut and fruit crops are often kept fallow, increasing the susceptibility to erosion (Kaspar and Singer, 2011). Incorporation of cover crops in a perennial orchard is one of the management practices to reduce soil erosion (Hatfield and Saur, 2011c). Establishment of a cover crop will reduce soil detachment which is caused by the impact of raindrops (Ram *et al*., 1960). Laflen *et al*. (1985) showed that the relationship between surface cover and erosion reduction is exponential, with a decrease in erosion as surface cover approaches 100%.

Coffee plantations with little ground cover have considerable soil erosion losses, especially when cultivated in highlands on steep slopes and in newly established plantations (Hartemink, 2007). According to Hashim *et al*. (1995), soil erosion losses (11 ton.ha^{-1}.yr^{-1}) in mono cropping cocoa in Malaysia could be reduced substantially with *Indigofera spicata* cover crops. Bornemisza (1982) showed that annual soil N losses due to erosion in Colombia exceeded the amount extracted by a good crop of coffee and could be reduced to less than 2% with cover crops. Keesstra *et al*. (2016) evaluated the effects of soil management practices (tillage, herbicides and covered with vegetation) on soil water erosion in apricot orchards. Covered plots had an average of 87% vegetative cover in the winter and 56 % in the summer. They reported that the vegetative cover, soil moisture and OM were significantly higher in covered plots compared to the other two treatments, whereas soil erosion was significantly higher in the herbicide plot treatment. Covered plot treatment had the lowest erosion rate (0.02 ton.ha^{-1}.yr^{-1}), followed by tillage treatment (0.51 ton.ha.yr^{-1})

and herbicide treatment (0.91 ton.ha^{-1}.yr^{-1}). Parlak *et al*. (2015) evaluated the effect of cover crops on soil erosion in a five-year old olive orchard, characterized as a sandy loam type. Cover crop treatments: i) vetch (*Vicia sativa*), ii) field pea (*Pisum arvense* L.), iii) field bean (*Vicia faba* L.), iv) vetch & wheat (*Triticum aestivum*) mixture and v), field pea & wheat mixture as well as an additional control plot (fallow) treatment. Total soil loss was significantly higher for the control plot (583.10 g.m^{-2}) (almost 12 times higher) compared to the field pea & wheat treatment (47.20 g.m^{-2}). Prosdocimi *et al.* (2015) reported that barley straw mulch (median straw cover of 59 %) effectively reduced the median soil erosion rate in an olive orchard from 2.81 to 0.63 ton.ha^{-1}. compared to control (bare) plot. Thus, the efficacy of cover crops to reduce erosion is species and soil dependent and will vary according to specific sites.

Soil Organic Carbon

Soil organic matter (SOM) is closely linked to soil productivity and its primary component is soil organic carbon (SOC) (Hatfield and Sauer, 2011d). In a perennial orchard, it is important to maintain and increase SOC content in order to ensure long-term productivity of that soil (Reeves, 1997). SOM plays an important role in soil function and has a dominant influence on many soil physical (water holding capacity), chemical (cation exchange capacity) and biological properties especially in the surface horizon (Brady and Weil, 2008c). According to Don *et al*. (2010) and Poeplau *et al*. (2011), cultivation of croplands leads to SOC losses of 30 to 40 %, compared to natural or semi-natural vegetation. Replacing a diverse ecosystem with a single crop specie, maintaining bare soils and applying poor farming practices, led to a loss in soil C and soil deterioration. Implementation of cover crops will lead to a higher plant diversity and density in an orchard, contributing to an increase in SOM via decomposing material of the dying cover crop, and stablising soil C in the work rows, through improving photosynthetic activity and rate of the orchard area (via the work rows) and providing additional root exudates in addition to the main crops (Álvarez *et al*., 1995; Atucha *et al*., 2012; Liu *et al*., 2013; Jones, 2018).

Organic C deposited into the soil are converted by microorganisms to stable polymers of C such as humus and glomalin (Leu, 2007). The conversion of C inputs into stable C is higher for root derived materials than for above ground biomass, this is why a diversity of cover crops with different plant architectures (foliage and root

systems) are important (Rasse *et al*., 2005; Jackson *et al*., 2017). Jackson *et al*. (2017) reported that stabilisation of root derived C was five times higher than that of above ground biomass and suggested that the stabilisation of root-derived C could even be higher in perennial-based ecosystems than in annual systems studied. According to Leu (2007), there are three steps that can be followed to increase soil C: i) the use of plants to increase soil C, ii) allowing microorganisms to convert soil C into stable forms and iii) avoiding farming techniques that diminishes soil C. Implementation of cover crops in a perennial orchard provides a living plant cover throughout the year and enhances microbial community by providing a favorable environment (Jones, 2008; Gargouri *et al*., 2013; Kim *et al*., 2020).

Organic matter in a peach orchard was significantly higher in a covered plot (vegetation) (1.78 %) compared to tillage (1.06 %) and herbicides (1.04 %) (Keestra *et al*., 2016). Demir *et al*. (2019) evaluated the effect of different cover crops on soil quality parameters and yield in an apricot orchard. Winter cover crop treatments included *Vicia villosa* (VV), V. *pannonica* (VP), P. *tanacetifolia* (PT) and a mixture of V. *pannonica* and *Triticale* (VPT), whereas F. *esculentum* (FE) was grown as a summer cover crop. The experiment also included: herbicide control (HC), bare control (BC) and mechanical control (MC). They reported that the OM level was significantly lower for HC (1.33 and 1.45 %), BC (1.61 and 1.56 %) and MC (1.85 and 1.87 %) (2015/2016) compared to the cover crop treatments. VV had the highest OM (2.32 and 2.55 %) for both years.

Guzmán *et al*. (2019) evaluated the impact of temporary cover crops on soil properties and vegetation communities in southern Spain vineyards. Commercial vineyards were used to evaluate two contrasting soil management strategies, which included moderate tillage and cover crops. The cover crop treatment improved SOC and macro aggregate stability by 73 and 29 % respectively, compared to bare soil vineyards with a moderate tillage intensity.

Castro *et al*. (2008) conducted a study on the effect of different olive-grove management systems on the organic C and N content of soil in Spain and found that cover crops increased the organic carbon (OC) content in the topsoil layer and reduced bulk density despite the sandy texture of the soil. They concluded that non-tillage (the removal of vegetation (bare soil) and application of herbicide in the autumn and glyphosate in spring) had the lowest OC levels (3.49 – 11.31 $g.kg^{-1}$) at all depths (0-30cm), and the tillage (3-4 annual passes with disc harrow) and cover crops

combined with mowing and harrowing, the highest soil C content increases, 6.30 – 12.09 g.kg^{-1} and 5.76 – 15.48 g.kg^{-1}, respectively, and provided a large amount of biomass.

Ramos *et al*. (2011) conducted a short-term study (2005-2006) in an almond orchard in Spain. Treatments included reduced tillage (RT), native vegetation (NV), cover crop (CC) and fencing with sheep (SF). CC and SF were the most beneficial and increased water soluble C (± 941 – 1030 µg.g^{-1}) and dehydrogenase activity (2.3 – 2.6 µg.g^{-1}).

Yang *et al.* (2019) reported on the long-term effect of cover cropping on seasonally effects of soil microbial C metabolism in an apple orchard. Cover crops were planted in spring in the work row, i) White clover (*Trifolium repens*) (AW), ii) Crown vetch (*Coronilla varia* L.) (AC), iii) *Dactylis glomerata* L. grass (AS) and iv) Birds foot trefoil (*Lotus corniculatus* L.) (AH) v) with Clean cultivation (CT) as the control. There was a significant increase in soil C for the cover crop treatments from May (Spring) to October (Autumn) (± 7 – 11 g.kg^{-1}), but no change in CT (± 6 g.kg^{-1}). Novara *et al*. (2019) evaluated the contribution of cover crops have on SOC sequestration in a sloping vineyard. A cover crop (*Vicia faba*) was compared to conventional soil tillage (CT). The cover crop had a higher SOC-level (9.52 g.kg^{-1}) in the slope area compared to CT (8.74 g.kg^{-1}), with a 9 % increase for SOC after five years of cover cropping. Cover crops can be applied successfully to improve soil C levels in the work row. However, it was not clear which cover crop/combinaion resulted in the highest C increase in the soil.

Soil Biology and Biodiversity

Soil microorganisms play an important role in the ecosystem and are involved in various processes such as OM decomposition, nutrient cycling and retention, soil aggregation, and forming symbiotic relationships with plants improving their abiotic stress tolerance and disease resistance (van Elsas *et al*., 2019). The microbial community is plant dependent (Jones, 2018). Cover crops augments plant diversity in the orchard, provides a higher vegetation density throughout the year, improves the photosynthetic productivity (improving soil C), moderates soil temperature and water content (Hatfield and Sauer, 2011d) and improve the quality and quantity of food available for microbes in an orchard (Hatfield and Sauer, 2011b). Plants exude specific

sugars, enzymes, phenols, amino acids, hormones (auxin and gibberellin) and other biological compounds which act as signals to soil microbes, thus the more diverse the plant species, the higher the diversity of microbial population and the more robust the soil ecosystem (Jones, 2018). A multi specie cover crop mixture (two or more species) increases microbial diversity especially beneficial soil microbes such as rhizosphere bacteria (*Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp., and *Pseudomonas* sp.) and mycorrhizal fungi (*Acaulospora morrowiae*, *Archaeospora trappei*, *Gigaspora gigantea*, and *Scutellospora calospora*), compared to monoculture (Hamel *et al*., 2005; Mazzola and Manici, 2012; Wartman *et al*., 2016; Jones, 2018).

Wartman *et al*. (2016) conducted a study in Southern Ontario, Canada to compare the effects of different levels of plant diversity and compost addition on soil microbial abundance, tree growth and soil properties. Treatments were as follow: i) Three different grasses, kentucky blue (*Festuca arundinacea cv. Schreb*.), creeping red fescue (*F. rubra L*.) and perennial rye grass (*Lolium perenne L*.) ii) The same as treatment one, but with an additional organic turkey litter compost iii) forest garden system (FGS), consisted out of nine different plant species, which includes comfrey (*Symphytum* x *uplandicum* cv. Blocking 14), chives (*Allium tuberosum* cv. *Rottler ex Spreng*), sorrel (*Rumex acetosa* L.), lupins (*Lupinus perennis* L.), white clover (*Trifolium repens* L.), mint (*Mentha* x *piperita* L. cv. Chocolate Mint), bergamot (*Monarda fistulosa* L.), onion (*Allium proliferum*) and Siberian pea shrub (*Caragana arborescens* Lam), each plant was covered with a 15 cm layer of coarse softwood chip mulch, iv) treatment 3 with additional organic turkey litter compost. All treatments increased total fungal abundance and decreased total bacterial abundance, without significant differences between treatments. Total bacterial abundance decreased in spring 2013 for treatment 1, 2, 3 and 4 from 9.99, 10.06, 10.08 and 10.17 to 9.21, 9.22, 9.22 and 9.29 ± 0.08 in fall 2014. The abundance of total fungi increased significantly over the two-year period. In spring 2013 for treatment 1, 2, 3 and 4 increased from 2.62, 2.83, 2.87 and 3.05, to 4.74, 4.69, 4.69 and 4.83, respectively in fall 2014. Their results indicated a higher total fungi to total bacteria ratio (F:B ratio) for all treatments in 2014 compared to 2013. According to Unger *et al*. (2012), continuous vegetative cover without tillage and varied complex matter inputs that contain a high C concentration are factors contributing to a higher F:B ratio.

Zhou *et al*. (2019) conducted a study in an apple orchard in Shenyang, China to determine the impact of cover crop shoot decomposition on soil microorganisms

with the following treatments: i) native mixed herb sward (NMS), ii) red clover sward (RCS) iii) ryegrass sward (RES) and each with a control. There was a significant difference in the total C and N content of the three treatments. Total C content, RCS > RES > NMS. Total N content, NMS > RES > RCS. The C:N ratio, RCS > RES > NMS. Shoot decomposition for all three treatments increased soil microbial activities over the five sampling times. Decomposition of NMS had the greatest effect on improving C metabolism of soil organisms compared to the other two treatments, whereas RES shoot decomposition had the least positive effect. Hättenschwiler and Jørgensen (2010) reported that the difference can be attributed the decomposition of mixed-type litter which was more efficient than that of a single litter type. In all treatments, shoot decomposition significantly promoted bacterial reproduction compared with the respective control groups. NMS compared to its control, 118.89 ± 7.19 $mg.kg^{-1}$ and 99.45 ± 9.60 $mg.kg^{-1}$, respectively. RCS compared to its control, 210.20 ± 5.36 $mg.kg^{-1}$ and 86.05 ± 5.31 $mg.kg^{-1}$, respectively. RES compared to its control, 98.96 ± 5.04 $mg.kg^{-1}$ and 84.13 ± 2.93 $mg.kg^{-1}$, respectively.

Comparisons with the control groups showed that decomposition of the shoots of NMS, RCS, and RES had three different effects on fungal reproduction, namely significantly negative, significantly positive, and non-significant, whereas comparison among the three treatments indicated significant differences in the content of fungal biomass in soils containing their decomposed shoots (RCS_{soil} > RES_{soil} > NMS_{soil}). A high C:N ratio in the litter is related to fungal proliferation, whereas a low C:N value is related to bacterial proliferation, and this finding is consistent with the significant positive correlation between fungal proliferation and C:N ratio observed in this study (Glaser *et al*., 2004; He *et al*., 2011).

Yang *et al*. (2019) quantified soil microbial C metabolism. They reported a significant difference between treatments for all three measurement dates (May, July and October). With regards to May (spring), AW (0.87), AC (0.89) and AS (0.90) were significantly higher compared to AH (0.59) and CT (0.58). With regards to July (summer), AW (0.71) and CT (0.65) were similar, but AW was significantly higher compared to AC (0.58), AS (0.50) and AH (0.47), whereas AC did not differ significantly from AS and AB. There was no significant difference between AS and AH. With regards to October (fall), AW (0.94) and AC (0.93) were significantly higher compared to AS (0.66), AH (0.64) and CT (0.54). There was no significant difference

between AS and AH. Cover crops significantly, but differentially, promoted soil microbial carbon metabolism during spring and fall.

Cui *et al*. (2015) conducted a study in a guava orchard, south of China, the soil was classified as a red soil, rich in aluminium (Al) and iron (Fe), with a low pH while deficient in soil OM. They monitored the phosphatase activities and organic P (P_O) fractions, and further detected the diversity of the alpha bacterial community. Treatments included: i) clean culture (CC), ii) cover crop treatment, *Paspalum natatu* (PN), iii) cover crop treatment with *arbuscular mycorrhizal* inoculation (PNA), iv) cover crop treatment, *Stylosanthes guianensis* (SG), and v) cover crop treatment with *Rhizobial* inoculation (SGR). Cropping alone or with microbial inoculation significantly affected moderately labile P_O (MLP_O) and moderately resistant P_O (FAP_O). Cover cropping together with microbial inoculation increased the activities of acidic phosphomonoesterase (ACP) (most dominated), neutral phosphomonoesterase (NP) and alkaline phosphomonoesterase (ALP) (most sensitive). ALP had a positive correlation with both MLP_O and FAP_O. Their results suggest that cover cropping with microbial inoculation in red soils can improve P_O hydrolysis *via* the promoted alpha bacterial community and ALP activity.

Radić *et al*. (2012) conducted a study on grapevines in Croatia, to determine if multiple hosts influenced the formation of the symbioses with arbuscular mycorrhiza fungi (AMF). Grapevine (*Vitis vinifera L. cv. Plavac mali*) and two selected weeds from Mediterranean Croatian vineyards, known as ribwort plantain (*Plantago lanceolate*) and dandelion (*Tanacetum cinerariifolium*) were examined in pot culture experiments, individually or combined. Combinations were as follows: i) one grapevine plant, ii) four grapevine plants, iii) one dandelion plant, iv) four dandelion plants, v) one ribwort plantain plants, vi) four ribwort plantain plants, vii) one grapevine plant and one dandelion plant, viii) one grapevine plant and one ribwort plantain plant, viiii) one grapevine plant, one dandelion plant and one ribwort plant. They concluded that grapevine and T. *cinerariifolium* individually and in combination resulted in a greater development of AMF in terms of spore production, extraradical mycelium length and root colonization compared with pots containing P. *lanceolata*. Herbaceous weed species potentially provided a wider spectrum of AMF for colonising grapevine roots and indicated the value of encouraging host plant diversity in vineyards.

Kim *et al*. (2020) conducted a meta-analysis of current research to fill the knowledge gap in cover cropping research on soil microbial abundance, activity and

diversity and compared cover cropping to bare fallow soil. Cover cropping significantly increased parameters of soil microbial abundance, activity, and diversity by 27, 22 and 2.5 % respectively, compared to bare fallow soil.

Zheng *et al*. (2018) conducted a cover crop trial in an apple orchard to explain the changes in the composition and activity of soil microbial communities and the microbial network after 9 years. Two main plots were as follows i) control treatment (bare soil) and ii) cover crop treatment (crown vetch, *Coronilla varia* L.) and the two subplots comprised out of no fertilizer and a NPK fertilizer treatment. The crown vetch was mowed three times during the season (July, August and September), where the residues were left on the soil surface as a mulch. The composition and activity of bacterial and fungal communities changed significantly throughout the trial, with cover crops having a greater effect than chemical fertilizer on the composition of soil microbial community. The relative abundance of potential genes involved with the degradation of cellulose, hemicellulose and cello-oligosaccharides increased significantly with the cover crops and therefore the SOC and total N contents were enhanced by the cover crop treatment with the increase of soil enzyme activities. In addition, yield was improved by cover crop treatment. Cover crops improved soil microbial parameters (abundance, activity and diversity) compared to bare/fallow soil. However, the efficiency of improving microbial parameters differed among cover crops as well as the timing of taking the samples (spring, summer, autumn and winter). Therfore no single recommendation can be made for application under local conditions and site specific trials may provide more guidance for local application.

Weed Suppression

Management of weeds are important to reduce competition with the main crop for water and nutrients, making the environment unfavorable for pathogens and pests, improving aesthetics of an orchard, while providing an economical return of a good quality fruit. Weeds are mostly pioneer species, preferring to establish in unproductive or recently disturbed soils where there is little competition for water and nutrients (Hammermeister, 2016). Cover crops can suppress weeds, because of their ability to compete for light, water, space and nutrients and produce allelopathic substances, which influences seed germination and weed growth (Farooq *et al*., 2011; Bezuidenhout *et al*., 2012). Cover crops can also displace weeds while they grow and

their residues (mulch) can suppress weeds further (Hoffman *et al*., 1996; Smeda and Weller, 1996; Moyer *et al*., 2000). Therefore, cover crops should be allowed to grow for as long as possible in order to suppress weeds for a longer period (Smeda and Putnam, 1988).

Masilionyte *et al*. (2017) conducted a study (2006 to 2012) to evaluate the effect of cover crops on weed suppression and volunteer plants in alternative farming systems. Treatments comprised: i) narrow-leaved lupine (*Lupinus angustifolius* L.) in mixture with oil radish (*Raphanus sativus* var. Oleiferus Metzg.), ii) white mustard (*Sinapis alba* L.), iii) white mustard in mixture with buckwheat (*Fagopyrum exculentum Moench*.) and iv) control (no cover crops). White mustard cultivated alone or in combination with buckwheat was more effective in smothering weeds than narrow-leaved lupine in combination with oil radish.

Tursun *et al*. (2018) used living, mowed and soil-incorporated cover crops for weed control in apricot orchards (*Prunus armeniaca* L.). Winter cover crop treatments included: i) hairy vetch (Vicia villosa), ii) Hungarian vetch (*V. pannonica*), iii) a mixture of V. *pannonica Crantz* and *Triticale* (*V. pannonica 70 % + Triticale 30 %*) and iv), lacy phacelia (*Phacelia tanacetifolia*), whereas the summer cover crop included buckwheat (*Fagopyrum esculentum*). Additional treatment included a herbicide control and mechanical control plot. Both the cover crop mowing and soil incorporation significantly decreased the weed density in apricot orchards. The most effective cover crops for mowing and soil incorporation, were V. *villosa*, V. *pannonica*, P. *tanacetifolia*, and *Triticale* with V. *pannonica*, whereas F. *esculentum* had the lowest effectiveness in reducing weed density. Mechanical weed control and glyphosate application were less effective than the cover crops.

Mennan *et al*. (2006) reported on alternative management systems for weed control in a hazelnut (*Corylus avellane L*.) orchard with: i) ryegrass (*Lolium multiflorum* Lam.), ii) hairy vetch (*Vicia villosa* Roth.), iii) red clover (*Trifolium pratense* L.) and iv), herbicide control (bare soil). Cover crop treatments reduced weed density, number of weed species emerging and total weed dry biomass compared to the control. Cover crop residues also effectively suppress weeds (*Urtica urens* L., *Convolvulus arvensis* L., *Chenopodium album* L., *Lapsana communis* L., and *Poa annua* L.).

It was evident that cover crops in fruit orchards effectively suppressed weeds (> 80%) and may serve as an alternative to herbicides in the work row. The choice between growing cover crops for weed suppression and or mulch versus the benefit

to incorporate it into the soil for microbial activity will depend on the priority of the producer at the specific site.

Pest and Disease Management

A sustainable approach to pest control is by introducing biological control, which relies on the modification of the environment or management practices to protect natural enemies within an ecosystem, enhancing their ability to suppress pest population by naturally occurring predators, parasitoids and pathogens (Barbosa, 1998; Pell *et al*., 2009). Cover crops in orchards are a form of habitat manipulation to conserve and increase beneficial natural enemy population and may serve as a form of surface management (Butler, 1986; Catzeflis, 1988; Bugg and Waddington, 1994; Sullivan, 2003; Li *et al*., 2005; Kou *et al*., 2010). Implementation of cover crops in a perennial orchard will create and improve the positive plant-soil feedback system, which leads to a decrease in disease incidence and an increase in plant productivity (van Elsas *et al*., 2002; Garbeva *et al*., 2004). The cover crops create a “dilution effect” (Keesing *et al*., 2010), making it more difficult for a pathogen to find a suitable host which will result in less frequent disease outbreaks. In California, cover crops are an important component of IPM (Costello and Daane, 1998). Cover crops are able to attract and maintain natural enemies (Earnshaw, 2004; Tscharntke *et al*., 2007) and may stabilize the ecosystem and bring pest-predator relationships into balance. Similar results were reported in apple orchards in China (Zhou *et al*., 2014). Evans *et al.* (1988) stated that the ‘Sirato’ legume (*Macroptillium atropurpureum*) in a banana plantation suppressed the burrowing nematode (*Radolphus similis*). Bugg and Dutcher (1993) reported that *Sesbania exaltata* in pecan orchards in southern Georgia controlled *aphidophagous coccinellidae* and pecan aphids. Irvin *et al*. (2021) evaluated the potential of flowering plants for enhancing *Syrphidae* hoverflies for biological control of *Diaphorina citri* in California. Alyssum (*Lobularia maritima*) had a short sowing to flowering time and attracted hoverflies efficiently and showed potential for biological control.

The establishment of cover crops to reduce pest and pathogen outbreak depends on the disease pressure of the specific site and applicability of the species adaptation and performance on site. Therefore, *ad hoc* cover crop combinations cannot be recommended to achieve sufficient pest and disease control under all

conditions and needs to be evaluated for each crop and environment for final recommendation.

Improving Soil Water Content and Moisture

Cover crops improves soil quality by contributing to total soil C, improving aggregate stability and soil structure, which increases soil water retention capacity and protect soil from detrimental effects such as soil erosion and water runoff which leads to soil degradation (Kuo *et al*., 1997; Strudley *et al*., 2008). It enhances water infiltration by creating open root channels, promoting earthworm activity and protecting soil surface structure (Brady and Weil, 2008a) and reducing direct evaporation and hence increasing soil root zone water availability (Zimmer and Zimmer-Durand, 2011).

Gabriel *et al*. (2019) conducted a long-term field study from 2006 to 2016 in Tajo River Basin, Spain. The field experiment consisted of a 10-year crop rotation with or without a winter cover crop between consecutive main summer crops. The cover crop used in rotation was Barley (*Hordeum vulgare* L.). The cover crop rotation resulted in an increase of soil micro- and macro porosity, improved soil structure and increased the soil water retention capacity. This latter improvement was mostly in the soil layer between 40 and 80 cm depth. The expected cover crop competition for water with the main crop (evapotranspiration) can be compensated for by an improvement of the water retention in the intermediate soil layers and a reduction of drainage losses. Thus, the implementation of cover crops may improve hydrologic functions of soil.

Demir and Isik (2020) evaluated the impact of different cover crops, mechanical cultivation and herbicide treatments on the soil quality variables in an apple orchard with coarse textured soil. Treatments consisted of: i) *Trifolium repens* L. ii) (TR), *Festuca rubra rubra* L. (FRR), iii) *Festuca arundinacea* (FA), iv) mixture of *Trifolium repens, Festuca rubra rubra*, *Festuca arundinacea* (TFFF), v) *Vicia villosa* (VV) and vi) *Trifolium meneghinianum* (TM). Other treatments included mechanical cultivation (MC), herbicide treatment (HC) and control without cover crops (C). MC, HC and C had a significantly lower OM and volumetric water content (VWC) (2013-2014), whereas VV had a significantly higher VWC in 2013 compared to the other treatments and in 2014, VV and TR were significantly higher in VWC compared to the others.

Demir *et al*. (2019) also reported that the VWC for HC (29.9 and 31.5 %), BC (29.8 and 30.9 %) and MC (30.8 and 32.1 %) (2015/2016) were significantly lower

compared to the cover crop treatments. VV had the highest VWC (37.1 and 38.8 %) for both years. Keestra *et al*. (2016) reported that the soil moisture content was significantly higher in the covered plot treatment (4.44 %) compared to tillage (3.47 %) and herbicide (3.5 %) treatment and Yang et al (2019) found that soil moisture in May (spring) and July (summer) was significantly lower for the living mulches (± 6.14 – 12.01 %) compared to clean cultivation (± 13.93 – 15.86 %). However, soil moisture for living mulches (± 16.25 – 16.49 %) was similar to the control (15.92 %) in October (autumn).

Ramos *et al*. (2010) evaluated the effect of cover crops under different management systems with frequent tillage in an almond orchard. Two cover crops, oats (O) and an Oat-vetch (OV) mixture were compared to a frequently tilled system (three to four tillages per year). O and OV had a significantly lower soil moisture, 2.4 – 5.0 $g.H_2O.100g^{-1}.Soil^{-1}$ and 2.7 – 5.2 $g.H_2O.100g^{-1}.Soil^{-1}$ compared to frequent tillage, 4.9 – 9.0 $g.\ H_2O.100g^{-1}.Soil^{-1}$. O and OV had a significantly higher wet aggregate stability, 62 % and 61 %, compared to tillage (44 %). Irmak *et al*. (2018) compared a cover crop treatment with and without maize with a bare plot and maize only. After 3 years (2013-2016) there were no significant differences between treatments for WHC, but the cover crop treatment increased the WHC with 6 % from 2014 to 2015.

Although it was clear that cover crops improved soil moisture compared to bare soil, the concern of water competition between cover crops and the main crop was an ongoing concern. Thus, it is recommended to plant annual cover crops only during the rainy season where irrigation is required in deciduous cultivation and use cover crop biomass as a mulch during the dry season to reduce possible competition for water between crops, until accurate information about the specific cover crop/s at that site can be provided in terms of WHC. Furthermore, it may require a more composite evaluation, i.e., life cycle analyses to incorporate all factors influencing water use and availability *in situ*.

Improving Soil Structure

Cover crops provide an additional vegetative cover and the crops residues contribute to SOM that improves soil aggregation and soil structure (Lichtfouse, 2010). Cover crops conserve soil moisture and regulate soil temperature, which favors beneficial

fungi that produces glomalin which enhance the stability of soil aggregates (Lichtfouse, 2010; Phillips, 2017). Demir and Isik (2020) reported that aggregate stability was significantly lower for MC (mechanical cultivation), HC (herbicide treatment) and C (control without cover crops) in comparison to cover crop treatments. Bulk density of soil in an apricot orchard was significantly lower in the covered plots (vegetation) (1.10 $g.cm^{-3}$) compared to tillage (1.27 $g.cm^{-3}$) and herbicide (1.45 $g.cm^{-3}$) treatments (Keestra *et al*., 2016). Cover crops contributed towards improving soil structure, however more time was required to improve soil productivity sigificantly to confirm the feasibility of introducing cover crops for this attribute. Additional research is proposed to identify suitable cover crop mixtures that can significantly improve soil structure in a short period, especially in the more arid regions of South Africa.

Nutrient Cycling

Cover crops are capable of improving nutrient cycling especially C, N and P cycling (Vukicevich *et al*., 2016; White *et al*., 2016; Wolf *et al*., 2018; Morugán-Coronado *et al*., 2020). Cover crops act as P scavengers and protect soluble P in strong P-fixing soils (Kuo *et al*., 2004). Soil bacterial and fungal communities produce extracellular enzymes to break down organic molecules into monomers, which is plant available. They are the principal drivers of soil P dynamics, by producing P-solubilizing compounds (e.g., carboxylate) in the rhizosphere, complementing P mobilization by roots (Schilling *et al*., 1998; Bünneman *et al*., 2011; Richardson and Simpson, 2011; Zheng *et al*., 2018).

Demir *et al*. (2019) found that the N level (%) was significantly lower for HC (herbicide control) (0.094 and 0.092 %), BC (bare control) (0.103 and 0.100 %) and MC (mechanical control) (0.120 and 0.112 %) compared to the cover crop treatments. VV had the highest N (0.173 and 0.175 %) for both years. Messiga *et al*. (2015) evaluated the response of soil quality to cover crops in a 'Leon Millot' vineyard in Nova Scotia, Canada, with: i) control (CONT) (no cover crop), ii) mixture of oats (*Avena sativa*), peas (*Pisum sativum* L.) and hairy vetch (OPV), iii) oats under seeded with red clover (ORCI) and iv), mixture of timothy (*Phleum pretense*), alsike (*Trifolium hybridum*) and red clover (TM). The soil mineral N (bloom period, mainly NO_3-form) was 23.56 $kg.ha^{-1}$ for OPV and 20.68 $kg.ha^{-1}$ for ORCI, whereas the CONT (16.38 $kg.ha^{-1}$) and TM (12.53 $kg.ha^{-1}$) treatment had the lowest. Soil mineral N (harvest,

mainly NH_4-form) was 21.95 kg.ha^{-1} for ORCI, but there was a reduction in N for OPV and TM (15.43 kg.ha^{-1}), CONT (9.10 kg.ha^{-1}).

The effect of integrated cover crop management on N, P and S release from above and below ground residues in a sandy-loam soil was studied by Hansen *et al*. (2021) for: i) buckwheat (*Fagopyrum esculentum*), ii) crimson clover (*Trifolium incarnatum*), iii) white lupin (*Lupinus albus*), iv) oilseed radish (*Raphanus sativus*), v) Italian ryegrass (*Lolium multiflorum*), vi) garden sorrel (*Rumex acetosa*) and vii) hairy vetch (*Vicia villosa*). Nutrient composition after 77 days of growth varied amongst cover crops. The legumes (clover, lupin and vetch) had the highest N concentration in both shoots and roots. Oilseed radish and sorrel had the highest P concentration (shoot and root), whereas oilseed radish had the highest S and K concentrations (shoot and root) and sorrel the highest Mg concentration (shoot and root). Lupin had the highest Mn concentrations (shot and root). Vetch had the highest concentrations of B (shoot), Zn (shoot and root) and Ca (shoot). The lignin level of buckwheat shoots (4.2 %) was the highest, whereas the other cover crops had relatively lower levels (0.6 – 2.5%). Vetch and sorrel had the highest N release after 80 days of incubation, whereas buckwheat released the lowest N. Intact soil with legumes resulted in N mineralization, whereas intact soil with non-legume cover crops resulted in immobilization. Sorrel had the highest shoot P concentration followed by oilseed radish. Sorrel shoots released 50 % of shoot P (highest fraction), while oilseed radish only released 31%. Intact soil with legumes released the highesbirdt fraction of added root P. Oilseed radish had the highest shoot S concentration followed by sorrel, however sorrel shoots released the highest fraction of added shoot S (34.3 %) followed by oilseed radish (28.2 %).

Wendling *et al*. (2016) evaluated the influence of root and leaf traits on the uptake of nutrients in twenty different cover crops. Berseem clover, faba bean, vetch, phacelia, daikon radish, sunflower and niger had the highest N, P and K levels in the shoots and daikon radish, the highest N, P and K levels in the roots. They recommended that species with high nutrient concentrations and high root length density should be used in the short term under high fertility conditions, whereas in lower fertility conditions, species with additional strategies (N-fixing) might be more beneficial.

Qian *et al*. (2015) evaluated the effect of different living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple

orchards. Their treatments were as follow: i) control (bare soil/ no mulch), ii) white clover, iii) crown vetch and iv), perennial ryegrass. The cover crops were mowed three times a year and used as a mulch in the tree row. Soil water content (%) of white clover and crown vetch were significantly higher compared to the control. Water content of crown vetch and rye were similar, whereas rye did not differ significantly from the control. The organic C (OC) levels of white clover and crown vetch were significantly higher compared to the other treatments, whereas the control had the lowest OC level. White clover and crown vetch had significantly higher total N (TN) levels compared to the other treatments. Ryegrass had a significantly lower mineral N ($mg.kg^{-1}$) level compared to the other treatments. White clover had a significantly higher available P, followed by crown vetch, compared to the other treatments. Ryegrass had a significantly higher available K, followed by crown vetch, white clover and control. Crown vetch had a significantly higher invertase activity, followed by white clover. Invertase activity was similar for the control and ryegrass. Urease activity of white clover and crown vetch were significantly higher compared to the other treatments. Alkaline phosphatase activity of white clover and crown vetch were significantly higher compared to the other treatments, whereas the control had the lowest activity. For the bacterial community diversity indices, ryegrass had a significantly higher Shannon index and richness index, followed by crown vetch and white clover. The control had the lowest bacterial activity and diversity.

Ball *et al*. (2020) performed an intra-row cover crop trial in a vineyard and used the following treatments: i) grass, ii) legume, iii) mixture of grass and legume and iv), the control (bare plot). The grass and mixture had a significantly higher OC for both bulk density and fine fraction. The mixture had a significantly higher total N (TN) for bulk density compared to the other treatments. Srivastava *et al*. (2007) reported that a citrus orchard intercropped with soybean and chickpea (legumes), produced a significantly higher yield (72.2 $kg.tree^{-1}$) compared no intercrops (68.5 $kg.tree^{-1}$). Higher levels of nutrients were found in the inter-cropped citrus orchard (2.35 % N, 0.13 % P, 2.08 % K, 86.5 ppm Fe, 71.1 ppm Mn, 22.2 ppm Cu, and 22.0 ppm Zn) compared to the orchard without cover crops (2.29 % N, 0.13 % P, 2.47 % K, 79.2 ppm Fe, 63.8 ppm Mn, 21.7 ppm Cu, and 23.2 ppm Zn).

Morugán-Coronado *et al*. (2020) conducted a meta-analysis of field studies on the impact of intercropping, tillage and fertilizer type on soil type and crop yield in fruit orchards under Mediterranean conditions. Alternative practices (crop diversification,

conservation tillage and organic fertilization) contributed to the improvement of soil quality and fertility and influenced yield, but they recommended annual cover crops as (winter and summer cover crop) instead of permanent crops, under no-tillage, to prevent a possible negative effect on soil P and N.

Cover crops assists with nutrient cycling during the growing season, through taking up and releasing minerals when they decompose at the end of their lifecycle making minerals more accessible to the main crop, however it is important to leave the cover crops in the orchard as a mulch to obtain the benefits of the released nutrients from the cover crops.

Reduction in Surface Water Runoff and Pollution

Cover crops have the potential of reducing the total runoff of water and leaching of nutrients and pesticides – reducing environment contamination (Hartwig and Ammon, 2002; Magdoff and van Es, 2009b). They intercept raindrops, slowing down water running over the soil surface, improve soil structure and reduce soil crusting. The roots bind soil and scavenge nutrients before it leaches (Magdoff and van Es, 2009b). Results from Keesstra *et al*. (2016) indicated that the covered plot treatment in a peach orchard had significantly less runoff, a lower sediment concentration and runoff coefficient compared to tillage and an herbicide treatment. Prosdocimi *et al*. (2015) reported that barley straw mulch effectively reduced the median sediment concentration in runoff from 9.8 to 3.0 $g.L^{-1}$ compared to control (bare plot). Gómez *et al*. (2009) reported that tillage had a higher water runoff (91.9 mm), soil loss (1.94 $kg.m^{-2}.year^{-1}$) and sediment concentration (17.3 $kg.m^{-1}$) compared to the cover crop treatments of 32.7 mm, 0.040 $kg.m^{-2}.year^{-1}$ and 1.9 $kg.m^{-3}$, respectively, in an olive orchard.

In areas with a high risk of fertiliser pollution, cover crops with high above and below ground biomass should be selected for remediation of excessive minerals to reduce environmental contamination.

The limitations of implementing cover crops in a perennial orchard

The positive attributes of cover crops outperform the limitations (Fageria *et al*., 2005). Incorporation of cover crops into farming practices should be considered as a long-term investment as initial expenses may be considerable – i.e., establishment, management practices, termination of cover crops and cost should be considered (Hoorman, 2009). The selection of suitable species/combination must comply with the main goal, such as improving soil structure, reducing erosion and water runoff and improving soil biology. The success of different cover crop species may be very specific to circumstances such as climate, soil type, farm management system therefore cover crop species may not perform equally under different conditions. Cover crops should be terminated in time, in order to prevent re-seeding of cover crops (Singh *et al*., 2016). General knowledge about the effects of cover crops on soil ecology, nutrient cycling and overall soil health is still relatively limited in South Africa, as it varies greatly between species, climates and crops – leaving a grey area in the industry.

Conclusion

The benefits of incorporating cover crops in perennial orchards affects the soil physical, chemical and biological domain. Although extensive research has been done on the topic in other countries, insufficient literature regarding performance and specie selection in deciduous orchards in South Africa is available. For a cover crop to be successful in a perennial orchard the positive outcomes must out-weight the negative outcomes. Management of cover crops in a perennial orchard is complex due to the focus on the primary crop and requires a more holistic approach to achieve the full value of a cover crop. However, it is important to remember that the incorporation of cover crops should be a long-term investment and management practices should incorporate this approach. The additional costs to establish a cover crop, the drive towards a more sustainable orchard and the individual needs for cover crop contributions in a specific site still requires to be determined under local conditions and provides an opportunity for further research.

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Paper 1: Evaluate the contribution of cover crop combinations towards soil characteristics in perennial crops.

Abstract

Cover cropping is used extensively in regenerative agriculture to build soil health and increase resilience to drought and other extreme environmental factors. We evaluated the effect of different cover crop combinations towards soil chemical composition, soil moisture, soil compaction, water holding capacity (WHC), microbial activity and plant performance of perennial crops under South African conditions. A two-year field study (Trial 1 and 2) was conducted in Stellenbosch (33°56'51.27"S 18°52'19.29"E) and a three-year field study (Trial 3) in Elgin, Western Cape (34°09’16.83”S, 19°02’28.01”E). Trial 1 (Multi species: forage rye, radish and white mustard) and trial 2 (Single specie: forage rye) comprised four treatments each: i) Control (natural vegetation), ii) Cover crop with no fertilizer application (MNFA; SNFA), iii) Cover crop with a single fertilizer application (MSFA; SSFA) and iv), Cover crop with a double fertilizer application (MDFA; SDFA). Trial 3 comprised five annual winter cover crop treatments: i) Phacelia (PC), ii) Forage rye & vetch (FRVC), iii) Forage radish & white mustard (FRWMC), iv) Forage barley & peas (FBPC) and v), Forage rye (FRC). Cover crops did not affect soil chemical composition and plant performance of perennial trees. The control and PC treatment of trial 1 and 3 respectively had a significantly lower CO_2 production in March/April 2021. The cover crop treatments of trial 1 and 2 provided a significantly higher biomass for mulching and soil moisture in October and November, compared to the control. Trial 3 only had a significant difference in soil moisture in September, before mulch was moved to the tree row. There were conflicting results between treatments with regard to biomass and moisture conservation in trial 3. Cover crops did not primarily influence soil WHC, but conserved soil moisture and provided a favorable environment for microbial community. Species selection would therefore influence results and therefore the aim for cover crop use must be specific and clear.

Keywords: Biomass, forage rye, soil microbial activity, soil waterholding capacity

1. Introduction

Cover cropping is used extensively in regenerative agriculture to build soil health and increase resilience to drought and other extreme environmental factors (Doran and Zeiss, 2000). In a perennial orchard, a cover crop is any living ground cover (Hartwig and Ammom, 2002) and may be annuals, biennials or perennials that include grasses, legumes or various broadleaf plants (Hammermeister, 2016) grown on bare soil in the work row, between tree rows. Incorporation of cover crops into a perennial orchard contributes to high specie richness and will promote a positive plant-soil feedback system in an orchard. Subsequently, this could lead to better plant performance, promoting beneficial microbial communities and reducing disease outbreaks (Keesing *et al*., 2010).

In conventional perennial orchards, weed suppression in the work row is usually maintained with herbicides (Lizek, 2014; Mia *et al*., 2020). In a more sustainable approach, cover crops can be planted to provide a leaf canopy and root activity in the work row (Moebius-Clune, 2016), whereby it will improve the photosynthetic capacity and rate of entire orchard and reduces the carbon dioxide (CO_2) levels per hectare/area (Jones, 2018). Cover crops also play an important role by providing various ecosystem services such as: enhance nutrient cycling (Lu *et al*., 2000; Dabney *et al*., 2001), protect the soil surface from wind and water erosion (Lu *et al*., 2000; Dabney *et al*., 2001; Magdoff and van Es, 2009; Kaspar and Singer, 2011; Hatfield and Saur, 2011), improve organic matter (OM) levels in soil (Álvarez *et al*., 1995; Dabney *et al*., 2001; Magdoff and van Es, 2009), primary improve water infiltration and secondary improve water holding capacity (WHC) (Dabney *et al*., 2001), reduce soil compaction (Chen and Weil, 2009), support beneficial microbial communities (Kaspar and Singer, 2011; van Elsas *et al*., 2019) provide a mulch (Bristow, 1998), reduce the rate and quantity run off and leaching of water of the field into waterways (Dabney *et al*., 2001) and contribute towards weed suppression (Lu *et al*., 2000; Dabney *et al*., 2001; Farooq *et al*., 2011; Bezuidenhout *et al*., 2012; Miglécz *et al*., 2015; MacLaren *et al*., 2019).

Cover crops can either fix, scavenge or trap nutrients, depending on the type of cover crop (Scholberg *et al*., 2010). Legume cover crops are able to fix nitrogen (N), whereas non-legume plants can only scavenge or trap soil N (Kladivko, 2016). Cover crops decompose slowly, therefore releasing nutrients gradually, making it available

for the main crop. Plant residue is decomposed by microbial communities and, as these microbes die and decay or are consumed by other soil organisms, they release nutrients into the soil in a plant-available form (Zimmer and Zimmer-Durand, 2011). Deep rooted plants such as forage rye (*Secale cereal*), forage radish (*Raphinus sativus*) and brassicas are able to scavenge nutrients from the deeper soil profiles and bring it to surface layers for crops with less developed roots that are concentrated in the topsoil (Dabney *et al*., 2001; Fageria *et al*., 2005). Cheng and Baumgartner (2004) evaluated the role of cover crops with arbuscular mycorrhizal (AM) fungi with N transfer in a vineyard. Both crops formed a symbiotic relationship with AM fungi and may be interconnected via common mycorrhizal networks (Phillips, 2017). AM fungi mediated N transfer from the cover crops to grapevines and N transfer was significantly higher from the grass (*Bromus hordeaceus*) than from the legume (*Medicago polymorpha*). In addition, Clark (2012) reported that grass type cover crops such as forage rye, forage barley, wheat and sorghum-sudangrass hybrids can conserve soil moisture. Soil where forage rye was left as a mulch after termination maintained a higher soil moisture than the soil where above ground rye was removed (Gallaher, 1977).

Van Huyssteen *et al*. (1983) evaluated the effect of cover crop management on soil conditions and weed control in a ‘Colombar’ vineyard in Oudtshoorn, South Africa. One half of the vineyard was planted with Wimmera ryegrass (*Lolium multiflorum*) and the other half was planted with vetch (*Vicia sativa*). They compared four treatments for both cover crops: i) bare soil (above ground plant material was cut and removed from plots), ii) growing cover crop - the cover crop was left to complete life cycle, iii) a single layer of residue - cover crops on these plots were sprayed with herbicide before bud burst and left as a mulch on the soil surface and iv), a double layer of residue - cover crops on these plots were also sprayed with glyphosate, with additional plant material removed from treatment 1. They found that soil temperature was the highest in treatment 1 (bare soil) and lowest in treatment 2 (cover crop treatment). When compared to bare soil, the mulches conserved water before flowering (November) of vines. After cover crops completed their lifecycle (treatment 2) they acted as effective mulches, especially Wimmera ryegrass. Water conservation was the lowest for treatment 1. They concluded that a mulch, from cover crops grown in the vineyard, effectively suppressed weeds and replaced pre-emergence herbicides. Fourie and Freitag (2010) found a difference in soil temperature between un-mulched and

mulched treatments in their evaluation of different soil management practices in a 'Chardonnay' vineyard near Robertson, South Africa. They reported that mulching minimized the diurnal variation in soil temperature (between 1.74 °C and 2.01 °C less than un-mulched treatment) and that the annual cover crop did not delay bud break and kept the soil temperatures below 25 °C.

Cover crops have the potential of increasing soil microbial activity, abundance and diversity (Gargouri *et al*., 2013; Kim *et al*., 2020), because they provide a favourable environment for microbial communities by providing a higher vegetation density and diversity during the year, as well as moderating soil temperature and water content in the orchard (Kaspar and Singer, 2011). Roots secrete organic compounds (sugars, enzymes, phenols, amino acids and hormones) which act as chemical/physiological signals for microbes and enhance soil microbial activity (Jones, 2008; Scholberg *et al*., 2010). According to Chaparro *et al*. (2012), there is increasing evidence that plants can shape the soil microbiome through the secretion of root exudates and nearly two thirds of plant C synthesis is transported to roots, stimulating microbial interaction (Phillips, 2017). Root soil contact is determined by three factors: root length, root branching and root hairs (Phillips, 2017), and actively growing feeder roots are important for nutrient uptake. A nutrient depletion zone is created by roots (Taiz, 2018b), however in roots that associate with mycorrhizae, it will enable the roots to mine nutrients in a wider area beyond the depletion zone. Different fungal networks form multiple affiliations with numerous plants, creating a common mycorrhizal network (Phillips, 2017) and, by increasing the diversity of plants in an orchard, this network can be increased exponentially. Kim *et al*. (2020) found that cover cropping significantly increased microbial abundance and all treatments led to an increase in fungal and a reduction in bacterial communities. In contrast to Zhou *et al*. (2019), who determined the impact of cover crop leaf decomposition in an apple orchard (lower C/N ratio) demonstrated that the cover crop combination efficiently promoted bacterial reproduction compared to the control.

McGowen *et al*. (2018) and Koerner *et al*. (2011) used the gas chromatograph (GC) to quantify CO_2 production whereas others (Demir *et al*., 2019; Demir and Isik, 2020) evaluated the effect of different cover crops on basal soil respiration (CO_2) with different techniques. Soil respiration (CO_2 levels) vary seasonally and thus are indicators of the labile fraction of C (Chahal and Van Eerd, 2020), which is useful for detecting the initial changes in the status of SOM (Salinas-Garcia *et al*., 1997).

Seasonal variability in labile fractions of C is primarily dependent on the quantity of cover crop residue produced, rhizodeposition during growth cycle of crop and soil moisture and temperature, which influences soil microbial activity and residue decomposition (Xiang *et al*., 2008).

Cover crops can be used as an alternative approach towards pest management via increasing plant diversity on site, increasing microbial diversity and minimizing the proliferation of soil borne pathogens (Vukicevich *et al*., 2016). Sánches *et al*. (2007) evaluated the influence of cover crops on soil properties and tree performance in an organic apple orchard in northern Patagonia. They compared four treatments: alfalfa (*Medicago sativa* L.) with fescue (*Festuca arundinacea Schribn*.), strawberry clover (*Trifolium fragiferum* L.), common vetch (*Vicia sativa* L.), with a control (natural vegetation of grasses and legumes with the soil disked twice in late winter). Tree growth (trunk cross sectional area and canopy volume) and yield were significantly lower in the control compared to the treatments in year 5 and 6. This also indicated the time span required to obtain the benefits of a cover crop in a perennial orchard.

Wooldridge *et al*. (2013) conducted a study in Elgin, South Africa, where they evaluated the effect of soil surface management practices on soil and tree parameters in a ‘Cripps Pink’/M7 apple orchard. They compared integrated production (IP) management practices and organic surface management practices. The organic management practice (compost, straw mulch and cover crop/weed) increased stem circumferences, pruning weight and total root number compared to the IP treatment. The IP treatments 1 (Work row: weeds are mowed; Tree row: herbicide and inorganic N) and 2 (Work row: cover crops and herbicides; Tree row: herbicide and inorganic N) showed significantly lower stem circumferences compared to the organic treatments 4 (Work row: permanent mulch and hand weeded; Tree row: compost and mulch as well as hand weeded), 5 (Work row: cover crops are mowed; Tree row: compost and mulch as well as hand weeded), 6 (Work row: cover crops are mowed; Tree row: compost, mulch and compost tea, as well as hand weeded), 7 (Work row: weeds are mowed; Tree row: compost, mulch and hand weeded) and 8 (Work row: weeds are mowed; Tree row: compost, straw mulch and compost tea as well as hand weeded), after eight seasons. Organic treatments 6 and 8 had a significantly higher root count compared to the organic treatment 4 and IP treatments 1, 2 and 3. However, in

contrast, yield efficiency was significantly lower in OM treatment due to excessive phosphorus (P) levels in the compost.

Soil compaction is a major threat to fertility of arable soils and food security (Colombi and Keller, 2019). Soil compaction can change the chemical, physical, biological, as well as the mechanical properties of soil (Hatting and Hatting, 1998) in the following ways: i) increase soil penetration resistance due to decreased space available for displacement of soil particles (Hamza and Anderson, 2005; Batey, 2009) and the low connectivity and continuity of pore space and ii), reduce water and air transport in the soil (Kuncoro *et al*., 2014; Keller *et al*., 2017), which in turn reduce oxygen concentrations in soil air (Colombi and Keller, 2019). These changes in soil physical conditions led to structural degradation. The movement of water and air becomes restricted with an increase in soil compaction and the volume that can be exploited by the roots becomes less, thus less water can be absorbed, subsequently inhibiting plant growth (Hatting and Hatting, 1998).

Root growth, especially the apical zone of roots, is affected by high penetration resistance (Bengough *et al*., 2011) and crops will experience critical levels of soil penetration resistance which can reduce yields (Colombi and Keller, 2019). Hatting and Hatting (1998) compiled a guideline on root growth and level of penetration resistance (kPa), which was adapted to local South African conditions (Table 1). This was used as baseline for analyses of root penetration in our trial. Chen and Weil (2009) evaluated the penetration of cover crop roots through compacted soils for three cover crops: forage rye and rapeseed (*Brassica napus*, cv. ‘Essex’) (taproot system) and forage rye (fibrous root system). They created three compaction levels (high, medium and no compaction) and found that forage radish had a significantly higher penetration than rapeseed and forage rye. As the work row in perennial crops experiences a substantial amount of tractor traffic during its lifespan, compaction in the work row edges seriously impacts tree root growth into the work row (Vogeler *et al*., 2006; Correa *et al*., 2019; Gordon, 2020). Thus, if introduction of a cover crop in the work row can reduce the compaction significantly, it may enhance/stimulate tree root growth in addition to available moisture in the work row.

In spite of numerous publications on the advantages of cover crops, reviewed papers on the i) direct and indirect potential of cover crops and ii), suitable cover crop combinations, for commercial fruit orchards under local conditions, are limited. A current deciduous fruit study on cover crops focuses on the influence of cover crop

diversity on the production and orchard ecology (Steenkamp, 2021). However, information on the performance of suitable cover crops species in deciduous fruit orchards under local conditions, with specific reference to the soil characteristics in the work rows, is lacking. Therefore, this study evaluated the contribution of different cover crop combinations, in the work row, of newly established fruit orchards, with reference to specific characteristics. Although a direct effect of cover crops on the young fruit trees at this early stage was not expected, selective vegetative parameters were also recorded for future reference.

1.2. Materials and Methods

Trials were conducted in Elgin (Glen Elgin; apple orchard) and Stellenbosch (Welgevallen Research Farm; plum orchard). Different cover crop combinations were selected to evaluate the contribution of these crops towards increasing soil fertility and plant performance under two climatically different areas (temperature and rainfall) and different soil types (clay and loamy clay) in perennial orchards.

1.2.1. Site Description

1.2.1.1. Welgevallen Research Farm (Site 1)

The trial site was located at the Welgevallen Research Farm (33°56'51.27"S 18°52'19.29"E) located in Stellenbosch**.** Stellenbosch has a Mediterranean climate, with a mean annual precipitation of 673 mm, most of which occurs during the winter months. The average maximum temperature of Stellenbosch is 22 ˚C and average minimum temperature is 10.9 ˚C (Stellenbosch climate: Average temperature, 2021). The ‘Laetitia’ (*Prunus domestica*) plum orchard, with ‘Songold’ (*Prunus domestica*) as cross pollinator, was established in September 2017, on Marianna rootstock. Planting distance is 1.2 m x 4 m. The soil type is characterized as an Oakleaf (MacVicar *et al*., 1991) and trees are irrigated using micro irrigation. Two trials were conducted, a multispecies trial (trial 1) and a single species trial (trial 2). The layout of both trials was a randomised complete block design (RCBD). Four treatments were replicated four times (Tables 2 and 3). Each plot was approximately 40 m^2 and was situated in the middle of the work row. The cover crop received no irrigation and was completely dependent on winter rainfall.

In order to decrease the compaction on the soil surface and ensure successful establishment of the cover crop, the soil was tilled with an 8 blade disc to a depth of 3 cm. Cover crops were hand sown, after the first winter rain (21 April 2020; 28 April 2021). Seed was mixed with soil (850 g per treatment combination; loamy sand: 70-80% sand and 10-15% clay) to ensure homogeneity during sowing. After sowing, seeds were covered via manual raking.

1.2.1.2. Glen Elgin Farm (Site 2)

The second trial site was located at Glen Elgin farm (34°09'16.83"S 19°02'28.01"E), located in Elgin. Elgin has a Mediterranean climate, with a mean annual precipitation of 949 mm, most of which occurs during winter months. The average maximum temperature of Elgin is 20 ˚C and average minimum is 11.1 ˚C (Elgin Grabouw climate: Average temperature, 2021). The 'Cripps Red' (*Malus domestica*) apple orchard was established in 2019 on MM109 with 'Mahana Red' (*Malus domestica*) as cross pollinator. Planting distance was 1.25 m x 3.5 m. The soil type was characterized as a Tukulu (MacVicar *et al*., 1991) and trees were irrigated using micro-jet irrigation. The layout of the trial was a RCBD, with the same treatment on both sides of the plant row. Five treatments were replicated five times (Table 4). Each plot was approximately 20 m^2 and was situated in the middle of the work row. The cover crop received no irrigation and was dependent on winter rainfall. This trial commenced in 2019/20 as a pilot trial, with limited data recording before it continued thereafter with more detailed data collection. This will be indicated in the results accordingly.

During the second season, an herbicide (Preeglone) was applied (March, 2020) to control kikuyu and other summer weeds. To decrease the compaction of soil surface and ensure successful establishment of the cover crop, soil was tilled with a rotavator tiller to a depth of 3 cm, before planting in March 2020 and 2021. Cover crops were hand sowed, after the first winter rain (26 March 2019; 24 March 2020; 24 March 2021). Seed was mixed with soil (2 kg per treatment combination; loamy sand: 70-80% sand and 10-15% clay) to ensure homogeneity during sowing.

1.2.2. Mineral Analyses

Soil physicochemical data was collected for both sites. Soil samples were collected in October 2019 and January 2021 for Glen Elgin and for Welgevallen Research Farm,

on 21 April 2020 and 28 April 2021. A composite sample of three subsamples per plot, was collected for analyses. Soil samples were taken at a depth of 30 cm with an auger and stored in a plastic bag at room temperature, away from direct sunlight, until analysed. Leaf samples of the fruit crops were collected for the orchards annually at the end of January according to standard procedures to serve as a reference point in future (Addendum A). Fruit tree leaf samples were collected according to standard protocols. Additional cover crop leaf mineral analyses were performed for Glen Elgin in October 2019 (1st season) and 2021 (3rd season). Mineral nutrient analyses were performed by commercial laboratory LABSERVE Analytical Services (Nelspruit, South Africa) for Welgevallen Research Farm and BEMLAB Pty. Ltd. (Strand, South Africa) for Glen Elgin, due to the customer preferences.

1.2.3. Soil Moisture

A Hydro-Sense II sensor (Campbell Scientific Incorporation, Technopark, Stellenbosch) was used to determine soil moisture. This diagnostic tool uses soil dielectric permittivity to estimate volumetric water capacity (VWC). Three measurements were recorded per replicate. Measurements were taken in a diagonal line. The rods of the sensor were fully inserted, vertically into the soil, to ensure quantifying the soil moisture of the top 20 cm. Volumetric water content was measured and water deficit was calculated by software for Welgevallen Research Farm (April 2020; October 2021) and Glen Elgin farm (March 2020; October 2021).

1.2.4. Soil Density

A soil penetrometer (Geotron systems (PTY.) LTD., Potchefstroom) was used to quantify soil density. Soil density was quantified up to 3000 kPa (Hatting and Hatting, 1998). Three measurements were recorded per replicate on both sites in a diagonal line. Soil density measurements were taken as follows: Welgevallen Research Farm - Before establishing cover crop (24 April 2020; 12 April 2021), mid-season when cover crops were established (16 July 2020; 16 July 2021) and at the end of the cover crop season (17 September 2020; 17 September 2021) and Glen Elgin - Before establishing cover crop (23 March 2020; 9 March 2021), mid-season when cover crops were established (30 June 2020; 21 June 2021) and at the end of the cover crop season (10 September 2020; 15 September 2021).

1.2.5. Water Holding Capacity

To determine the soil water holding capacity (WHC), one composite soil sample was collected from three randomly selected locations per replicate, for both sites (Welgevallen Research Farm: 16 July 2020; 7 July 2021; 8 September 2021 and Glen Elgin: 28 July 2020; 7 July 2021; 9 September 2021). Using an auger, soil samples were collected from the top 30 cm within the work row**.** Soil samples were transported in sealed plastic bags in a cooled, covered container, to reduce moisture loss. Samples were collected two to four days after a rain event during July 2020 and 2021, as well as for September 2021, to ensure an even distribution of precipitation in the soil profile, while allowing enough time for drainage to avoid waterlogged soil. Soil samples of 100 g were then allowed to dry naturally until it reached its final dry weight, after which it was oven dried at 65 °C for 12 hours. This was used to calculate the soil moisture content. This is a variation of the method used by Priha and Smolander (1999), where soil samples are drenched for two hours and then drained for two hours. This method was altered to avoid soil structural disruptions that may alter the WHC. The method was adjusted from the one used in Davenport (2019).

1.2.6. Microbial Activity

Carbon dioxide (CO_2) analyses were performed with the gas chromatography technique (SOP, Department of Horticulture Science, Stellenbosch University) to quantify the microbial activity of the soil. Soil samples from both sites (Welgevallen Research Farm: 21 April and 17 September 2020; 31 March and 8 September 2021 and Glen Elgin: 18 March and 8 September 2020; 17 March and 9 September 2021) were collected from the top 30 cm within the work row, using an auger. Soil samples were kept at 2- 4°C until processed. The protocol selected for analyses was the Agilent 6890N Gas Chromatograph (GC) fitted with a TCD (Thermal Conductivity Detector) detector, and helium as carrier gas to detect CO_2 levels and oxygen (O_2) levels present in soil samples. Ten grams of soil was added to a 20 ml sealed test tube and allowed to respire for 30 minutes. After 30 minutes, a syringe was used to extract gas (CO_2, O_2 and Ethylene) from a sealed test tube. The gas was injected into GC, measuring the CO_2, O_2 and ethylene levels. In 2020, this analysis was performed on 20 March 2020 for Glen Elgin, 9 July 2020 for Welgevallen Research Farm and again 22

September 2020 for both trial sites. In 2021, this analysis was performed on 8 April 2021 and again 16 September 2021, for both trial sites.

1.2.7. Plant Performance

On both sites, one tree per replicate as selected at random to represent tree performance. Trunk diameter, as well vegetative growth of two representative one-year-old branches were measured at the beginning of April 2020 and 2021. The first yield of the plum orchard was quantified in 2021.

Statistical Analyses

SAS Enterprise Guide 7.1 statistical software version 9.4 (SAS Institute Incorporation, Cary, North Carolina, United States of America), was used to perform one-way Analysis of Variance (ANOVA) using general linear models (GLM) with LSD- Fisher as comparison method. Means were separated at a 5 % significance level. An additional regression analysis was performed to describe the change in soil density over depth using the NLIN Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). A power curve (Density = a*Depth^{b}) with density as dependent variable and depth as independent variable was fitted for each experimental unit (treatment*replication combination). The estimated depth at which a density of 3000 kPa was reached was calculated for each experimental unit using the regression parameters obtained. Estimated regression parameters and depth to 3000 kPa was subjected to ANOVA using the GLM (General Linear Models) Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA) to test if any treatment significantly affected the rate of density change and depth to 3000 kPa. A Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality (Shapiro, 1965). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

1.3. Results

1.3.1. Mineral Analyses

1.3.1.1. Welgevallen Research Farm

For trial 1, there was no significant difference between treatments for any of the parameters for either of the seasons (Table 5).

For trial 2, there was a significant difference between treatments in 2020 (Table 6). The double fertilizer (SDFA) treatment had a significantly higher P than the no fertilizer (SNFA) and single fertilizer (SSFA) treatments, but was similar to the control. The control, SNFA and SSFA did not differ significantly from each other. Calcium (Ca) of the SDFA was significantly higher than the other three treatments. The control, SNFA and SSFA did not differ significantly. Copper (Cu) of the SDFA was significantly higher compared to the other three treatments, whilst the control, SNFA and SSFA did not differ significantly. Zinc (Zn) of the SDFA was significantly higher than SNFA and SSFA, but was similar to the control and the control, SNFA and SSFA did not differ significantly from each other. The electric conductivity (EC) was significantly higher in SDFA compared to the other three treatments. The control, SNFA and SSFA did not differ significantly. There was no significant difference between treatments in 2021.

1.3.1.2. Glen Elgin Farm

Soil analyses: There was no significant difference between treatments for any of the parameters, for either of the seasons (Table 7).

Cover crop mineral analyses: In 2019, there was a significant difference between treatments (Table 8). N of the FRWMC was significantly higher compared to the other four treatments. FRVC was significantly lower compared to PC, FBPC and FRC. PC, FBPC and FRC did not differ significantly from each other. Potassium (K) of the PC was significantly higher compared to the other four treatments. K in FBPC and FRC was significantly lower compared to the other three treatments. FRVC and FRWMC did not differ significantly from each other. Ca of the PC was significantly higher compared to the other four treatments. Ca in FRWMC was significantly higher than FRVC, FBPC and FRC with FRVC, FBPC and FRC not differing significantly from each other. With regards to Magnesium (Mg), PC and FRWMC were significantly

higher than the other three treatments. FRVC, FBPC and FRC did not differ significantly from each other. Sodium (Na) of the PC and FBPC were significantly higher than FRVC. PC, FRWMC, FBPC and FRC did not differ significantly from each other. However, FRVC and FRC did not differ significantly from each other. With regards to Zn, FRWM was significantly higher compared to the other four treatments. PC, FRVC, FBPC and FRC did not differ significantly from each other. Boron (B) in the PC and FRWMC were significantly higher compared to the other three treatments. FRVC, FBPC and FRC did not differ significantly from each other.

In 2021, sufficient plant material for PC was lacking at the time of analyses (Table 8). FRWMC had a significantly higher Ca, Mg, Na and B compared to the other treatments. FRVC, FBPC and FRC did not differ significantly from each other for these parameters.

1.3.2. Soil Moisture

Volumetric water content (VWC) and water deficit were quantified at both sites.

1.3.2.1. Welgevallen Research Farm

In trial 1, there was a significant difference between treatments in October 2020. The control had a significantly lower VWC and a significantly higher water deficit compared to the other three treatments (Table 9). No fertilizer (MNFA), single fertilizer (MSFA) and double fertilizer (MDFA) treatments did not differ significantly from each other for either parameter. In November 2020, the control had a significantly lower VWC and a significantly higher water deficit compared to the other three treatments. MNFA, MSFA and MDFA treatment did not differ significantly from each other for either parameter. MNFA, MSFA and MDFA had a higher VWC for October (1.97 %, 1.93 % and 1.93 %) and November (16.50 %, 17.29 % and 18.15 %) and a lower soil water deficit for October (0.77 mm, 3.41 mm and 3.96 mm) and November (-4.88 mm, -6.37 mm and -7.15 mm), respectively. In October 2021, the control had a significantly lower VWC and a significantly higher water deficit (16.42 mm) compared to the other three treatments. MNFA, MSFA and MDFA treatment did not differ significantly from each other for either parameter. MNFA, MSFA and MDFA had a higher VWC (12.09 %, 12.94 % and 10.64 %) and a lower soil water deficit (3.97 mm, 2.27 mm and 6.87 mm).

In trial 2, the control also had a significantly lower VWC and higher water deficit compared to the other three treatments in October 2020 (Table 10). SNFA, SSFA and

SDFA treatment did not differ significantly from each other for either parameter. In November 2020, the control had a significantly lower VWC (and a significantly higher water deficit compared to the other three treatments. SNFA, SSFA and SDFA treatment did not differ significantly from each other for either parameter. SNFA, SSFA and SDFA had a higher VWC for October (14.75 %, 13.07 % and 15.28 %) and November (19.26 %, 17.79 % and 15.28 %) and a lower soil water deficit for October (0.51 mm, 2.03 mm and -2.45 mm) and November (-10.35 mm, -7.40 mm and -9.66 mm). In December 2020, the control had a significantly lower VWC compared to SSFA and SDFA treatment. The control and SNFA treatment did not differ significantly from each other. SNFA, SSFA and SDFA treatment did not differ significantly from each other for the VWC parameter. The control had a significantly higher water deficit compared to SSFA treatment. The control, SNFA and SDFA treatment did not differ significantly from each other. SNFA, SSFA and SDFA treatment did not differ significantly from each other. During the second season (2021), there was a significant difference between treatments for June and October 2021. In June 2021, the control had a significantly lower VWC and higher water deficit compared to the other three treatments. SNFA, SSFA and SDFA treatment did not differ significantly from each other for either parameter. SNFA, SSFA and SDFA had a higher VWC (13.51 %, 12.40 % and 11.82 %) and a lower soil water deficit (1.15 mm, 3.34 mm and 4.54 mm). In October 2021, the control had a significantly lower VWC and higher soil water deficit compared to the other three treatments. SSFA had a significantly lower VWC and higher soil water deficit compared to SDFA treatment. SNFA did not differ significantly from SSFA and SDFA for either parameter. SNFA and SDFA had a higher VWC (10.92 % and 12.31 %) and a lower soil water deficit (6.33 mm and 3.55 mm).

1.3.2.2. Glen Elgin Farm

In trial 3, FRWMC had a significantly lower VWC and higher soil water deficit compared to PC and FRC in September 2020 (Table 11). FRWMC, FRVC and FBPC did not differ significantly from each other for either parameter. PC, FRVC and FBPC treatment did not differ significantly from each other for either parameter. PC, FBPC and FRC treatment did not differ significantly from each other for either parameter. In September 2021, PC and FRC had a significantly higher VWC and a significantly lower water deficit compared to FRVC and FRWMC treatment. PC, FBPC and FRC

treatment did not differ significantly from each other for either parameter. FRVC, FRWMC and FBPC treatment did not differ significantly from each other for either parameter.

1.3.3. Soil Density

1.3.3.1. Welgevallen Research Farm

Maximum depth of penetration (≤ 3000 kPa) is indicated for both trials 1 and 2 in Tables 12 and 13 respectively. For trial 1, there was no significant difference between the treatments for either 2020 or 2021. For trial 2, the only significant difference between treatments occurred in April 2020. The SDFA treatment had a significantly lower maximum depth of penetration compared to the other three treatments. The control, SNFA and SSFA did not differ significantly from each other.

The change in soil density over depth is indicated in Figures 1 to 10 for both trials 1 and 2. There were no significant differences between the treatments for either of the seasons for either of the trials, except for September 2021 for trial 2 (Figure 10). SNFA density was significantly higher compared to the control and SDFA. The control, SSFA and SDFA did not differ significantly from each other.

1.3.3.2. Glen Elgin Farm

There was no significant difference between the treatments for maximum depth of penetration or the change in soil density over depth for either 2020 or 2021 (Table 14; Figures 11 to 16).

1.3.4. Water Holding Capacity

1.3.4.1. Welgevallen Research Farm

No significant differences between treatments were reported for WHC, for either of the trials or seasons (Table 15 and 16).

1.3.4.2. Glen Elgin Farm

There was a significant difference between the treatments for July 2020 (Table 17). PC, FRWMC and FRC had a significantly higher WHC compared to FBPC. PC, FRVC,

FRWMC and FRC did not differ significantly from each other, and neither did FRVC and FBPC. There was no significant difference between treatments for July and September 2021.

1.3.5. Microbial Activity

1.3.5.1. Welgevallen Research Farm

For trial 1, there was no significant difference between the treatments, for either of the seasons, except for CO_2 levels in April 2021 (Table 18), when the control had a significantly lower CO_2 level compared to the other three treatments. MNFA, MSFA and MDFA did not differ significantly from each other. For trial 2, there was no significant difference between the treatments, for either season (Table 19).

1.3.5.2. Glen Elgin Farm

There was no significant difference between the treatments, for either of the seasons, except for CO_2 levels in March 2021 (Table 20) when PC had a significantly lower CO_2 level compared to the other four treatments. The other treatments, i.e., FRVC, FRWMC, FBPC and FRC did not differ significantly from each other.

1.3.6. Plant performance

1.3.6.1. Welgevallen Research Farm

There were no significant differences between treatments for any of the parameters, for either of the trials or seasons (Table 21 and 22).

1.3.6.2. Glen Elgin Farm

There was no significant difference between treatments for any of the parameters for either of the seasons (Table 23).

1.4. Discussion

1.4.1. Mineral Analyses

1.4.1.1. Welgevallen Research Farm

In trial 1, there was no significant difference between treatments for either of the seasons. However, according to the industry norms of LABSERVE, the following minerals were i) below optimal range: P-level for all of the treatments (2020/2021), S-level for the control in 2020 and for all treatments in 2021 and C-level for all of the treatments in 2021 and ii), above optimal range: Cu-level for all of the treatments in 2020 and Zn-level for MDFA in 2020. Their levels were within the range in 2021. The change in mineral levels, although not significant, indicates a natural variation in soil mineral levels during the study.

In trial 2, there was a significant difference between treatments in 2020. SDFA had a significantly higher EC, Ca and Cu compared to the other treatments. SDFA had a significantly higher P and Zn compared to SNFA and SSFA. The control did not differ significantly from SNFA, SSFA and SDFA. There was no significant difference between treatments for 2021. However, P and sulphur (S) soil levels were below optimal range for all treatments (2020/2021), whereas Zn and Cu soil levels were above optimal range for all treatments in 2020 according to LABSERVE. The control Cu-level was still above optimal range in 2021, this indicated that all cover crops decreased Cu levels.

Soil pH (KCl) (5.2 – 6.0) for both trial 1 and 2, was within optimal range according to LABSERVE (5.2 – 6.5), for both sample dates and the treatments did not differ significantly from each other. These results corresponded with findings from Qian *et al*. (2015) in an apple orchard, where they found a non-significant decline in soil pH. Soil C levels for both trials did not increase significantly over the two-year study period and contradicted findings by Fourie (2012) who reported improved SOC levels within the first three years of the trial, with SOC levels of cover crop and mulch treatments exceeding 9 % after 10 years. According to Blanco-Canqui *et al*. (2015) cover crops increase SOC stocks, however, the magnitude depends on the cover crop biomass, how long cover crops have been used and the initial soil C level. Although our cover crop treatments for both trials produced a higher biomass compared to the control (Paper 3), we did not find a significant difference for SOC among cover crop

treatments and the control. Although differences in EC, P, Ca, Cu and Zn occurred between treatments in 2020 for trial 2, there was no difference in 2021, therefore cover crops did not significantly influence soil mineral composition. Fourie (2012) reported similar results, with differences between treatments for P, Ca and Mg, however, they did not observe a significant trend in the years during which they measured (2000/03). Although no significant difference was found for S-levels for either trial, control levels in trial 1 were below optimal range in 2020 and below optimal range for all treatments in 2021. Establishment and growth of white mustard and forage radish was good in 2020, but not in 2021. The low S-level could partly explain the poor establishment of white mustard and forage radish.

1.4.1.2. Glen Elgin Farm

In trial 3, similar to trial 1, there was no significant difference between treatments for either of the seasons (2019-2021). However, according to BEMLAB industry norms, the following minerals were i), above optimal range: S, K and P soil levels for all of the treatments (2019/2021), soil pH for all treatments in 2019 and C levels were very high for all treatments in 2021 and ii) below optimal range: Ca for all treatments in 2019, except for PC and FRWMC and in 2021, for all treatments except for FRWMC. Therefore, cover crops did not significantly influence the soil mineral composition, however there was a natural variation in soil mineral levels during the study.

Soil pH (KCl), was above optimal range in 2019, but within optimal range for all treatments in 2021. Over time all cover crop treatments influenced soil pH (2019: 6.04; 2021: 5.60 – 5.90). However, no significant difference was found between treatments, confirming results from trial 1 and 2 and reported by Qian *et al*. (2015). Soil C levels (3.43 – 3.68 %) for 2021 were higher compared to the results of trial 1 (0.64 – 0.73 %) and 2 (0.74 – 0.76 %), as well as reported in sandy clay soils in Robertson (0.43 – 1.21 %) (Fourie, 2012), however this is not directly related to cover crops, but probably indicates soil differences at the different sites.

Although there was no significant difference among treatments for S, K and P, levels were above optimal range for both sampling dates (BEMLAB). Our P-levels (15.1 to 35.9 $mg.kg^{-1}$) were similar to those reported previously for an organic treatment (134 $mg.kg^{-1}$) in an apple orchard, in Elgin (Wooldridge *et al*., 2013), but higher compared to Quin *et al*. (2015), who conducted their trial on a dark loessial soil

and used the Olsen method. Qian *et al*. (2015) also reported that their living mulches significantly increased soil P and K levels. In this trial, PC also had lower Ca (8.5 – 14.8 cmol.kg^{-1}), Mg (1.9 – 3.0 cmol.kg^{-1}) and Na (0.1 - 0.2 cmol.kg^{-1}) levels, but a higher K level (1.4 – 2.4 cmol.kg^{-1}) compared to the phacelia treatment, as reported by Demir and Isik (2020), i.e., respectively 17.1 - 17.9 cmol.kg^{-1}, 5.8 - 7.0 cmol.kg^{-1}, 0.2 - 0.3 cmol.kg^{-1} and 1.2 - 1.3 cmol.kg^{-1}. However, their soil texture was classified as a clay, slightly alkaline with low OM content – implying that cover crops will perform differently and is site specific.

Foliar analyses

In 2019, there was a significant difference between treatments for cover crop leaf mineral analyses, implying that some crop species are better in taking certain minerals up in their foliage. FRWMC had a significantly higher level for N and Zn compared to the other treatments, which might indicate that they are effective in scavenging and taking up N and Zn from the soil. FRVC had the lowest level of N level, which one will not expect due to the legume being present in the mixture. PC and FRWMC had a significantly higher level for Mg and B compared to the other treatments, which might imply that these treatments are more effective in taking up these nutrients under the specific trial site conditions. PC had a significantly higher level for K and Ca compared to the other treatments, implying that they are effective in scavenging these two minerals. FRVC and FRWMC had similar levels for K, as did FBPC and FRC. FRWMC had a significantly higher Ca level than FRVC, FBPC and FRC. The difference in nutrient acquisition might be due to a difference in plant architecture and the difference in mineral requirement for different species. FRWMC had a significantly higher Na – level compared to FRVC and FRC. PC and FBPC did not differ significantly from FRWMC and FRC. FRWMC might be a good option to use in soils with high Na – levels.

In 2021, there was a significant difference between treatments for cover crop leaf mineral analyses. However, PC was not included in analyses because there was no PC present at time of sampling. FRWMC had a significantly higher level for Ca, Mg, B and Na compared to the other treatments that did not differ significantly from one another.

FRWMC was the crop with a significantly higher foliar nutrient content than all other treatments (2019 and 2021). One would assume a pure legume or legume mixture would contain a higher level of N in their biomass due to their ability to fixate atmospheric N (Thorup-Kristensen *et al*., 2003). However, this was not evident in our legume-grass mixture treatment. FRWMC had high N levels but did not differ significantly from the rest of the treatments, as was the case in 2019. The legume-grass mixtures N-level ranged from 1.6 – 3.4 %, which was similar to the upper level, reported by Ovalle *et al*. (2007) in a vineyard (2.91 – 3.15 %). Forage radish root architecture allows deep penetration into the soil profile, enabling scavenging of N from deeper soil layers. This is a possible explanation for the higher N levels in FRWMC.

Mineral levels for PC with regard to N (3.3 %), P (3.3 %), K (5.2%), Ca (4.6%) and Mg (0.4 %) where higher than reported by Wendling *et al*. (2016), 2.13 %, 0.48 %, 4.21 %, 2.93 % and 0.13 %, respectively. This difference might be attributed to the type of soil and climatic conditions. The FRWMC treatment was a *Brassica* mixture, whereas Wendling *et al*. (2016) evaluated radish and white mustard separately. The N (3.3 – 4.8 %) and Mg (0.5 %) levels were higher than their radish and white mustard treatments. Their values for radish were 1.6 – 2.2 % and 0.2 %, respectively. Their values for white mustard were 1.4 % and 0.02 %, respectively. However, our K (3.5 %) and Ca (2.5 – 2.7 %) levels were higher than their white mustard treatment, but lower than their radish treatment. Their values for white mustard were 2.1 % and 2.1 %, respectively. Their values for radish were 3.9 – 4.3 % and 3.9 – 4.3 %, respectively.

The FBPC treatment was a legume-grass mixture, whereas Wendling *et al.* (2016) evaluated peas only. However, our N (2.6 – 3.4 %), P (0.3 – 0.4 %), K (2.7 – 2.9 %), Ca (0.5 – 0.6 %) and Mg (0.2 %) levels were lower than their pea treatment, with values 3.6 %, 0.5 %, 3.2 %, 1.9 % and 0.3 %, respectively.

The FRVC treatment was a legume-grass mixture, whereas Wendling et al. (2016) only evaluated vetch. However, our N (1.6 – 1.9 %), P (0.3 %) and Ca (0.6 – 0.7 %) levels were lower than their vetch treatment. Their values were, 3.6 %, 0.4 % and 1.3 %. Mg and K levels were similar. These differences might partly be due to the different crop combinations as well as the type of soil and planting conditions.

Nutrient uptake by cover crops are influenced by various factors. Soil type is one of the main factors influencing nutrient uptake, because it plays an important role in the establishment and growth of a cover crop. The type of cover crop also plays a

role, because each cover crop has a specific growth architecture enabling acquisition of nutrients at different soil levels and results will vary between a monoculture and multispecies cover crop mixture.

1.4.2. Soil Moisture

Soil water content influences plant growth and development, as well as affects various physiological processes (Taiz *et al*., 2018a). The results indicated that there was a significant difference between treatments for both soil moisture parameters (VWC and soil moisture deficit) during early and late spring.

1.4.2.1. Welgevallen Research Farm

The cover crop treatments in trial 1 (MNFA, MSFA and MDFA) conserved soil moisture better compared to the control during October 2020 and 2021 and November 2020. This is most likely due to the amount of mulch that the cover crops treatment provided which is reported in Paper 3.

The cover crop treatments in trial 2 (SNFA, SSFA and SDFA) conserved soil moisture better compared to the control in October and November 2020. However, in December there was a different trend, the control did not differ significantly from SNFA and SDFA water deficit, but was drier, whereas SSFA had a significantly lower soil water deficit (wetter) compared to the control. In 2021, there was significant differences between treatments in June and October for trial 2. The control had a significantly lower VWC and higher soil water deficit than the other treatments. The cover crop treatments (SNFA, SSFA and SDFA) conserved soil moisture better compared to the control. This is most likely due to the amount of mulch that the different cover crops treatments provided as was shown in Paper 3, where the control had a lower biomass compared to SNFA, SSFA and SDFA. However, after two years there was an additional difference between the cover crop treatments (SNFA, SSFA and SDFA) in October. SDFA had a significantly higher VWC and a lower water deficit (wetter) compared to SSFA, but biomasses did not differ significantly (Paper 3). SNFA soil moisture was similar to SSFA and SDFA. The significant difference in soil moisture between SDFA and SSFA could therefore not be attributed to biomass.

Cover crops left as a mulch had a higher soil moisture content compared to the control plot in both trials confirmed similar results by various papers (Van Huyssteen

et al., 1983; Kühn and Pedersen, 2009; Keestra *et al*., 2016; Demir *et al*., 2019; Demir and Isik, 2020). The higher soil moisture content in cover crop treatments is most likely due to the mulches reducing soil evaporation by providing an isolation layer between the soil surface and the atmosphere (Zribi *et al*., 2014; Liao *et al*., 2021). We can therefore conclude that cover crops had a positive effect on soil moisture, during spring (October and November) when the dry summer conditions begin, and plum trees start to grow. What we could not confirm yet is the effect of the higher soil moisture content of the work row on the young plum trees due to the assumption that their root systems have not rich the work row.

1.4.2.2. Glen Elgin Farm

In trial 3, the cover crops were mowed in September and moved onto the tree row at the end of September/ beginning of October to serve as a mulch in the tree row. There were significant differences between treatments for both soil moisture parameters (VWC and soil water deficit) during September 2020 and 2021. In 2020, FRWMC had a lower VWC and higher soil water deficit, whereas FRC had a higher VWC and lower soil water deficit. This difference could not be attributed to biomass, because the biomasses of FRWMC and FRC were similar, whereas PC had the lowest biomass (Paper 3). In 2021, FRVC had a lower VWC and higher soil water deficit, whereas FRC had a higher VWC and lower soil water deficit. The difference in soil moisture conservation could not only be attributed to biomass, because although FRVC and FRC differed according to wet weight, dry weights were similar and their biomasses were also significantly higher compared to the other treatments (Paper 3). From trial 3, the effect of a cover crop (mulch) in work row versus removal of the biomass was illustrated. In September, there was a difference in moisture conservation between treatments in the work row and after removal, there was no difference in moisture conservation in October. However, contradictions between treatments in terms of soil moisture conservation and biomass still need to be clarified before final conclusions can be drawn.

1.4.3. Soil Density

1.4.3.1. Welgevallen Research Farm

In trial 1, there was no significant difference between treatments for maximum depth of penetration or the change in soil density over depth, for either 2020 or 2021, implying that the cover crops did not primary effect soil density. However, additional analyses were conducted to describe the change in soil compaction until a resistance of 3000 kPa was reached, giving one a better indication of how steep the correlation is between maximum depth of penetration and soil compaction.

In trial 2, SDFA had a significantly shallower maximum depth of penetration compared to the other treatments in April 2020. However, no significant differences were found for the remainder of the trial, which might be a treatment effect, however it is important to remember that soil compaction and soil biological, physical and chemical properties influence each other and it is difficult to characterize the effects of soil compaction by considering individual soil properties only, due to the interaction of various factors on soil strength (Chen *et al*., 2014). In September 2021, SNFA had a significantly steeper slope in terms of soil density over depth compared to the control and SDFA, however the slopes of SNFA and SSFA were similar. The steeper slope of SNFA, implies that 3000 kPa are reached quicker and the crops experience more stress in terms of soil compaction, compared to the control and SDFA.

1.4.3.2. Glen Elgin Farm

In trial 3, there was no significant difference between the treatments for maximum depth of penetration or the change in soil density over depth for either 2020 or 2021. Maximum depth of penetration and the change in soil density over depth before reaching 3000 kPa were similar among all treatments. Implying that the cover crops did not have a primary effect on soil density in this trial.

1.4.4. Water Holding Capacity

1.4.4.1. Welgevallen Research Farm

No significant differences between treatments were reported for WHC, for either of the trials for both seasons. This was in contrast with Davenport (2019) who reported a significantly higher WHC in the living mulch treatment (*Tradescantia*) compared to the

control (bare soil). However, our control consisted of natural vegetation (weeds) and thus the contrast between treatments may have been less. Additional analyses were performed to investigate possible trends between WHC and various parameters (Addendum A). No relationship was found with biomass production - implying that there should be other factors influencing WHC. No clear conclusion can be made regarding the effect of cover crops on WHC for both trials, due to similar results between treatments over two seasons.

1.4.4.2. Glen Elgin Farm

In trial 3, there was a significant difference between treatments in July 2020**.** PC, FRWMC and FRC had a significantly higher WHC compared to FBPC. PC, FRVC, FRWMC and FRC treatment did not differ significantly, as neither did FRVC and FBPC. Water holding capacity was ranked in descending order as follows: PC > FRWMC > FRC > FRVC > FBPC. However, no significant difference was found during the second season. Nevertheless, there was an increasing trend in soil WHC for PC (± 1.11 %), FRVC (± 7.76 %), FRWMC (± 3.8 %), FBPC (± 6.65 %) and FRC (± 5.32 %) from 2020 to 2021 (July). The decreasing trend in WHC from July to September 2021 is largely attributed to the difference in soil moisture conditions as a result of the Mediterranean climate. Soil was saturated in July 2021 (Precipitation: 104.75 mm) and naturally dried out towards September 2021 (Precipitation: 18.22 mm). Thus, based on our results, cover crops did not primarily determine soil WHC and that there are additional factors influencing WHC.

1.4.5. Microbial Activity

1.4.5.1. Welgevallen Research Farm

In trial 1, there were no significant differences between treatments in 2020. In April 2021, the control had a significantly lower CO_2 level compared to the other treatments. The CO_2 levels were similar among treatments for September 2021. CO_2 values from trial 1 were much higher (± 1250 to 2220 ppm) compared to McGowen *et al.* (2018) (± 7 to 36 ppm), Demir *et al*. (2019) (± 119 to 415 ppm) and Demir and Isik (2020) (± 23 to 98 ppm). However, it corresponded with Koerner *et al*. (2011) that ranged from 579 – 11 326 ppm, with approximately 80 % of the samples between 1000 – 2000 ppm.

The first significant difference between treatments was observed in April 2021, confirming observations of Demir and Isik (2020) who reported significantly higher CO_2 levels only in the second season of their study. Cover crop treatments (MNFA, MSFA and MDFA) promoted microbial activity in terms of CO_2 levels compared to the control (natural vegetation). However, we did not find a significant difference at the beginning of the trial (April 2020) or at the end of the cover crop life cycle (before termination) in September for either 2020 or 2021. The significant difference in April 2021, may be attributed to a higher above and below ground biomass of the cover crops in 2020 compared to the control (Paper 3), providing a favorable and long-term environment for the microbial community to decompose organic material, but this needs to be confirmed.

In trial 2, there were no significant differences between the treatments for either 2020 or 2021. CO_2 levels (± 1190 to 2190 ppm) were much higher compared to other researchers (McGowen *et al.*, 2018; Demir *et al*., 2019; Demir and Isik, 2020), but similar to trial 1 and Koerner *et al*. (2011). However, no clear conclusion can be made regarding the effect of a cover crop (single specie) on soil microbial activity.

1.4.5.2. Glen Elgin Farm

In trial 3, there were no significant differences between treatments in 2020. PC had a significantly lower CO_2 level in March 2021 compared to the other treatments. The CO_2 levels of FRVC, FRWMC, FBPC and FRC were similar. The CO_2 levels were similar among treatments for September 2021. CO_2 levels (± 1540 to 4225 ppm) were much higher compared to some recorded (McGowen *et al*., 2018; Demir *et al*., 2019; Demir and isik, 2020), but similar to trial 1 and 2 and Koerner *et al*. (2011). Thus, microbial activity quantified in terms of CO_2 levels differed significantly among the different cover crop treatments in April 2021. However, we did not find a significant difference at the beginning of the trial (March 2020) or at the end of the cover crop life cycle (before termination) in September for 2020 and 2021. The significant difference in March 2021, might be attributed to the fact that the cover crop treatments (FRVC, FRWMC, FBPC and FRC) had a higher above and below ground biomass in 2020, compared to the PC (Paper 3).

1.4.6. Plant Performance

1.4.6.1. Welgevallen Research Farm

There were no significant differences between treatments, for either of the seasons, with regard to plant performance (trunk diameter, average shoot length, yield and yield efficiency). This corresponded with findings from Sánches *et al*. (2007) and Kühn and Pederson (2009) but contradicts yield improvement reported by others (Demir *et al*., 2019; Demir and Isik, 2020). Trunk diameter increased and average shoot length decreased from 2020 to 2021. Trunk diameter increased and average shoot length decreased for our four-year old plum trees from 2020 to 2021. This also occurred in a nine-year old apple orchard of Sánches *et al*. (2007). The cover crops did not have a negative effect on the growth of plum trees during the study, however this should be monitored in the long term.

1.4.6.2. Glen Elgin Farm

There were no significant differences between treatments, for either of the seasons with regard to plant performance (trunk diameter and average shoot length). Trunk diameter increased and average shoot length decreased for our two-year old apple trees from 2020 to 2021, indicating normal tree growth. This corresponded with our findings from trial 1 and 2 as well as from Sánches *et al.* (2007) and Kühn and Pederson (2009). These results confirmed again that the cover crops did not have a negative effect on the growth of apple trees during the study, however this should be monitored in the long term.

1.5. Conclusion

Cover crops did not have a negative effect on the chemical composition of soil. The mineral composition did not change drastically during the study, except for trial 2 where we noticed a reduction in soil Ca level for the cover crop treatments and an increase for the control. Indicating a cover crop effect. However, it was still within optimal range. It is important to monitor Ca levels when using forage rye as a single specie cover crop. Cover crops did not have an effect on P - levels of soil, due to P-level remaining below optimal range for trial 1 and 2 and above optimal range for trial

3. Forage rye reduced Cu levels more efficiently (within optimal range) than the control (natural vegetation).

A poor establishment of white mustard (brassica) was noticed, this might be due to the S-level being below optimal range in 2021 for trial 1. The pH remained stable for trial 1 and 2, however the pH decreased (to a more favorable pH) for all treatments of trial 3, which is most likely a cover crop effect. According to literature and the industry it takes time to build soil C. Although the cover crop treatments of both trial 1 and 2 produced a higher biomass compared to their control, the SOC of all treatments did not differ significantly – implying that biomass and its contribution to OM of soil is not the main factor influencing C levels in soil. Soil C-levels for both trial 1 and 2 were below optimal range in 2021, we did not expect to see this (below optimal range) after two years. Soil C - level of trial 3 was very high, however a significantly higher biomass did not deliver a significantly higher C – level. A difference in location, climate and type of cover crops made it difficult to compare soil mineral analyses with other researchers.

The Brassica mixture (FRWMC) had a higher nutrient content in their foliage, implying that they were more effective in scavenging nutrients. Legume-grass mixtures did not have the highest N content in their foliage and thus it is recommended to inoculate legumes with the correct strains, enhancing symbiotic relationship and N-fixation.

Cover crops contribute towards nutrient cycling in the orchard by obtaining nutrients through various strategies and providing additional C through photosynthesis, after they complete their life cycle and serve as a mulch in the orchard they get broken down by the microbial community and the nutrients are released back into the soil in a plant available form. Enabling the farmer to use nutrients present in soil more efficiently.

Cover crops did not have a negative effect on soil moisture during the season and contributed towards soil moisture conservation during spring (September to November) when the fruit trees start to grow actively. Soil moisture measurements should be taken from August to October, because this is when one will most likely find a significant difference among cover crops and how effectively they conserve soil moisture. Cover crop treatments from trial 1 and 2, conserved soil moisture better in October and November (after mulching in work row) compared to their control. This is most likely attributed to their higher biomass production. However, after two

years we found an additional difference amongst cover crop treatments in October for trial 2. The soil of SDFA was wetter than SSFA, however their biomass was similar and thus the additional difference could not be attributed to biomass. In trial 3, the cover crops were mowed in September and moved over to the tree row at the end of September/ beginning of October, to serve as a mulch in the tree row. There was a significant difference between treatments for soil moisture in September for both years. The difference in soil moisture could not be attributed to biomass which was seen for both years. In 2020 the soil of FRWMC was wetter compared to the soil of FRC, however their biomass was similar and in 2021, the soil of FRVC was wetter compared to the soil of FRC, however their dry biomass was similar and significantly higher compared to the other treatments. A possible explanation could be a difference in root architecture, however the contradictions between cover crop treatments in terms of soil moisture conservation and biomass still needs further investigation. The positive effect of cover crops was more prominent in trial 1 and 2 when you compare it to a control. In trial 3, we saw the positive effect of a cover crop mulch in the work row versus the removal of the biomass from the work row, soil moisture conservation only occurred for one month. However, it was not possible to confirm what the effect of a higher soil moisture content in the work row will have on the young fruit tree.

Although the findings varied between treatments, penetrometer data added additional information about the quantification of soil compaction. The additional analysis describes the data more effectively in terms of how soil compaction changes over time before reaching 3000 kPa. One of the observations made during the study is that the penetrometer penetrated the soil easier where cover crops were planted. To compare data during the season, a different protocol should be followed to ensure similar soil moisture conditions at each evaluation date. According to literature various factors influence soil compaction and thus a possible future study would be to evaluate the interaction of various physical, chemical and biological properties on soil strength in a perennial orchard under South African conditions.

Quantifying WHC is season bound due to the big difference between a saturated and unsaturated soil water profile. The control for both trial 1 and 2 had a lower WHC (ns) in September 2021 when the soil profile was unsaturated. The WHC was higher (ns) in July 2021 for trial 3. Thus, cover crops did contribute towards improving WHC. However, based on our results, cover crops did not primarily

determine soil WHC during the two-year study and that there are additional factors influencing WHC. It is possible that our study stopped too early to see the significantly positive effect of cover crops on WHC.

CO_2 gives one an understanding of the seasonal variability in labile fractions of C, which is primarily dependent on the quantity of cover crop residue produced, rhizodeposition during growth cycle of crop and soil moisture and temperature, which influences soil microbial activity and residue decomposition. This was evident in our study, where we found a significant difference in CO_2 production in March and April 2021, during the second year of the study for trial 1 and 3. The control and PC treatment from trial 1 and 3, respectively had a significantly lower CO_2 production in 2021 and a significantly lower biomass in 2020. However, there was no significant difference at 5 % for trial 2 (single species trial), making the interpretation more complicated. A possible explanation might be due to a monoculture of forage rye (lower plant diversity), but this needs further investigation.

However, our CO_2 values (ppm) were higher compared to literature, indicating the effect of the actual technique (sampling and analyses procedures), the environment and crop selection. Therefore, the same technique should be selected to follow changes over time. From our results it was evident that cover crops improved microbial activity through providing a favourable environment, by conserving soil moisture and regulating diurnal changes of temperature (according to literature and a visual observation), providing food via rhizodeposition during the winter and providing plant material to breakdown during spring and summer (after cover crop cycle is completed). Future study would be to standardize our GC-method, making it easier to interpret and making it a viable commercial test for farmers. Cover crops did not have a negative effect on plant performance, however we did not expect to see differences this early in the trial and these measurements served as a baseline to track how cover crops influences plant performance in the long term if the study should continue.

Soil is a complex system consisting of physical, chemical and biological components interacting with each other. Cover crops did play an important role in shaping the soil “system/environment’ and having an effect on the parameters we quantified, however they were not the primary factor responsible for the change in soil. Future study would be to monitor the change in these parameters and how cover crops change them in the long-term.

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Table 1: A guideline compiled by Hatting and Hatting (1998) on root growth and level of penetration resistance (kPa) which was adapted to local South African conditions.

Penetration resistance (kPa)	Description
0 - 500	Soil is extremely unconsolidated with no compaction.
500 - 1000	The soil is sufficiently unconsolidated not to limit root growth.
1000 - 1500	Root growth may be affected in some soils but not necessarily the yield.
1500 - 2000	Root growth may be severely limited and loss in yield can be attributed to compaction.
2000 - 3000	Soil compaction is severe and specialized corrective treatment is necessary. Root development for most plants is severely inhibited with limited or no roots in this area.
>3000	In general, no root development is possible except in clayey soil where cracks and planes of weakness are exploited.

Table 2: Treatment details for trial 1 (multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, during 2020 and 2021.

Treatment	Cover crop	Treatment description
Control	Fallow / Natural weeds	Natural weeds.
No fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop.
Single fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop with single fertilizer application. First season: 21 April 2020 @ 1 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg per plot (Commercial organic fertilizer, 5:1:2).
Double fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop with two fertilizer applications. First season: 21 April 2020 @ 1 Kg and 19 May 2020 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg and 26 May 2021 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2).

Table 3: Treatment details for trial 2 (single specie), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, during 2020 and 2021.

Treatment	Cover crop	Treatment description
Control	Fallow / Natural weeds.	Natural weeds.
No fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop.
Single fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop with single fertilizer application. First season: 21 April 2020 @ 1 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg per plot (Commercial organic fertilizer, 5:1:2).
Double fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop with two fertilizer applications. First season: 21 April 2020 @ 1 Kg and 19 May 2020 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg and 26 May 2021 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2).

Table 4: Cover crop treatment details for trial 3, an apple orchard, on Glen Elgin farm, Elgin, during 2020 and 2021.

Treatment	Cover crop	Treatment description
Phacelia	Phacelia (*Phacelia Tanacetifolia)* (0.1 kg/replicate).	Single specie treatment. No additional fertilizer.
Forage rye & Vetch	Forage rye (*Secale cereale*) (0.8 kg/replicate); Vetch (*Vicia villosa*) (0.2 kg/replicate).	Multiple specie treatment consisting of a legume and non-legume plant. No additional fertilizer.
Forage radish & White mustard	Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.14 kg/replicate).	Multiple specie treatment consisting only out of Brassicaceae family. No additional fertilizer.
Forage barley & Peas	Forage Barley (*Hordeum vulgare*) (0.8 kg/replicate); Forage peas (*Pisum sativum*) (1 kg/replicate).	Multiple specie treatment consisting of a legume and non-legume plant. No additional fertilizer.
Forage rye	Forage rye (*Secale cereale*) (1.2 kg/replicate).	Single specie treatment. No additional fertilizer.

Table 5: Soil mineral analyses for trial 1 (multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, for April 2020 and 2021. Values in a column followed by different letters are significantly different (P ≤ 0.05).

Treatment	pH (KCl)	EC (mS/cm)	C (%)	P (mg/kg)	K (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	S (mg/kg)	B (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
	2020														
Control	6.01ns	0.46ns	0.94ns	13.59ns	377.20 ns	0.96ns	4.72ns	1.05ns	0.23ns	12.89ns	1.61ns	78.75ns	13.37ns	11.91ns	10.20ns
No fertiliser (MNFA)	5.82	0.45	0.82	12.04	363.17	0.93	4.05	0.94	0.24	15.57	1.52	76.16	12.72	10.13	8.84
Single fertiliser (MSFA)	5.99	0.49	0.92	13.46	329.38	0.84	4.64	0.88	0.26	19.78	1.53	71.97	10.21	11.54	9.97
Double fertiliser (MDFA)	6.02	0.54	0.93	14.58	403.65	1.03	5.26	1.04	0.23	21.47	1.83	85.63	15.41	12.14	11.19
P - Value	**0.7542**	**0.6306**	**0.5238**	**0.3885**	**0.7153**	**0.7153**	**0.5577**	**0.6166**	**0.0667**	**0.6658**	**0.4885**	**0.1917**	**0.3409**	**0.5994**	**0.3794**
	2021														
Control	5.55ns	0.26ns	0.73ns	13.85ns	299.53ns	0.76ns	4.66ns	0.98ns	0.12ns	8.25ns	1.49ns	28.05ns	16.34ns	8.91ns	6.11ns
No fertiliser (MNFA)	5.32	0.23	0.65	11.33	337.26	0.86	4.15	1.15	0.13	7.81	1.37	30.53	16.11	8.73	6.16
Single fertiliser (MSFA)	5.44	0.27	0.64	12.96	279.02	0.72	4.61	1.03	0.12	14.78	1.33	29.79	11.73	9.32	6.46
Double fertiliser (MDFA)	5.67	0.27	0.67	13.85	352.64	0.90	5.34	1.26	0.17	7.19	1.59	31.31	19.31	9.31	7.19
P - Value	**0.3072**	**0.4802**	**0.4759**	**0.7896**	**0.6052**	**0.6052**	**0.5783**	**0.3580**	**0.0768**	**0.3881**	**0.3360**	**0.7759**	**0.3936**	**0.9399**	**0.5849**

Table 6: Soil mineral analyses for trial 2 (single species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, for April 2020 and 2021. Values in a column followed by different letters are significantly different (P ≤ 0.05).

Treatment	pH (KCl)	EC (mS/cm)	C (%)	P (mg/kg)	K (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	B (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	S (mg/kg)
	2020														
Control	5.92ns	0.37b	0.97ns	15.12ab	312.80ns	0.80ns	4.67b	1.03ns	0.23ns	1.62ns	90.61ns	14.94ns	12.00b	12.97ab	10.44ns
No fertiliser (SNFA)	5.86	0.35b	0.92	14.22b	341.28	0.87	4.43b	1.05	0.21	1.41	89.78	16.07	11.61b	10.72b	8.94
Single fertiliser (SSFA)	6.03	0.37b	0.87	13.97b	337.80	0.86	4.48b	0.89	0.22	1.56	83.29	11.40	10.70b	10.93b	9.88
Double fertiliser (SDFA)	5.98	0.49a	1.04	17.59a	360.16	0.92	5.55a	1.17	0.23	1.83	103.64	18.71	14.35a	14.88a	9.47
P - Value	**0.7372**	**0.0040**	**0.1966**	**0.0469**	**0.7579**	**0.7579**	**0.0456**	**0.4482**	**0.4528**	**0.1634**	**0.2523**	**0.2907**	**0.0224**	**0.0185**	**0.9505**
	2021														
Control	5.30ns	0.22ns	0.76ns	13.79ns	271.15ns	0.69ns	4.80ns	1.27ns	0.14ns	1.59ns	36.19ns	15.04ns	10.66ns	8.66ns	6.17ns
No fertiliser (SNFA)	5.29	0.23	0.74	14.57	331.20	0.85	4.05	1.21	0.13	1.31	35.01	15.15	8.43	8.80	6.71
Single fertiliser (SSFA)	5.23	0.21	0.74	14.38	291.80	0.75	3.98	1.07	0.12	1.31	35.41	10.16	9.22	6.86	7.24
Double fertiliser (SDFA)	5.43	0.26	0.74	15.55	308.16	0.79	4.82	1.21	0.13	1.61	33.39	15.68	10.15	8.69	5.80
P - Value	**0.5309**	**0.3982**	**0.9741**	**0.9361**	**0.7169**	**0.7169**	**0.0939**	**0.6622**	**0.3496**	**0.3055**	**0.8791**	**0.2506**	**0.3643**	**0.4796**	**0.7203**

*Industry norms according to Labserve:

pH = 5.2 – 6.5; C = > 0.75 %; P (Ambic I) = 20 – 80 mg.kg^{-1}; K = > 40 mg.kg^{-1}; S = 15 – 40 mg.kg^{-1}; B = 1 - 4 mg.kg^{-1}; Fe = 10 – 250 mg.kg^{-1}

Mn = 10 – 250 mg.kg^{-1}; Cu = 1 – 10 mg.kg^{-1}; Zn = 2 -10 mg.kg^{-1}.

Table 7: Soil mineral analyses for trial 3, in an apple orchard, on Glen Elgin farm, Elgin, for October 2019 and January 2021. Values in a column followed by different letters are significantly different (P ≤ 0.05).

Treatment	2019							
	pH (KCl)	C (%)	P (mg/kg)	S (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)
Phacelia (PC)	6.44ns	-	209.34ns	27.34ns	2.40ns	14.78ns	3.01ns	0.26ns
Forage rye and Vetch (FRVC)	6.10	-	131.74	16.68	1.64	10.40	2.66	0.21
Forage radish and White mustard (FRWMC)	6.14	-	139.31	22.15	1.56	11.35	2.38	0.25
Forage barley and Peas (FBPC)	6.20	-	160.51	19.94	2.01	11.62	2.57	0.27
Forage rye (FRC)	6.04	-	120.22	12.84	1.53	10.06	2.46	0.21
P - value	**0.2650**	**-**	**0.5064**	**0.1047**	**0.2313**	**0.1402**	**0.4304**	**0.1886**
	2021							
Phacelia (PC)	5.90ns	3.68ns	120.76ns	12.30ns	1.46ns	8.48ns	1.90ns	0.15ns
Forage rye and Vetch (FRVC)	5.60	3.43	74.60	11.14	0.94	7.16	1.72	0.15
Forage radish and White mustard (FRWMC)	5.76	3.59	108.72	11.24	0.97	8.12	1.68	0.15
Forage barley and Peas (FBPC)	5.84	3.49	82.26	10.06	1.09	7.84	1.70	0.15
Forage rye (FRC)	5.64	3.53	91.60	10.90	1.09	7.18	1.52	0.15
P - value	**0.4593**	**0.8887**	**0.5039**	**0.6795**	**0.1134**	**0.2056**	**0.1686**	**0.9658**

*Industry norms according to Bemlab:

pH = 5.5. – 6.0; P (Bray II) = 30 $mg.kg^{-1}$; S = 5 – 10 $mg.kg^{-1}$; Na = < 7 %; K = 3 – 5 %; Ca = 70 -75 %; Mg = 12 -15 %

K sand (45 – 50 $mg.kg^{-1}$), loam (60 – 70 $mg.kg^{-1}$) and clay (80 – 90 $mg.kg^{-1}$)

C = < 0.05 % (low), 0.5 – 1.5 % (medium), 1.5 – 3 % (high) and > 3 % (very high).

Table 8: Cover crop mineral analyses for trial 3, in an apple orchard, on Glen Elgin farm, Elgin, for October 2019 and 2021. Values in a column followed by different letters are significantly different (P ≤ 0.05).

Treatment	2019										
	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	(%)						(mg/kg)				
Phacelia (PC)	3.3b	0.5ns	5.2a	4.6a	0.4a	628.6ab	35.8ns	348.0ns	8.0ns	28.8b	42.8a
Forage rye and Vetch (FRVC)	1.6c	0.3	3.6b	0.6c	0.2b	106.4c	19.6	116.4	7.2	31.0b	4.8b
Forage radish and White mustard (FRWMC)	4.8a	0.5	3.5b	2.7b	0.5a	991.0a	30.0	116.8	7.8	105.4a	38.6a
Forage barley and Peas (FBPC)	3.4b	0.4	2.7c	0.6c	0.2b	572.6ab	20.0	230.8	8.2	43.6b	10.2b
Forage rye (FRC)	3.2b	0.4	2.4c	0.8c	0.2b	337.0bc	23.6	140.2	9.0	36.8b	10.4b
P - value	**0.0011**	**0.0996**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0052**	**0.0619**	**0.0869**	**0.5409**	**< 0.0001**	**< 0.0001**
	2021										
Phacelia (PC)	*	*	*	*	*	*	*	*	*	*	*
Forage rye and Vetch (FRVC)	2.9ns	0.3ns	2.9ns	0.7b	0.2b	671.4b	70.2ns	155.6ns	8.7ns	54.3ns	14.6b
Forage radish and White mustard (FRWMC)	3.3	0.4	3.5	2.5a	0.5a	4214.0a	82.6	205.8	6.5	53.6	34.6a
Forage barley and Peas (FBPC)	2.6	0.3	2.9	0.5b	0.2	420.8b	69.7	131.2	7.4	45.0	8.8b
Forage rye (FRC)	2.6	0.3	2.6	0.5b	0.2	383.6b	64.3	116.2	6.3	39.9	6.5b
P - value	**0.4339**	**0.6277**	**0.1234**	**< 0.0001**	**< 0.0001**	**0.0006**	**0.6981**	**0.1140**	**0.4998**	**0.1739**	**0.0004**

Table 9: Quantification of soil moisture volumetric water content (VWC) and water deficit (DEF) for all four treatments for trial 1 (multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, for April 2020 to October 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)
	April (2020)		May (2020)		June (2020)		July (2020)		August (2020)	
Control	14.35ns	-3.35ns	16.81ns	1.52ns	24.67ns	-21.15ns	25.03ns	-21.91ns	26.04ns	-23.91ns
No Fertiliser (MNFA)	16.69	-5.17	10.71	6.77	45.24	-26.75	25.77	-25.49	27.42	-26.64
Single Fertiliser (MSFA)	14.00	0.34	12.17	3.81	25.83	-23.51	26.06	-23.97	26.96	-25.74
Double fertiliser (MDFA)	15.34	-0.45	12.57	3.00	27.72	-27.25	25.53	-19.55	26.58	-24.14
P - Value	**0.5822**	**0.1396**	**0.6424**	**0.9156**	**0.3159**	**0.3486**	**0.9190**	**0.3879**	**0.8105**	**0.7444**
	September (2020)		October (2020)		November (2020)		December (2020)		January (2021)	
Control	28.25ns	-28.37ns	1.65b	21.16a	5.31b	17.46a	2.14ns	24.10ns	1.07ns	26.12ns
No Fertiliser (MNFA)	29.10	-29.96	1.97a	0.77b	16.50a	- 4.88b	4.15	19.88	2.13	23.89
Single Fertiliser (MSFA)	27.98	-27.90	1.93a	3.41b	17.29a	- 6.37b	4.26	20.01	2.27	23.66
Double fertiliser (MDFA)	29.03	-29.89	1.93a	3.96b	18.15a	- 7.15b	5.09	18.15	2.57	23.01
P-value	**0.8709**	**0.8926**	**0.0142**	**0.018**	**< 0.0001**	**0.0001**	**0.3190**	**0.3450**	**0.3790**	**0.3560**
	February (2021)		March (2021)		April (2021)		May (2021)		June (2021)	
Control	3.13ns	21.89ns	9.18ns	9.80ns	4.32ns	19.54ns	4.91ns	18.37ns	9.17ns	9.83ns
No Fertiliser (MNFA)	3.05	22.02	15.42	-2.64	4.69	17.15	4.02	20.09	12.71	2.76
Single Fertiliser (MSFA)	3.47	21.20	16.33	-4.46	5.39	21.71	3.56	21.00	11.53	5.12
Double fertiliser (MDFA)	3.62	20.40	17.76	-7.38	7.80	12.56	4.72	17.63	10.84	6.40
P - Value	**0.9780**	**0.9370**	**0.1394**	**0.1393**	**0.3791**	**0.4827**	**0.4434**	**0.2618**	**0.6730**	**0.6716**
	July (2021)		August (2021)		September (2021)		October (2021)			
Control	26.40ns	-24.62ns	10.06ns	-9.99ns	8.75ns	10.62ns	5.88b	16.42a		
No Fertiliser (MNFA)	27.69	-27.17	20.76	-13.38	8.56	11.07	12.09a	3.97b		
Single Fertiliser (MSFA)	27.46	-26.75	18.98	-12.12	6.15	15.87	12.94a	2.27b		
Double fertiliser (MDFA)	28.14	-28.11	19.58	-10.96	9.81	8.56	10.64a	6.87b		
P - Value	**0.7059**	**0.7046**	**0.8282**	**0.7936**	**0.3855**	**0.3835**	**0.0006**	**0.0006**		

Table 10: Quantification of soil moisture volumetric water content (VWC) and water deficit (DEF) all four treatments for trial 2 (single species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, for April 2020 to November 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)
	April (2020)		May (2020)		June (2020)		July (2020)		August (2020)	
Control	18.53ns	-7.41ns	13.11ns	1.96ns	26.09ns	-24.03ns	26.16ns	-24,18ns	27.09ns	-26.00ns
No fertiliser (SNFA)	17.28	-6.13	13.67	1.55	26.56	-2.95	26.51	-24,85	28.65	-29.09
Single fertiliser (SSFA)	16.72	-5.23	11.91	4.34	25.92	-23.68	26.75	-24,85	26.76	-25.91
Double fertiliser (SDFA)	16.47	-4.16	13.39	-0.30	26.53	-24.89	24.17	-23,85	28.36	-28.91
P-value	**0.3389**	**0.6826**	**0.9590**	**0.9343**	**0.9803**	**0.9820**	**0.6093**	**0.9835**	**0.6008**	**0.6053**
	September (2020)		October (2020)		November (2020)		December (2020)		January (2021)	
Control	28.54ns	-28.89ns	2.53b	23.10a	8.63b	10.94a	2.00b	24.31a	2.15ns	23.91ns
No fertiliser (SNFA)	29.55	-31.25	14.75a	0.51b	19.26a	-10.35b	6.47ab	15.21ab	2.37	23.40
Single fertiliser (SSFA)	27.38	-25.73	13.07a	2.03b	17.79a	-7.40b	10.08a	8.01b	2.61	22.90
Double fertiliser (SDFA)	28.49	-28.82	15.28a	-2.45b	18.28a	-9.66b	10.96a	15.12ab	2.45	23.25
P-value	**0.7728**	**0.6224**	**0.0041**	**0.0042**	**0.0030**	**0.0020**	**0.0161**	**0.0220**	**0.9810**	**0.9760**
	February (2021)		March (2021)		April (2021)		May (2021)		June (2021)	
Control	3.07ns	21.65ns	13.30ns	1.58ns	5.05ns	18.05ns	5.93ns	16.32ns	8.72b	10.72a
No fertiliser (SNFA)	4.17	19.85	18.27	-8.35	6.90	14.33	6.19	15.77	13.51a	1.15b
Single fertiliser (SSFA)	2.90	22.36	15.36	-2.54	4.82	18.48	5.01	18.13	12.40a	3.34b
Double fertiliser (SDFA)	2.92	22.36	17.20	-6.20	5.67	16.86	6.50	15.14	11.82a	4.54b
P-value	**0.7870**	**0.8010**	**0.4901**	**0.4926**	**0.7713**	**0.7700**	**0.7390**	**0.7359**	**0.0302**	**0.0300**
	July (2021)		August (2021)		September (2021)		October (2021)			
Control	29.73ns	-31.25ns	20.56ns	-13.56ns	10.94ns	6.28ns	4.19c	19.77a		
No fertiliser (SNFA)	31.69	-35.18	21.41	-14.65	10.73	6.72	10.92ab	6.33bc		
Single fertiliser (SSFA)	25.54	-22.92	20.48	-12.76	10.61	6.91	8.91b	10.35b		
Double fertiliser (SDFA)	27.49	-26.83	20.13	-12.10	9.23	9.69	12.31a	3.55c		
P-value	**0.1344**	**0.1367**	**0.8927**	**0.8882**	**0.9002**	**0.9008**	**0.0019**	**0.0019**		

Table 11: Quantification of soil moisture volumetric water content (VWC) and water deficit (DEF) for trial 3 (single species), in an apple orchard, on Glen Elgin farm, Elgin, for March 2020 to November 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)	VWC (%)	DEF (mm)
	March (2020)		April (2020)		May (2020)		June (2020)		July (2020)	
Phacelia	5.77ns	29.54ns	-	-	25.48ns	-9.87ns	29.39ns	-17.71ns	20.88ns	-0.68ns
Forage rye and Vetch	5.51	30.04	-	-	26.35	-11.64	29.91	-18.74	19.36	2.34
Forage radish and White mustard	4.80	31.01	-	-	23.71	-6.36	27.82	-14.55	19.39	2.01
Forage barley and Peas	5.09	30.88	-	-	25.32	-9.58	27.99	-14.77	19.78	1.63
Forage rye	5.73	29.58	-	-	27.08	-13.10	29.24	-16.80	20.00	1.05
P-value	**0.6555**	**0.8052**	**-**	**-**	**0.3041**	**0.3032**	**0.4013**	**0.3329**	**0.7390**	**0.7562**
	August (2020)		September (2020)		October (2020)		November (2020)		December (2020)	
Phacelia	29.84ns	-18.60ns	28.74ab	-16.41bc	10.50ns	19.97ns	22.37ns	-5.95ns	14.11ns	11.06ns
Forage rye and Vetch	29.09	-17.18	25.74bc	-10.37ab	8.99	23.07	19.85	-0.31	12.93	15.21
Forage radish and White mustard	28.13	-15.12	25.10c	-9.13a	8.86	23.23	20.13	0.98	11.00	21.33
Forage barley and Peas	29.38	-17.69	26.44abc	-11.81abc	10.94	19.22	20.87	-0.29	12.11	16.86
Forage rye	27.31	-13.57	29.44a	-17.88c	10.73	19.61	21.86	-3.31	18.48	8.85
P-value	**0.6587**	**0.6452**	**0.0438**	**0.0441**	**0.7421**	**0.7461**	**0.3110**	**0.0690**	**0.1670**	**0.0870**
	January (2021)		February (2021)		March (2021)		April (2021)		May (2021)	
Phacelia	14.85ns	11.35ns	12.48ns	16.11ns	12.63ns	15.95ns	24.84ns	12.36ns	21.64ns	-2.24ns
Forage rye and Vetch	15.60	9.89	14.59	11.86	12.80	14.61	15.08	10.97	21.79	-3.34
Forage radish and White mustard	15.25	10.62	11.41	18.23	13.97	13.14	13.21	14.66	23.12	-5.19
Forage barley and Peas	14.60	11.82	10.91	19.28	11.51	17.72	14.84	11.35	21.41	-1.76
Forage rye	14.11	12.44	12.35	16.40	16.30	8.44	14.15	12.34	23.36	-5.62
P-value	**0.9880**	**0.9930**	**0.0550**	**0.0520**	**0.2740**	**0.3010**	**0.6608**	**0.6462**	**0.6990**	**0.7288**
	June (2021)		July (2021)		August (2021)		September (2021)		October (2021)	
Phacelia	15.31ns	7.62ns	25.10ns	-7.10ns	24.32ns	7.55ns	16.06a	8.87b	13.30ns	14.47ns
Forage rye and Vetch	15.42	10.23	20.63	0.35	23.22	-5.35	12.90b	15.27a	11.68	17.71
Forage radish and White mustard	14.74	11.77	20.69	-0.33	22.88	-4.70	13.13b	14.81a	11.16	18.70
Forage barley and Peas	15.42	10.22	22.65	-4.23	21.77	-2.46	14.49ab	12.08ab	12.98	15.11
Forage rye	16.58	7.94	23.11	-6.33	23.08	-5.09	16.13a	8.79b	14.91	11.23
P-value	**0.8997**	**0.8997**	**0.1462**	**0.0620**	**0.8701**	**0.8723**	**0.0308**	**0.0315**	**0.1329**	**0.1357**

Table 12: Maximum depth of penetration (≤ 3000 kPa) for trial 1 (multi species). Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Maximum depth of penetration (cm)					
	April 2020	**July 2020**	**September 2020**	**April 2021**	**July 2021**	**September 2021**
Control	9.5ns	42.0ns	45.2ns	8.3ns	*	5.7ns
No fertiliser (MNFA)	9.3	46.0	52.3	8.3	*	6.0
Single fertiliser (MSFA)	13.0	70.50	66.0	8.0	*	6.0
Double fertiliser (MDFA)	11.7	68.30	62.5	7.8	*	6.8
P-Value	**0.1213**	**0.0584**	**0.1192**	**0.9532**	*****	**0.6150**

*Equipment failure July 2021

Table 13: Maximum depth of penetration (≤ 3000 kPa) for trial 2 (single species). Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Maximum depth of penetration (cm)					
	April 2020	**July 2020**	**September 2020**	**April 2021**	**July 2021**	**September 2021**
Control	12.7a	42.0ns	46.0ns	7.5ns	*	6.2ns
No fertiliser (SNFA)	12.5a	57.7	50.0	8.0	*	6.8
Single fertiliser (SSFA)	13.5a	47.3	61.3	7.3	*	6.3
Double fertiliser (SDFA)	11.3b	41.5	49.5	9.3	*	6.5
P-Value	**0.0136**	**0.8417**	**0.5049**	**0.1898**	*****	**0.9868**

*Equipment failure July 2021

Table 14: Maximum depth of penetration (≤ 3000 kPa) for trial 3. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Maximum depth of penetration (cm)					
	March 2020	June 2020	September 2020	March 2021	June 2021	September 2021
Phacelia (PC)	9.0ns	29.0ns	14.8ns	7.4ns	12.4ns	9.8ns
Forage rye and Vetch (FRVC)	7.6	29.2	23.6	9.0	11.8	14.8
Forage radish and White mustard (FRWMC)	8.0	32.8	22.4	9.0	10.6	8.2
Forage barley and Peas (FBPC)	8.4	24.6	19.4	7.4	11.6	13.8
Forage rye (FRC)	8.2	29.0	24.8	8.2	19.2	11.8
P-Value	**0.6736**	**0.9307**	**0.8114**	**0.5541**	**0.5085**	**0.1415**

Table 15: Quantification of water holding capacity of soil for trial 1 (multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Water Holding Capacity (%)		
	July 2020	July 2021	September 2021
Control	14.99ns	13.96ns	10.83ns
No fertiliser (MNFA)	12.62	14.30	13.06
Single fertiliser (MSFA)	14.62	14.04	12.06
Double fertiliser (MDFA)	14.04	14.96	12.42
P-Value	**0.9269**	**0.9018**	**0.5622**

Table 16: Quantification of water holding capacity of soil for trial 2 (Single species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Water Holding Capacity (%)		
	July 2020	July 2021	September 2021
Control	19.23ns	12.99ns	12.06ns
No fertiliser (SNFA)	14.99	14.36	13.03
Single fertiliser (SSFA)	10.65	14.66	12.38
Double fertiliser (SDFA)	16.12	14.67	13.64
P-Value	**0.0602**	**0.7790**	**0.6357**

Table 17: Quantification of water holding capacity of soil for all five treatments for trial 3, in an apple orchard, on Glen Elgin farm, Elgin for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Water Holding Capacity (%)		
	July 2020	July 2021	September 2021
Phacelia (PC)	16.59a	17.70ns	16.04ns
Forage rye and Vetch (FRVC)	13.68ab	21.44	16.83
Forage radish and White mustard (FRWMC)	14.49a	18.29	16.85
Forage barley and Peas (FBPC)	9.98b	16.63	16.30
Forage rye (FRC)	14.03a	19.35	17.39
P-Value	**0.0398**	**0.5860**	**0.6284**

Table 18: Microbial activity, expressed as CO_2 as concentration in soil, for all four treatments for trial 1 (multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different ($P \leq 0.05$).

Treatment	2020				2021			
	April		September		April		September	
	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)
Control	0.17ns	1744ns	0.21ns	2156ns	0.08b	748b	0.18ns	18210ns
No fertilizer (MNFA)	0.17	1693	0.18	1878	0.16a	1588a	0.21	21350
Single fertilizer (MSFA)	0.18	1784	0.19	1950	0.13a	1273a	0.22	2211
Double fertilizer (MDFA)	0.18	1827	0.21	2114	0.15a	1525a	0.21	2080
P - value	**0.4108**		**0.8830**		**0.0071**		**0.8023**	

Table 19: Microbial activity, expressed as CO_2 as concentration in soil, for all four treatments for trial 2 (single species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different ($P \leq 0.05$).

Treatment	2020				2021			
	April		September		April		September	
	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)
Control	0.18ns	1819ns	0.16ns	1684ns	0.12ns	1190ns	0.21ns	2174ns
No fertilizer (SNFA)	0.19	1934	0.16	1614	0.18	1834	0.20	2113
Single fertilizer (SSFA)	0.16	1639	0.18	1867	0.19	1854	0.22	2182
Double fertilizer (SDFA)	0.16	1605	0.17	1679	0.18	1808	0.21	2058
P - value	**0.4046**		**0.8999**		**0.0887**		**0.7308**	

Table 20: Microbial activity, expressed as CO_2 as concentration in soil, for all five treatments for trial 3, in an apple orchard, on Glen Elgin farm, Elgin for 2020 and 2021. Values in a column followed by a different letter are significantly different ($P \leq 0.05$).

Treatment	2020				2021			
	March		September		March		September	
	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)
Phacelia (PC)	0.23ns	2366ns	0.17ns	1742ns	0.15b	1549b	0.19ns	1982ns
Forage rye and Vetch (FRVC)	0.20	2042	0.38	3866	0.21a	2048a	0.21	2099
Forage radish and White mustard (FRWMC)	0.19	1973	0.34	3363	0.21a	2109a	0.22	2174
Forage barley and Peas (FBPC)	0.19	1977	0.20	1972	0.23a	2275a	0.20	2024
Forage rye (FRC)	0.14	1468	0.42	4225	0.22a	2177a	0.20	2010
P - value	**0.0809**		**0.5673**		**0.0283**		**0.8369**	

Table 21: Plant performance as expressed in trunk diameter and average shoot length for all four treatments, trial 1 and 2 combined, in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	April 2020		April 2021	
	Trunk diameter (cm)	Ave shoot length (cm)	Trunk diameter (cm)	Ave shoot length (cm)
Control	18.55ns	38.12ns	21.80ns	23.13ns
No fertiliser	18.37	42.37	21.13	21.50
Single fertiliser	17.75	39.63	22.63	24.13
Double fertiliser	17.47	51.63	21.13	31.13
P-Value	**0.9355**	**0.3842**	**0.8563**	**0.2810**

*Ave shoot = average shoot length

Table 22: Quantification of plant performance by evaluating yield and determining yield efficiency for all four treatments, trial 1 and 2 combined, in the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	2021	
	Yield (Kg)	Yield efficiency (kg/cm^2)
Control	13.56ns	0.04ns
No fertiliser	14.13	0.04
Single fertiliser	9.62	0.03
Double fertiliser	10.34	0.03
P-Value	**0.5348**	**0.5489**

Table 23: Plant performance as expressed in trunk diameter and average shoot length for all five treatments, trial 3, in an apple orchard, on Glen Elgin farm, Elgin for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05).

Treatment	March 2020		March 2021	
	Trunk diameter (cm)	Ave shoot length (cm)	Trunk diameter (cm)	Ave shoot length (cm)
Phacelia (PC)	11.20ns	37.70ns	13.74ns	28.60ns
Forage rye and Vetch (FRVC)	10.70	35.10	13.74	23.10
Forage radish and White mustard (FRWMC)	10.96	38.80	13.28	24.90
Forage barley and Peas (FBPC)	10.80	34.10	13.40	23.90
Forage rye (FRC)	10.64	39.30	13.24	24.10
P-Value	**0.8195**	**0.8662**	**0.9741**	**0.3014**

*Ave shoot = Average shoot length

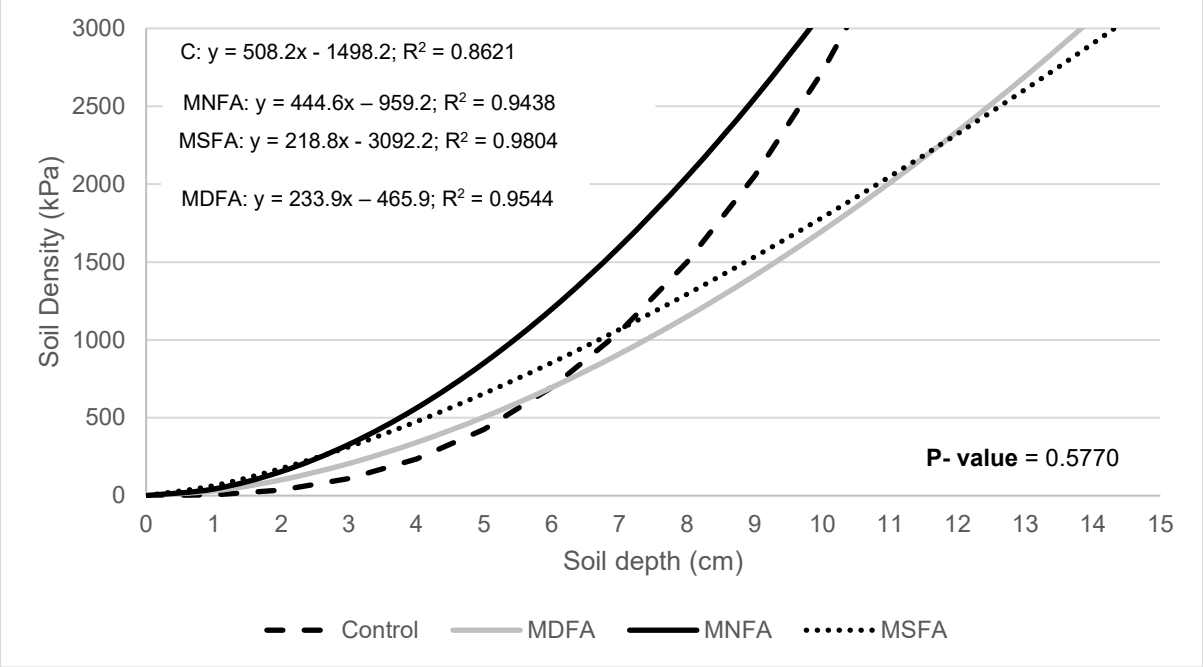

Figure 1. The change is soil density over depth for trial 1 (multi species) in April 2020, Welgevallen Research Farm, Stellenbosch.

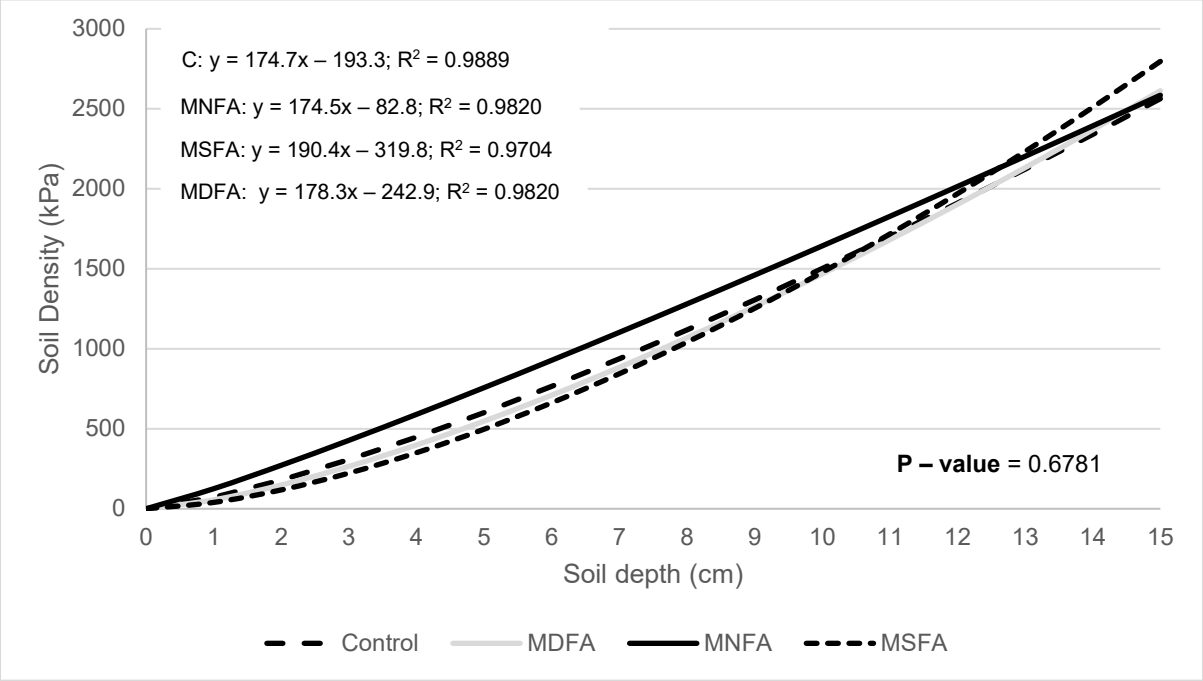

Figure 2. The change is soil density over depth for trial 1 (multi species) in July 2020, Welgevallen Research Farm, Stellenbosch.

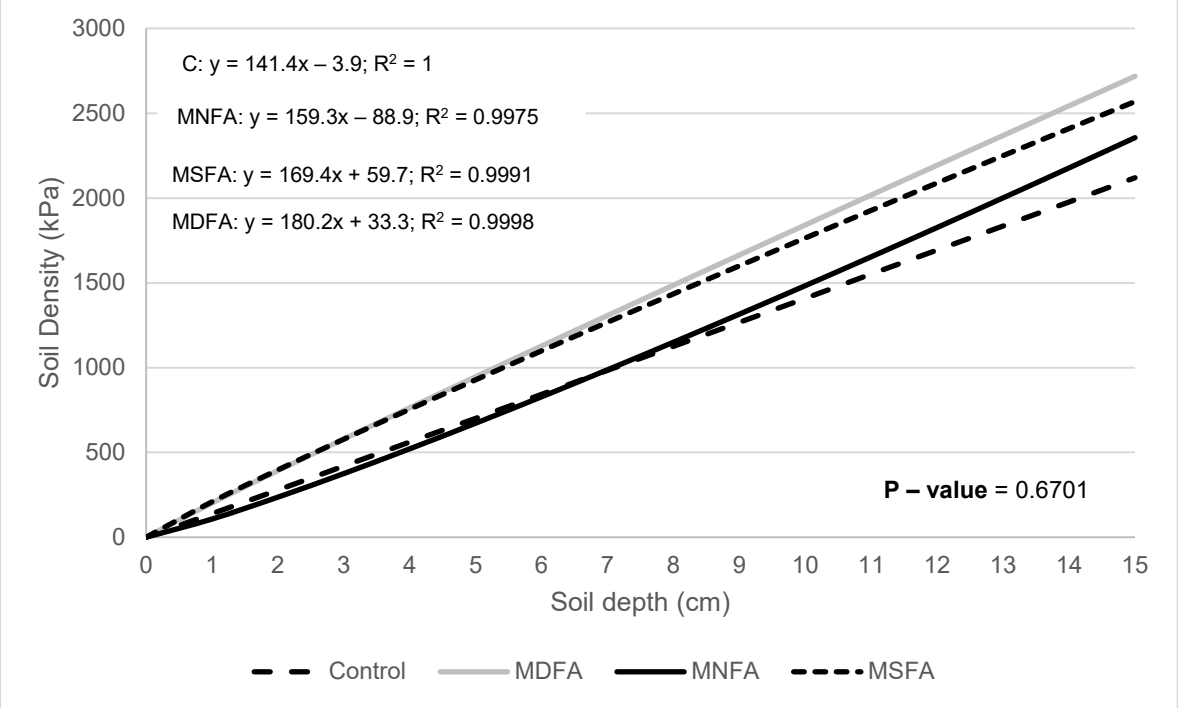

Figure 3. The change is soil density over depth for trial 1 (multi species) in September 2020, Welgevallen Research Farm, Stellenbosch.

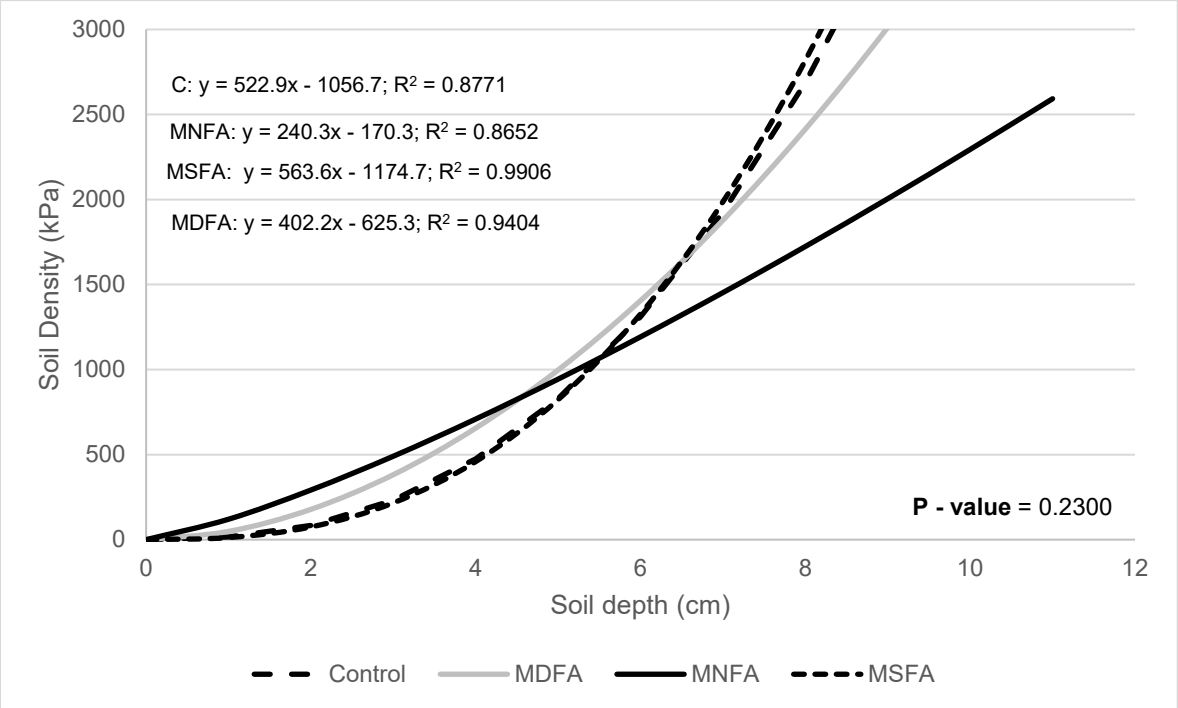

Figure 4. The change is soil density over depth for trial 1 (multi species) in April 2021, Welgevallen Research Farm, Stellenbosch.

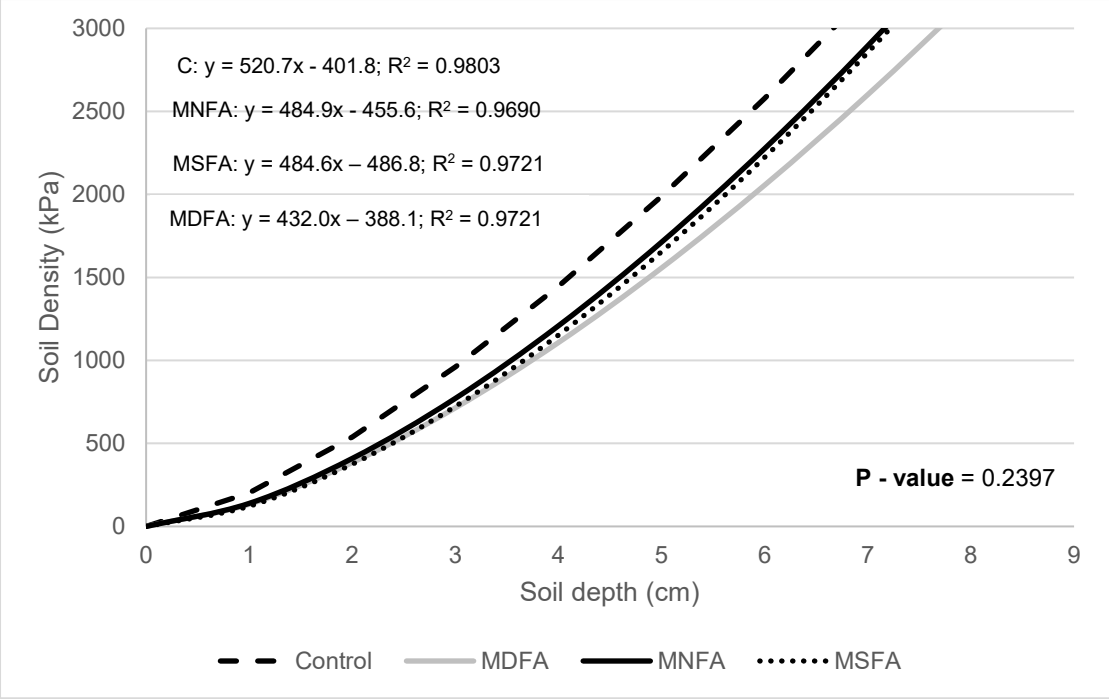

Figure 5. The change is soil density over depth for trial 1 (multi species) in September 2021, Welgevallen Research Farm, Stellenbosch.

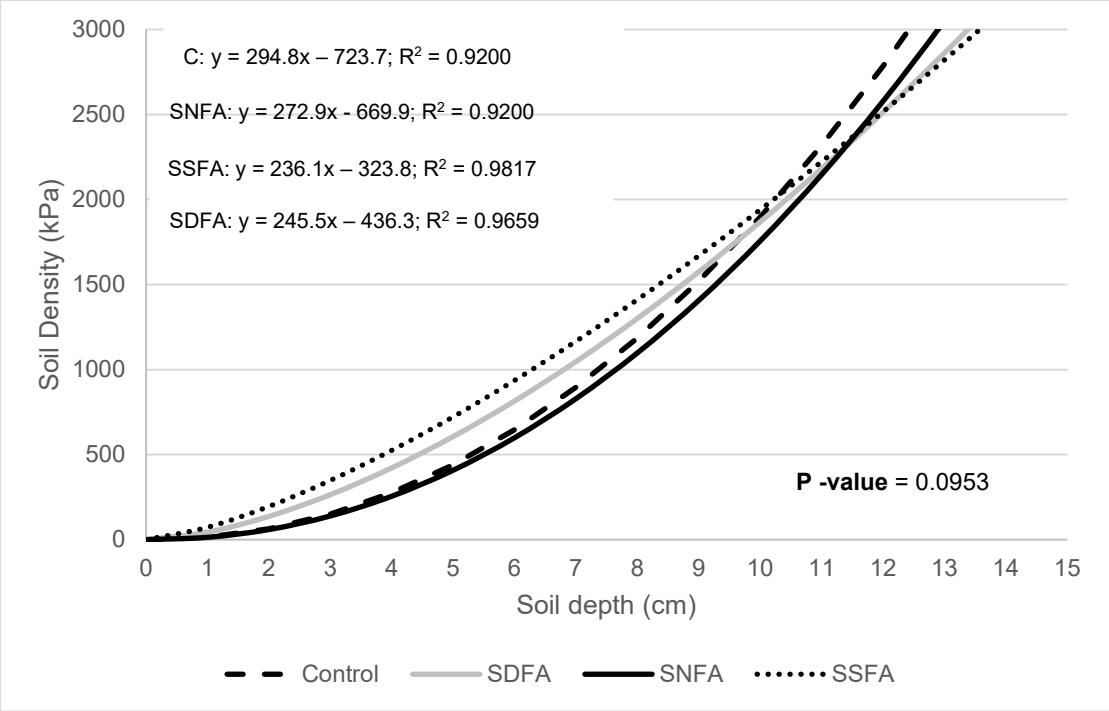

Figure 6. The change is soil density over depth for trial 2 (single species) in April 2020, Welgevallen Research Farm, Stellenbosch.

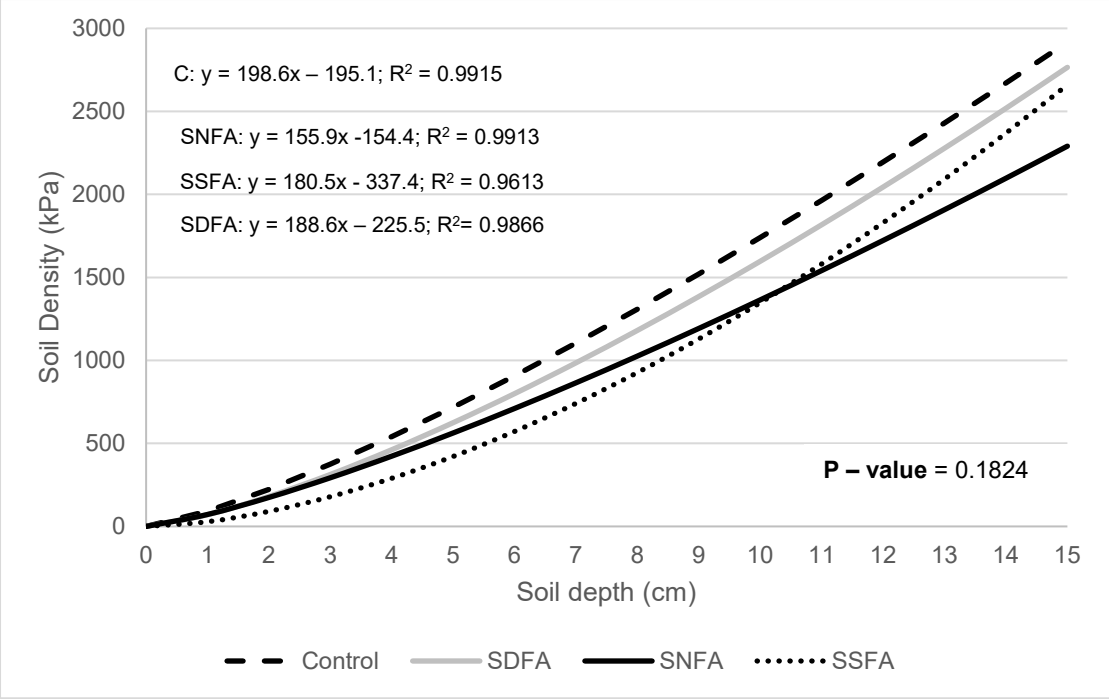

Figure 7. The change is soil density over depth for trial 2 (single species) in July 2020, Welgevallen Research Farm, Stellenbosch.

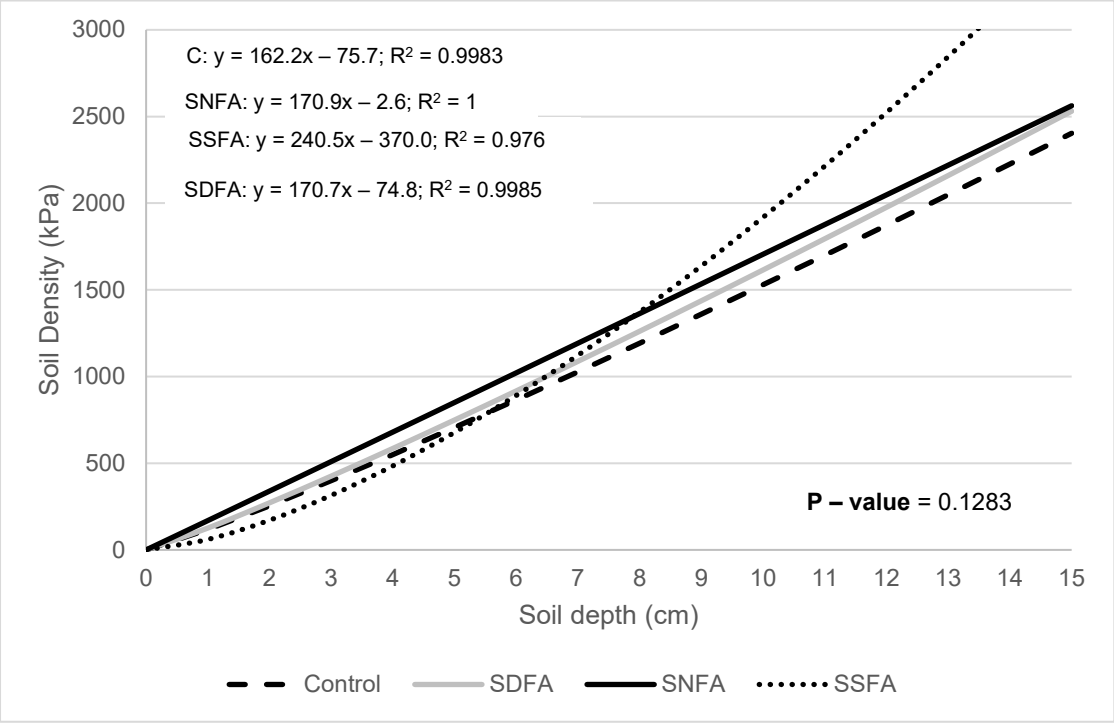

Figure 8. The change is soil density over depth for trial 2 (single species) in September 2020, Welgevallen Research Farm, Stellenbosch.

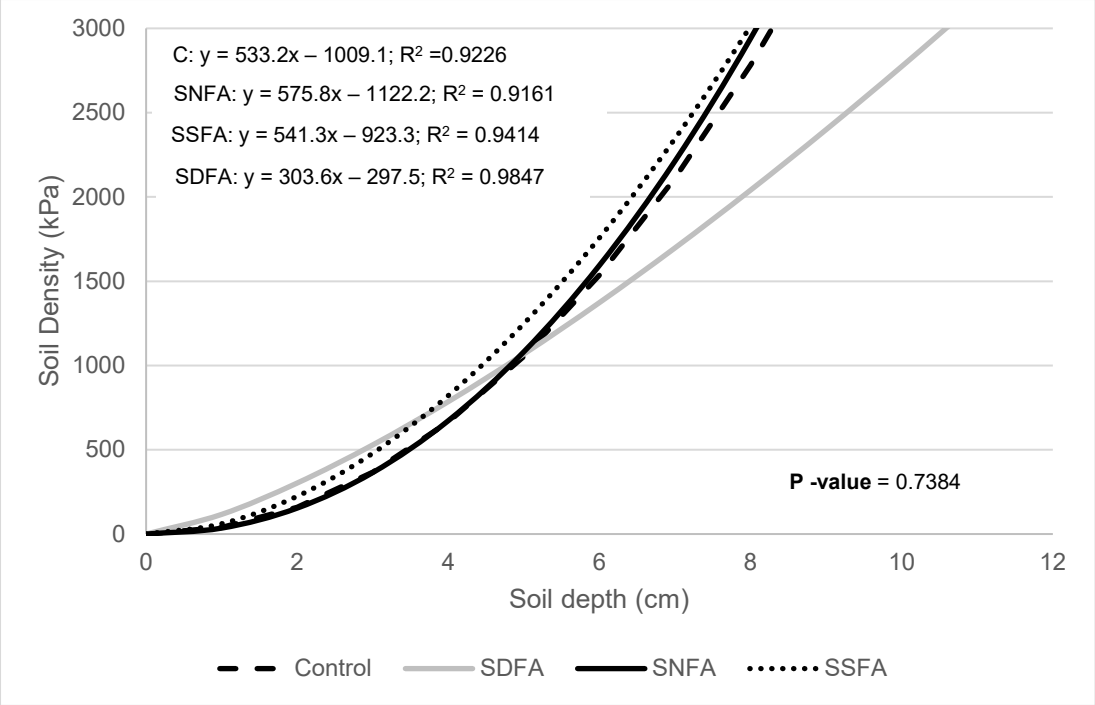

Figure 9. The change is soil density over depth for trial 2 (single species) in April 2021, Welgevallen Research Farm, Stellenbosch.

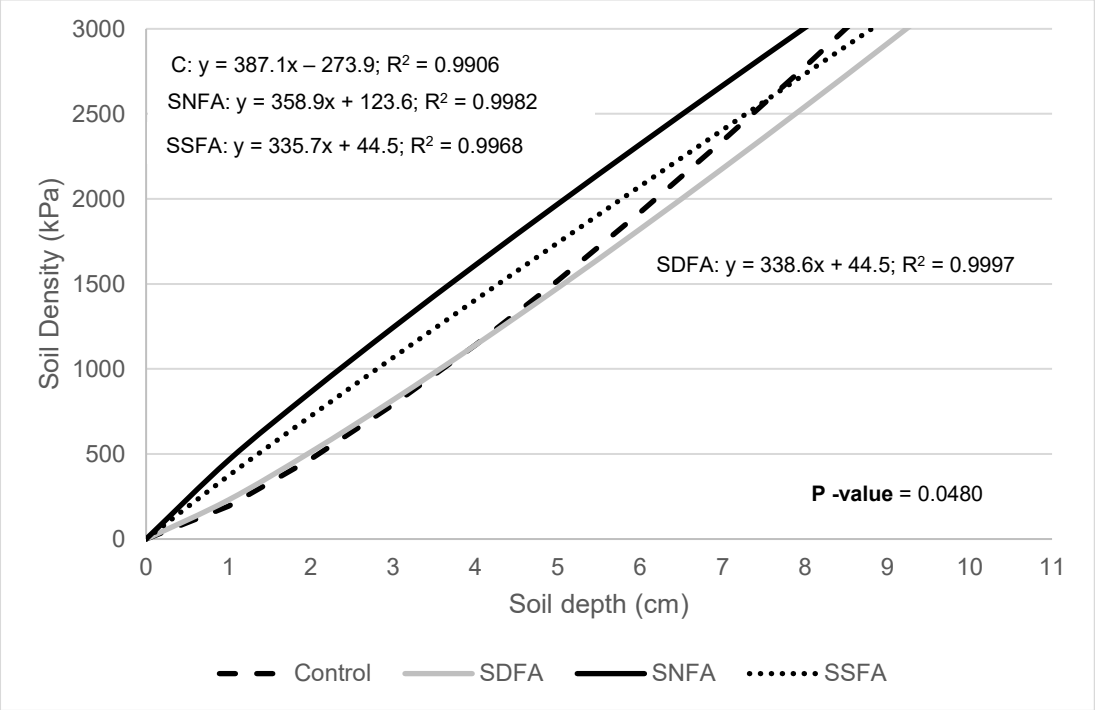

Figure 10. The change is soil density over depth for trial 2 (single species) in September 2021, Welgevallen Research Farm, Stellenbosch.

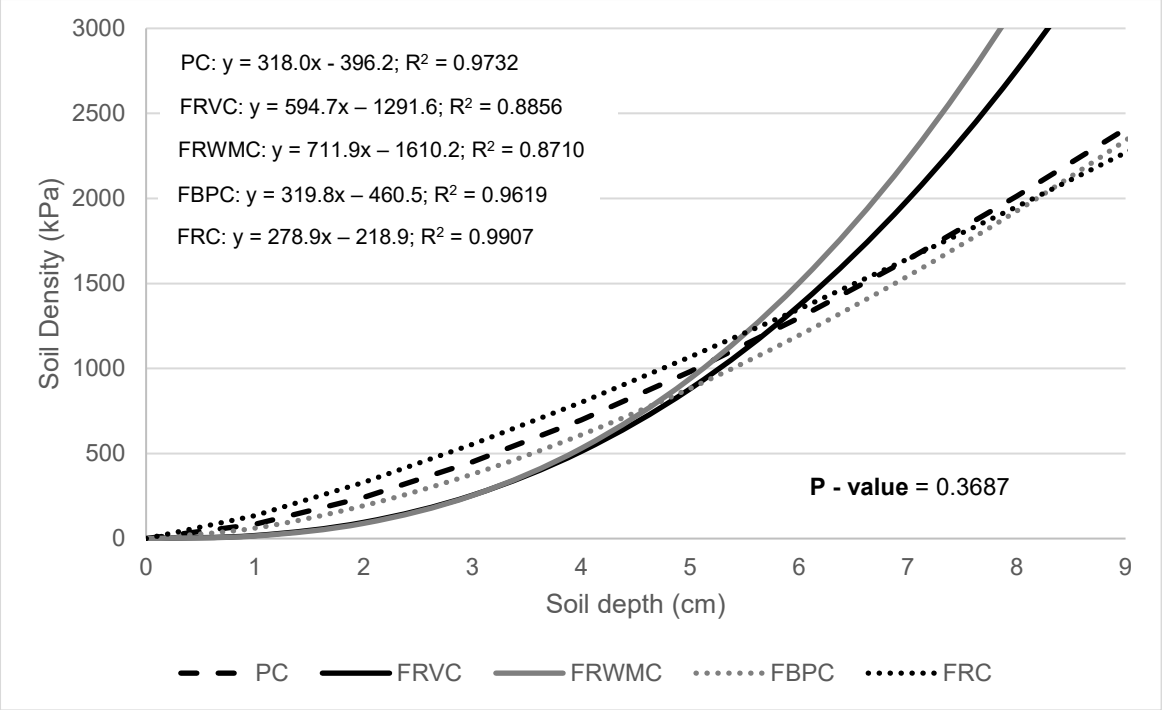

Figure 11. The change is soil density over depth for trial 3 in March 2020, Glen Elgin farm, Elgin.

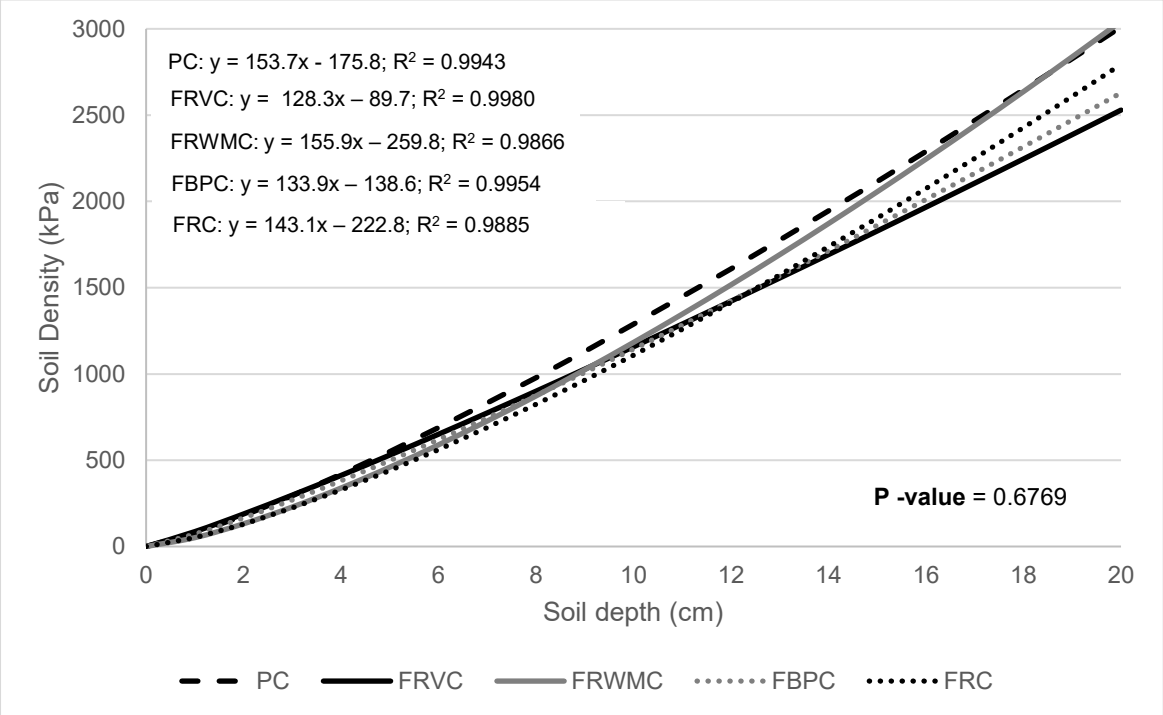

Figure 12. The change is soil density over depth for trial 3 in June 2020, Glen Elgin farm, Elgin.

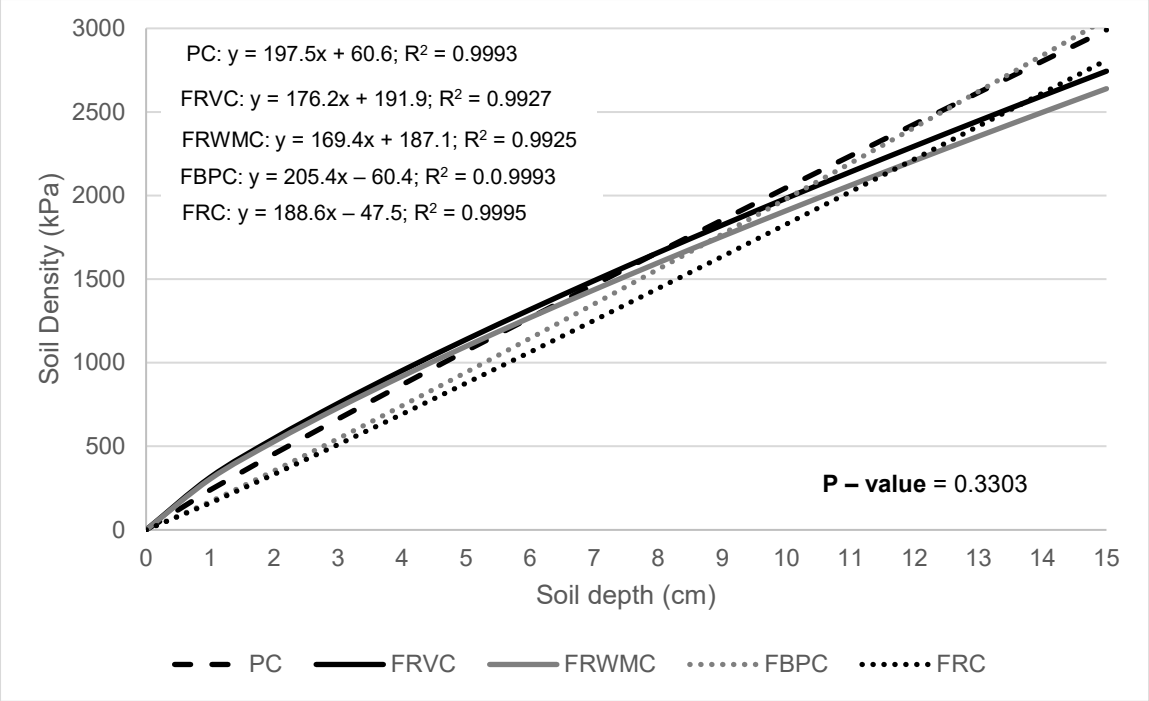

Figure 13. The change is soil density over depth for trial 3 in September 2020, Glen Elgin farm, Elgin.

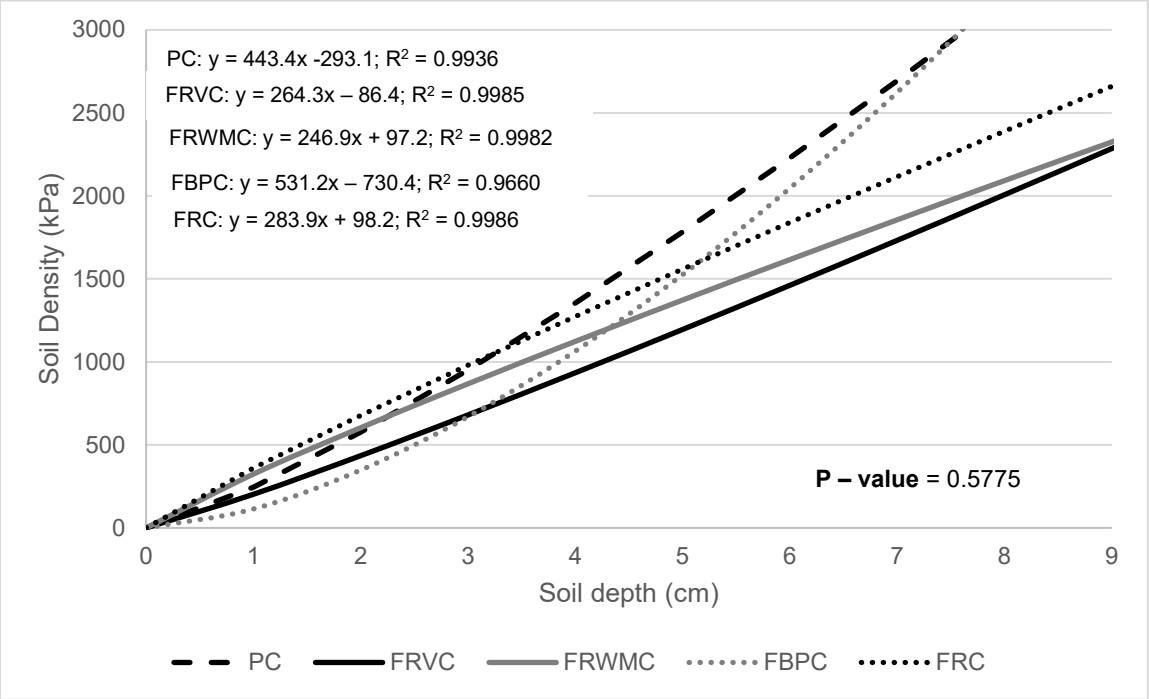

Figure 14. The change is soil density over depth for trial 3 in March 2021, Glen Elgin farm, Elgin.

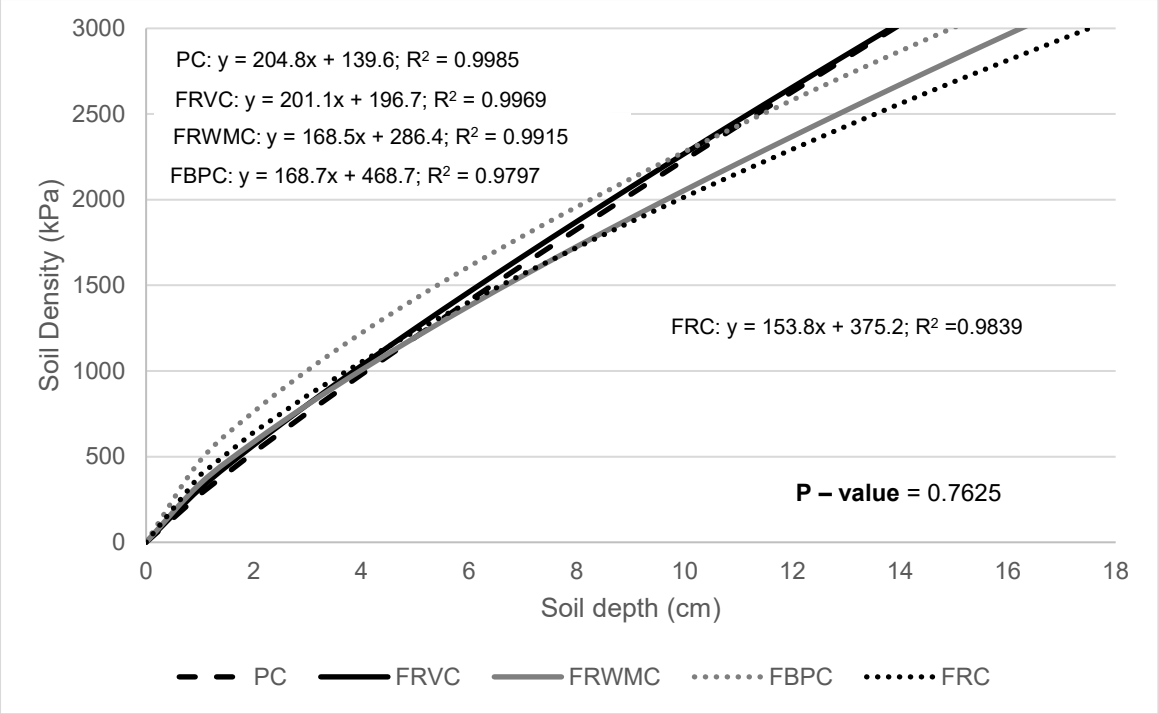

Figure 15. The change is soil density over depth for trial 3 in June 2021, Glen Elgin farm, Elgin.

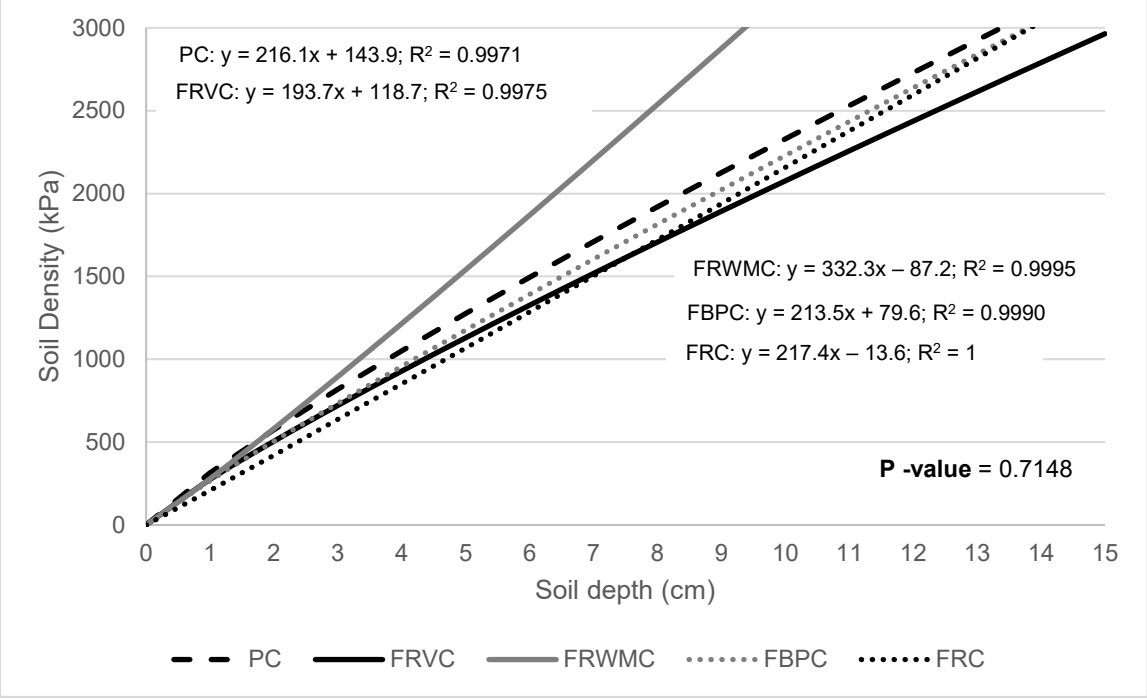

Figure 16. The change is soil density over depth for trial 3 in September 2021, Glen Elgin farm, Elgin.

Paper 2: Comparison of three local analysis techniques to quantify soil microbial diversity and activity for different cover crop combinations in perennial crops.

Abstract

Soil microbial communities play an important role in agriculture. They provide various biological services that are important for the maintenance of soil quality. Cover crops have several benefits, i.e., stimulation and promotion of microbial community activity through provision of a favourable environment and as a food source. There is an increasing need for robust, reliable and resilient biological indicators for the monitoring/quantification of soil quality in commercial orchards. Different cover crop combinations were selected to evaluate three locally available techniques (molecular fingerprinting, CO_2-burst test and gas chromatograph technique) that quantify soil microbial activity. The study was conducted in an apple orchard, in Elgin, Western Cape (34°09'16.83"S 19°02'28.01"E). The trial consisted of five annual winter cover crop treatments: i) Phacelia (PC) ii) Forage rye and vetch (FRVC) iii) Forage radish and white mustard (FRWMC) iv) Forage barley and peas (FBPC) v) Forage rye (RFC). One analysis was carried out in March, before the crop was established in the work row, and one in September, at the end of the lifespan of the crops. All treatments were dependent on natural rainfall and the study was conducted in the second cycle of cover cropping at this site. All three techniques indicated high microbial activity for all treatments, with some additional information in the molecular fingerprint technique. Most treatments indicated an increase (ns) in CO_2 release from March to September, supporting the increased soil biological activity via different treatments, which were quantified through CO_2 production. Molecular fingerprinting also indicated treatment effects on promoting fungal or bacterial communities. The CO_2-burst test indicated an increase in CO_2 production in all treatments, except FBPC. Gas chromatography indicated an increase in CO_2 production, except for PC and FBPC. Molecular fingerprinting technique indicated microbial community shifts from one season to the next, whereas CO_2 techniques primarily indicated whether microbial activity occurred in the soil.

Key words: Molecular fingerprinting, CO_2-burst test, gas chromatography, legumes, microbiology, soil biology

2.1. Introduction

Soil quality (Doran and Parkin, 1994; van Elsas *et al*., 2019a) and soil health (Moebius-Clune *et al*., 2017; Woodyard and Kladivko, 2017) are related to three parameters: the physical, chemical and biological properties of soil. Doran and Park (1994) introduced the concept of soil quality and defined it as "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health." In the past, the main focus was on the chemical and physical aspects of the soil, with less focus on the invisible part of soil biota, neglecting the microbiome (Schloter *et al*., 2018). Microbial communities in soil provide several biological functions that are important for life on earth, as well as for maintenance of soil quality (Bastida *et al*., 2008; Bünemann *et al*., 2018; Díaz-López *et al*., 2019). It is therefore important to develop robust, reliable and resilient biological indicators for the monitoring of soil quality (Schloter *et al*., 2018)

There is a wide spectrum of approaches to assess and quantify biological properties of soils due to vast microbial communities consisting of various microscopic (bacteria, fungi, algae, protozoa, viruses) and macroscopic (earthworms, nematodes, mites and insects) organisms with unique functions. In-field approaches include: earthworm population determination and cotton strip assays, traps for macro- and meso-fauna organisms, as well as quantifying soil aggregation and evaluating soil odour. Laboratory analyses include soil chemical mineral analyses for quantification of the soil organic matter (SOM) or carbon content (%C) and quantification of the nematode and microbial communities (i.e. abundance, diversity and activity) (Hmielowski, 2018).

Nematodes occur naturally in the soil and can be grouped as plant parasitic, free-living and entomopathogenic nematodes, all of which have the potential to indicate ecosystem disturbances (Stone *et al*., 2016; Knox *et al*., 2020; Daramola *et al*., 2021). Stones *et al*. (2016) evaluated a European scale biological program, using the nematode community to serve as a biological indicator for the monitoring of soil quality. They used a traditional morphological assessment identifying feeding group levels, as well as a molecular approach, for rapid nematode community structure assessment. These two methods were designed for rapid screening of a large number of samples and are coarse tools to investigate the structure of nematode communities. They were able to determine that nematode communities differed between

biogeographical zones and between different land uses within biogeographical zones. Locally, a diagnostic method using nematode dynamics as a biological indicator of soil health was developed by a private laboratory Nemlab (Groenfontein Farm, Klapmuts). Soil samples are analysed to determine a nematode diversity index that indicates soil health. Soil samples are collected throughout the year, at a depth of 10 to 50 cm, while there is active root growth and it is important to avoid wet and powdery dry soil conditions. A sample consists of 2 kg soil with 10 g roots. A colonizer persister scale (cp-value) is used and forms the basis for three indices, i.e., structural index (SI), enrichment index (EI) and channel index (CI). Minoshima *et al*. (2007) conducted a study focusing on the effect of conservation tillage on soil food webs and C dynamics and one of the parameters was the determination of the nematode community. They quantified the microbial community by determining the values of SI, EI, CI and basal index (BI).

Microbial communities are sensitive to various factors such as soil temperature, moisture, pH and nutrient supply, which will influence qualification and quantification and need to be considered for interpretation of the results (Fierer, 2017; van Elsas *et al*., 2019a). Microorganisms are classified into four classes according to their temperature preference: Psychrophile (Temperature range -5 to 20 °C, with optimal growth at 15 °C), Mesophile (Temperature range 15 to 45 °C, with optimal growth at 37 °C), Thermophile (Temperature range 40 to 70 °C, with optimal growth at 60 °C) and lastly, Hyperthermophile (Temperature range 65 to 95 °C, with optimal growth at 85 °C) (van Elsas *et al*., 2019a). According to Brady and Weil (2008b), a microbial community is most active between 20 to 40 °C and temperature extremes suppress their activity. Pietikäinen *et al*. (2004) compared the effect of temperature on soil respiration and bacterial and fungal growth rates. Fungal and bacterial growth rates were found to be optimal around 25 to 30 °C, while at higher temperatures, lower values were found, with a more pronounced decrease in growth rate for the fungal community. At temperatures below 3 °C, most microorganisms ceased their metabolic activity (Brady and Weil, 2008b).

Microbial groups have different physiological preferences for soil pH and can survive in a pH range of 3 - 4 units from their optimal pH range (Jin and Kirk, 2018; van Elsas *et al*., 2019a). These groups include: acidophiles (preferring pH < 5), neutrophils (prefer pH between 5 and 9) and alkaliphiles (preferring pH > 9) (Jin and Kirk, 2018; van Elsas *et al*., 2019a). Soil pH influences biotic factors such as the ratio

of fungi to bacteria in soil (Rousk *et al*., 2009) and according to Brady and Weil (2008b), a lower pH allows fungi to become more dominant, whereas bacteria proliferate at a higher pH.

Soil microbial activity is directly correlated with soil respiration and can be quantified by measuring the carbon dioxide (CO_2) levels respired by microbial communities (Krasil'nikov, 1958; Ryan and Law, 2005; Brady and Weil, 2008a; Sciarappa *et al*., 2017; van Elsas *et al*., 2019b). Krasil'nikov (1958) stated that various experiments showed that, as soon as the activity of microorganisms was hindered, the release of CO_2 decreased. Seemingly, this is a reliable indicator of microbial metabolic activity and the process of microbial mineralization is indicative of the level of biological soil function that releases plant nutrients (Brady and Weil, 2008a; Sciarappa *et al*., 2017).

Carbon dioxide levels (soil respiration) vary seasonally and thus, are indicators of the labile fraction of C (Chahal and van Eerd, 2020), which is useful for detecting initial changes in the status of SOM (Salinas-Garcia *et al*., 1997). Seasonal variability in labile fractions of C is primarily dependent on the quantity of cover crop residue produced, rhizodeposition during growth cycle of crop, and soil moisture and temperature, which influences soil microbial activity and residue decomposition (Xiang *et al*., 2008). A better understanding of seasonal dynamics of labile C will assist in improving agricultural management practices.

Techniques such as the Solvita CO_2-Burst test, traditional alkali trap and titration method, quantify CO_2 respiration of the microbial communities in soil (Haney *et al*., 2008). Gas chromatography and substrate-induced respiration (SIR) determine the amount of CO_2 before and after addition of a substrate (Campbell *et al*., 2003). However, sampling for analyses varies from taking the soil sample to preparing the soil sample for analysis and have implications when interpreting results. Standardizing respiration methods will contribute towards meaningful results (Hmielowski, 2018; Franzluebbers and Veum, 2020).

The Solvita CO_2-Burst test quantifies CO_2 respiration of rewetted soils, after it was dried and sieved after collection (McGowen *et al*., 2018; Moore *et al*., 2019). The technique of drying and rewetting soil represents SIR, where the natural release of intracellular compounds occurs due to osmotic shock from microbial cells, resulting in an increased/released availability of previously unavailable organic C and N (Fierer and Schimel, 2002). With this analysis, sample preparation requires a 24 h incubation

period following re-wetting. Thereafter, a colorimetric gel paddle that reacts with the CO_2 burst effect is inserted into the container with soil sample. A specific digital colour reader is used to quantify the CO_2 (Fungenzi, 2015; McGowen *et al*., 2018). One of the advantages of the Solvita CO_2 burst test is that it is easy to use and supplies accurate indication of CO_2 release within a 24 h period, with the option of extending the incubation period (Doran *et al*., 1997; McGowen *et al*., 2018; Tech Memos - Solvita, 2021). However, it is more costly than the alkaline trap method (McGowen *et al*., 2018). The Solvita technique indicates microbial activity, but does not specify the type of bacterial and fungal communities present or the ratio between fungi and bacteria. Solvita CO_2 burst test mineralization rates are scored on a 0-5 scale: low from 0 to 1.9, moderate 2.0 to 3.9, and high to excessive from 4.0 to 5.0 (Solvita® guidelines 2013, Ward Laboratories). Sciarappa *et al*. (2017) assessed soil health in highbush blueberry plants, using the Solvita CO_2-burst test. After a three-year period (2013-2015), an average CO_2 value of 36.7 CO_2 ppm was obtained, averaging 3.08 on the Solvita mineralization scale, with 3.0 representing good fertility and microbial activity. A survey of native forest soil (New Jersey Pine Barrens forest with wild blueberry understory) and conventional blueberry farms was conducted in 2015. Native forest soil had similar biological respiration rates compared to organic orchards, with 41.2 CO_2 ppm and averaging 3.20 on the rating scale, whereas marginal ratings of 6.06 CO_2 ppm, with an average of 1.2 on the rating scale in conventional blueberry farm soils. The SOM of the organic orchard and native forest was higher compared to the conventional orchard.

Gas chromatography (GC) separates and analyses compounds that can be vaporised without decomposition. Relative amounts of a compound in a mixture can then be determined using a standard. One of the disadvantages of this method is the initial cost of establishing a laboratory with the specialised equipment. McGowen *et al*. (2018) refined the method to assess soil microbial respiration through direct GC analysis of headspace CO_2 concentration and compared it with Solvita, as well as assessed the effect of soil drying temperature on CO_2 production. In their method, soil samples were collected from the top 10 cm of soil, sealed in a plastic bag and placed on ice, following sampling and during transportation. Soil samples were stored at 4 ºC until processed. In preparation for analysis, soil samples were sieved and 5 g of soil was added to a 20-ml glass vial and re-wetted with 1.25 ml deionized water, before being sealed with a butyl septa and metal collar. Thereafter, samples were incubated

at room temperature (22 ºC) for 24 h - before analyses with the GC. According to McGowen *et al*. (2018), their approach reduced the cost, as well as increased the rate of analysis for commercial and research laboratories.

Biolog Ecoplates (Biolog Inc., Hayward, CA, USA) analyse and determine the functional diversity of the bacterial community in soil by measuring the ability of bacteria to metabolize carbon substrates (Uphoff *et al*., 2006; Sofo and Ricciuti, 2019). An EcoPlate is a 96-well microplate, comprising 31 C sources and one blank well (control), repeated three times (Campbell *et al*., 2003; Xu *et al*., 2015; Sofo and Ricciuti, 2019). This method involves extraction of microbial community from soil samples into an aqueous suspension and combining the bacterial suspension and C substrates into each microplate (Campbell *et al*., 2003; Sofo and Ricciuti, 2019). The utilization rates of C substrates in the wells are quantified spectrophotometrically, by the colour development of a redox indicator dye (reduction of water-soluble colourless triphenyl tetrazolium chloride to purple triphenyl formazan) (Campbell *et al*., 2003; Sofo and Ricciuti, 2019). Measurement occurs at two optical density (OD) filters, 590 nm to evaluate colour development and turbidity values and 750 nm to measure turbidity values only (Sofo and Ricciuti, 2019). Each bacterial community has a characteristic reaction pattern with different optical density values for different carbon compounds. This technique focuses on metabolic fingerprinting (Campbell *et al*., 2003), because each bacterial community has a characteristic reaction pattern with different optical density values for different C compounds (Zak *et al*., 1994). The metabolic fingerprint and specific pattern of colour development on the microplate are then compared with the Biolog database to identify the microorganisms present (Widmer *et al*., 2001).

Biologs also quantify the change in community structure and functional diversity (Preston-Mafham *et al*., 2002; Feigl *et al*., 2017), and were adapted to study a wide range of changes in the soil environment (Sofo and Ricciuti, 2019). It calculates indices of bacterial functional diversity including the richness index, Shannon's diversity index and Shannon's evenness index (Zak *et al*., 1994; Xu *et al*., 2015). Limitations of Biologs include: i) the collection and storage of samples extracted and dilution of microbial community prior to inoculating the plates (Campbell *et al*., 2003; Uphoff *et al*., 2006; Sofo and Ricciuti, 2019) and ii), the requirement that substrates are soluble, readily metabolized and present at high concentrations to allow determination of community level physiological profiling (CLPP) (Campbell *et al*., 2003). In contrast,

Biolog EcoPlate only determines the bacterial community and discriminates against the fungal community. Moreover, this method is bias towards organisms that are easy to extract, able to grow within a nutrient rich and neutral pH, aqueous environment of the test well (Smalla *et al*., 1998). The rate of colour development is dependent on bacterial inoculum density and, consequently, long lag phases (72 – 168 hours) occur with the Biolog-CLLP technique (Campbell *et al*., 2003). Sofo and Ricciuti (2019) proposed a standardized and accurate step-by-step method for estimating functional diversity of a soil bacterial community by using the Biolog EcoPlates assay.

The Micro-respirometry system (MicroResp) was developed by modifying the BIOLOG technique (Degens and Harris, 1997; Campbell *et al*., 2003). In the approach of Degens and Harris (1997), soils were contained in separate containers, whereas Campbell *et al*. (2003) miniaturized the process of measuring soil respiration and SIR in a microtiter plate (MTP) system, allowing the CO_2 reactions to be carried out with conventional automated plate readers. MicroResp involves a whole soil method, based on SIR, measuring the short-term responsiveness of the microbial community to the addition of a substrate that closely reflects the potential of community metabolic activity (Campbell *et al*., 2003; Uphoff *et al*., 2006). Micro-respirometry method is a unique microplate-based respiration system that allows analysis up to 96 soil samples, can be read with existing plate readers, facilitates measurements over a short period of time (i.e. 4 to 6 h), and does not require the extraction and culturing of organisms (Campbell *et al*., 2003). When preparing the soil sample, it is important to sieve and mix soil thoroughly in order to obtain a representative sample and by adjusting the moisture to ensure a homogenous distribution. This method enables analyses of various combinations of substrates and controls with high levels of replication and sample numbers that can be tested quickly and economically. According to Campbell *et al*. (2003), this method reflects activity rather than growth and sets new possibilities for CLPP to assess microbial community structures and the whole soil method has more advantages compared to the soil extraction method for determining CLPP.

Stable Isotope Probing (SIP) is a molecular method used to detect the activity of microbes in soil. The power of SIP is its ability to link biochemical pathways and metabolic activity of a microbial community with its phylogenetic composition. SIP supplies substrates labelled with the heavier and non-radioactive isotopes of common elements ($^{13}C/^{15}N/^{2}H/^{15}N/^{18}O/^{34/36}S$) to microorganisms. When these microorganisms consume and metabolize these labelled substrates, they actively assimilate and

incorporate these isotopes into a variety of biomarkers such as carbohydrates, proteins, lipids, nucleic acids and metabolites, marking the cells actively consuming the given compound (Redmond and Valentine, 2009; van Elsas *et al*., 2019c). The biological markers, labelled with isotopes, can be partitioned selectively by density gradient centrifugation and processed to identify the microbe with active metabolism from which the biomarker originated. When designing an experiment with the incorporation of SIP, careful consideration should be given to various factors in order to determine the technique most suitable for the experimental goal (van Elsas *et al*., 2019c). Firstly, the type of molecule that will be extracted from the soil, i.e., DNA, RNA, protein and phospholipid fatty acid (PLFAs). Secondly, which stable isotope to use and lastly, the type of incubation suited for the experiment. Incubation techniques vary and should be adapted to the goal of the study. There are currently four incubation techniques: homogenized soil, soil slurries, intact soil cores and in-field incubations.

The microbial community produces extracellular enzymes to obtain nutrients such as C, N and phosphate (P) from complex organic compounds (Jackson *et al*., 2013) by hydrolyzing complex polymers into smaller useable subunits that can be taken into the cell (Merino *et al*., 2016). Microbial enzymes are also involved in various processes such as nutrient cycling, decomposition of organic matter (OM) and mineralization of C, N, P and other essential nutrients (Khare and Yadav, 2017). Measuring the activity of microbial extracellular enzymes in soils can provide insight into the rate of ecosystem level processes such as nutrient mineralization and OM decomposition (Jackson *et al*., 2013). The colorimetric analyses of extracellular enzyme activity in soil and sediment protocol created by Jackson *et al*. (2013), focused on the measurement of activity of beta(ß)-glucosidase, NAGase and phosphatase because these enzymes are tied to C, N and P cycling, respectively. The protocol can be adapted to measure other extracellular enzymes by using different artificial substrates. Quantifying microbial activity with determination of enzymatic status of soil enables the identification of certain enzymes that are specific to a bacterial or fungal community, as well as indicates the productivity of the ecosystem processes. Therefore, it is possible to determine the mineralization potential of microbial communities in soil and link microbial activity to the C or N cycle in orchards. Enzymatic testing is labour intensive because it requires field as well as laboratory actions and is costly to perform.

Fluorescein Diacetate (3',6'-diacetylfluorescein) (FDA) also determines microbial activity (Schnürer and Rosswall, 1982; Green *et al*., 2006). FDA is hydrolyzed by various enzymes such as proteases, lipases and esterases (Schnürer and Rosswall, 1982), producing fluorescein, which can be quantified by spectrometry or fluorometry. The Schürer and Rosswall FDA method is the most frequently used method to determine FDA hydrolysis activity in the soil, but was developed for pure microbial cultures as opposed to soil samples. Green *et al*. (2006) developed a simpler, rapid and precise method to assay FDA, using the Schürer and Rosswall (1982) method. Their optimised method was developed for the quantitative determination of FDA hydrolytic activity and was validated against existing enzymatic activities. This method has various advantages such as using static incubation, requiring less solvent to terminate the hydrolysis and covering a large range of activity.

Microbial abundance and diversity indicate the number of microbial species in a local area or region. Microbial abundance and function can be determined by various techniques: microscopic counts, viable plate counts, microbial biomass, fluorescence in situ hybridization technique (FISH) and *in situ* PCR. FISH detects and quantifies bacterial communities in aquatic, as well as soil environments, based on the hybridization of ribosomal RNA with fluorescent oligonucleotide probes for the detection of single cells in complex samples (van Elsas *et al*., 2019c).

Soil microbial biomass (SMB) is an active component of the terrestrial ecosystem and measures the living component of soil, which regulates important functions and properties related to soil and environmental qualities (Bölter, 1994; Islam and Wright, 2015). SMB is an index of soil fertility and ecosystem productivity (Singh and Gupta, 2018). According to Islam and Wright (2015), there is no standard method for measuring SMB. Direct methods such as microscopic counts and standard plate counts can be used to quantify microbial biomass (Islam and Wright, 2015; Bloem *et al*., 2006). Direct counting quantifies microbial abundance and diversity. Direct counting quantifies the number of bacterial cells in a soil sample through counting bacterial cells on a glass slide, using an epifluorescence microscope after staining samples with DNA-binding fluorochromes such as SYBR green, arcridine orange, or 4',6-diamidino-2-phenylindole (DAPI) (Muthukrishnan *et al*., 2017; van Elsas *et al*., 2019c). Preparation of the sample involves dispersing a known amount of soil in a known volume of water over a known area on a glass, before counting microbial cells, using fluorochromes (Bloem *et al*., 1995). It is an easy, reliable and inexpensive

method requiring minimum equipment for the estimation of bacterial abundance (Kirchman *et al*., 1982). One of the disadvantages is that both living and dead cells are counted in fungi and it is only applicable for fungi spore counting.

Standard plate counts, or viable counts, quantify the number of actively growing cells in a sample. It involves dilution of the soil samples, plating a portion of the dilutions onto an appropriate culture medium, and incubating plates under ideal conditions in order for colonies to form. After incubation, colonies are counted and the original number of viable cells can be calculated and are reported as colony-forming units (CFU). This is easy to perform, but very time consuming and not all bacteria can be cultured (Bloem *et al*., 2006).

SMB is also assessed indirectly, via biochemical, chemical and physiological approaches, for determination of a specific cell constituent such as C, N, P, S and phospholipids of microbes. These methods include the chloroform fumigation extraction technique (CFE), substrate induced respiration (SIR) and phospholipid fatty acid analysis (PLFA) (Witt *et al*., 2000; Setia *et al*., 2012; Willers *et al*., 2015). CFE is the most commonly used to quantify microbial biomass in soils (Witt *et al*., 2000; Setia *et al*., 2012). Soil samples are exposed to chloroform vapour for 24 h or more, inducing lysis of microbial cells (Setia *et al*., 2012), as well as the subsequent release of microbial constituents (Witt *et al*., 2000) such as microbial biomass carbon (MBC) (Vance *et al*., 1987) and microbial biomass nitrogen (MBN) (Brookes *et al*., 1985). After fumigation, fumigated soil samples and non-fumigated soil samples are extracted with potassium sulphate (K_2SO_4) (Setia *et al*., 2012) and the difference between these two serves as a measure of the chloroform labile C, which is then multiplied by a factor to determine the MBC. This technique can result in erroneous values when attempted by inexperienced users and has a number of limitations (Setia *et al*., 2012). With the direct extraction method, soil extraction and chloroform exposure steps are combined (Gregorich *et al*., 1990). Setia *et al*. (2012) found that chloroform labile C concentrations measured by both methods were comparable, but the direct extraction method is quicker and has a lower variability among replicates.

Phospholipid derived fatty acid (PLFA) analysis can be implemented to determine SMB and give insight into the functional status and structure of the microbial community (Willers *et al*., 2015). PLFA is an essential structural component of microbial membranes and can serve as a biomarker. This analysis is a culture independent technique (Willer *et al*., 2015), based on the extraction of phospholipid

fatty acids from microbial cells present in soil sample (Islam and Wright, 2015). After extraction, lipids are separated into neutral lipids, glycolipids and phospholipids. The phospholipids undergo alkaline methano-lysis to produce fatty acid methyl esters (FAME) (Blackwood and Buyer, 2004; Willer *et al*., 2015) which are analysed by gas chromatography (GC), coupled with a mass spectrometer (MS) detector (Guckert *et al*., 1985; Willer *et al*., 2015) or a flame ionization detector (FID) (Bailey *et al*., 2002).

In spite of the numerous available techniques to quantify microbial activity in soils, selection of the appropriate analysis for on-farm quantification of 'soil health' remains a challenge. The request for an unbiased, affordable assessment of soil biological indicators with a consistent outcome that characterizes changes in soil 'health' is required for an agricultural sector that is in transition. The aim of this study was to compare results from three local 'soil health' analyses techniques that quantify microbial activity in soil. The trial site comprised cover crop treatments in a commercial deciduous fruit orchard. In this paper, we focused on molecular fingerprinting analysis (Sporatec.co.za, Stellenbosch, South Africa) and a Solvita CO_2 Burst test (Biocult (PTY) LTD., Somerset West, South Africa) conducted by commercial laboratories and gas chromatography, conducted at the department of Horticultural Science, Stellenbosch University.

2.2. Materials and Methods

Different cover crop combinations were selected to evaluate three locally available techniques that quantify soil microbial activity.

2.2.1. Trial Site Description

The trial was conducted in an apple orchard at Glen Elgin farm (34°09'16.83"S 19°02'28.01"E), located in Elgin, Western Cape. The 'Cripps Red' (*Malus domestica*) apple orchard was established in 2019, on MM109 rootstock, with 'Mahana Red' (*Malus domestica*) as cross pollinator. Planting distance was 1.25 m x 3.5 m. Trees were irrigated using micro-jet irrigation. The layout of the trial was a randomised complete block design (RCBD). Five treatments were replicated five times (Table 1). Each plot was approximately 20 m^2 and was situated in the middle of the work row. The cover crop received no irrigation and was dependent on winter rainfall. Preeglone

was applied to control kikuyu and other summer weeds, before planting cover crops. Cover crops were hand sowed, after the first winter rain (March 2019 and 2020) and the seed was mixed with soil (2 kg per treatment combination; loamy sand: 70-80% sand and 10-15% clay) to ensure homogeneity during sowing. Sampling for technique comparisons was conducted only in 2020, representing two years of cover cropping.

2.2.2. Microbial Activity/Biomass

Soil samples were collected per replicate, using a composite sample of three subsamples per plot. Soil samples were collected (18 March 2020; 8 September 2020) from the top 10 cm (Solvita CO_2 Burst test method) or 30 cm (Molecular fingerprinting analysis and Gas chromatography method) in the work row, using an auger. Soil samples were stored in a plastic bag at room temperature, away from direct sunlight, until analysis. Subsamples were used to perform the three analyses as follows:

2.2.2.1. Molecular fingerprinting analysis

Soil microbial community analyses were conducted by a commercial laboratory, Sporatec (Sporatec.co.za, Stellenbosch University). This technique uses molecular fingerprinting to generate a profile for a soil sample and indicates the diversity and monitor changes in the soil microbial community. A sample of 0.35 g soil was used for DNA extraction, using the Zymo research Fecal/Soil Microbe DNA MicroPrep™ kit (Zymo research, United States of America). Polymerase chain reactions (PCR) were performed on the extracted DNA for both bacterial and fungal communities. All samples were analysed in triplicate and pooled after it was separated on a 1% agarose gel stained with ethidium bromide and visualised under ultraviolet light. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was used to determine the bacterial and fungal community, but at this stage, information of the specific primers and target sites are still confidential. Pooled samples were run on an ABI 3010xl Genetic Analyser to obtain an electropherogram of different fragment lengths and fluorescent intensities. Fluorescence intensities were converted to electropherograms using the Genemapper 5 software. The peaks on the electropherogram represent different fragments of different sizes, termed operational taxonomic units (OTU), and the heights of the peaks indicate relative abundance of the fragments (Brink, 2018; Brink *et al*., 2019). A Shannon Index for bacterial and fungal community was quantified in this analysis.

2.2.2.2. CO_2 burst test

The microbial activity of soil was quantified via CO_2 levels in the soil, using the Solvita CO_2 burst test, conducted by a commercial laboratory (Biocult (PTY) LTD., Somerset West, South Africa). Soil samples dried in an oven for 24 h at 40 °C. After reaching a certain moisture (less than 3 %), the standard Solvita protocol was followed to determine the CO_2 levels of individual samples. The Solvita CO_2 burst test quantifies the CO_2 respiration from dried, sieved soil once rewetted. A gel paddle was placed in a beaker with the soil sample and sealed for 24 h. The gel paddle changes colour in response to the amount of CO_2 released that are quantified with a digital colour reader (DCR) (Haney *et al*., 2008). The results were compared to the standard benchmark of ppm of CO_2 readings ($mg.L^{-1}$) indicating lowest level of microbial activity. Solvita provides an additional mineralization scale, which is indicative of the level of biological function.

2.2.2.3. Gas chromatography technique

A carbon dioxide (CO_2) analysis was performed with the gas chromatography technique (SOP, Department of Horticulture Science, Stellenbosch University). The protocol used for analyses was the Agilent 6890N Gas Chromatograph (GC) fitted with a TCD (Thermal Conductivity Detector) detector and helium as carrier gas to detect CO_2 and O_2 levels in soil samples. Ten grams of soil was added to a 20 ml sealed test tube and allowed to respire for 30 min. After 30 min., a syringe was used to extract gas (CO_2, O_2 and Ethylene) from a sealed test tube. The gas was injected into GC, measuring the CO_2, O_2 and ethylene levels. Analyses were performed on 20 March 2020 and again 22 September 2020.

Statistical Analyses

Trials were performed using a randomised complete block design (RCBD) comprising five treatments that were repeated five times. The general linear models (GLM) procedure of Enterprise Guide 7.1 (SAS Institute Incorporation, Cary, North Carolina, United States of America) was used to perform a one-way Analysis of Variance (ANOVA). Means were separated at a 5 % significance level using Fischer's LSD test.

2.3. Results

2.3.1. Microbial Activity/Biomass

2.3.1.1. Molecular fingerprinting analyses

There were no significant differences between treatments for either the bacterial or fungal Shannon Index (SI), irrespective of sampling date (Table 2 and 3).

2.3.1.2. CO_2 burst test

There was no significant difference in the C released as CO_2 between the treatments, for either of the sampling dates (Table 4).

2.3.1.3. Gas chromatography technique

There was no significant difference in CO_2 levels between the treatments for either of the sampling dates (Table 5).

2.4. Discussion

2.4.1. Microbial Activity/Biomass

2.4.1.1. Molecular fingerprinting analyses

There were no significant differences between treatments for both the fungal or bacterial SI, during the season, but the fungal SI ranged from 1.8 to 2.4 and the bacterial SI ranged from 3.2 to 3.6, indicating high microbial community according to Sporatec (2021). These SI values typically lie between 1.5 and 3.5, but can exceed 4 on rare occasions (Sporatec, 2021). No specific indicators for soil quality under local conditions have been developed yet (personal communication, C Brink, Sporatec). SI values were lower than reported by Wei *et al*. (2021) (fungal 3.81 – 4.95 and bacterial 5.47 – 6.64) and Ma *et al*. (2021) (fungal 3.10 – 3.25 and bacterial 4.48 – 4.75), but higher compared to Yang *et al*. (2019) (bacterial 2.5 – 3.1). This may partly be due to the different cover crop species, soil and environmental conditions. This technique was successful in quantifying the increase in microbial population, however it was not successful to differentiate on microscale between the potential differences between cover crop treatments at this moment.

2.4.1.2. CO_2 burst test

According to Sciarappa *et al*. (2017), high respiration levels of the Solvita CO_2 burst test correlate positively with high microbial activities, and ample supply of OM, which is readily available and favourable environmental conditions in the root zone. Our results, expressed as the CO_2-burst test /Solvita interpretation according to mineralization scale, varied as follows: i) PC 76.64 ppm/4.03 (March) and 101.32 ppm/4.44 (September), ii) FRVC 79.90 ppm/4.15 (March) and 102.16 ppm/4.45 (September), iii) FRWMC 73.70 ppm/4.06 (March) and 96.32 ppm/4.39 (September), iv) FBPC 87.16 ppm/4.39 (March) and 87.02 ppm/4.26 (September) and v), FRC 76.56 ppm/4.12 (March) and 101.16 ppm/4.42 (September). Although there were no significant differences between treatments for the CO_2 – burst test, results indicated high soil microbial activity on both sampling dates and corresponded with the findings of Sciarappa *et al*. (2017), with 36.7 CO_2 pp/3.08, with 3.0 representing a good fertility and microbial activity. However, they did find a significant difference between the organic and conventional orchards, with the conventional farm showing marginal values of 6.08 ppm/1.2.

Although there were no significant differences between treatments, most results, except the FBPC treatment, indicated an increase (ns) in CO_2 release from March to September, which supports the fact that cover crops increased soil biological activity, which can be quantified through CO_2 production. The increase in CO2 release from March (no cover crop) to September (full cover crop) supports the fact that cover crops increased soil biological activity after planting. Therefore, environmental conditions during the season, increased soil moisture and a possible higher SOC in soil aggregates in September should be considered as additional factors influencing CO_2 production and be investigated further. Although Solvita was not repeated the following season, SOC (3.43 – 3.68 %) was quantified in paper 1.

2.4.1.3. Gas chromatography technique

There were no significant differences between CO_2 levels for treatments for either of the sampling dates. CO_2 values (± 1460 – 4225 ppm) were comparable with Koerner *et al*. (2011) (579 – 11 326 ppm) who reported that 80 % of the samples ranged between 1000 – 2000 ppm. In contrast, the CO_2 values were much higher than those of McGowen *et al*. (2018). They reported CO_2 values from 7 to 36 ppm, with GC results

similar to Solvita CO_2 burst test results. However, procedures of soil sampling and analyses, as well as respiration times that differed between the studies, could partially explain the variation in actual CO_2 levels. Other studies (Demir and Isik, 2020; Demir *et al*., 2019) also reported CO_2 values ranging from 53 to 415 ppm, but again different techniques were used to quantify CO_2 levels. Moreover, we observed a tendency towards increasing CO_2 levels for FRVC (± 0.19 %), FRWMC (± 0.14 %), FBPC (± 0.01 %) and FRC (± 0.27 %), in contrast with a decreasing trend observed for PC (± 0.06 %) from March to September (ns). Trends was similar as reported by the Solvita CO_2 burst test, except for PC, therefore furter quantification is required.

2.5. Conclusion

In this paper, three commercial and locally available methods were evaluated for the quantification of soil microbial diversity and activity in newly established perennial orchards. Different cover crop combinations were selected to conduct these analyses**.** Initial measurements in March represents no cover crop and September represents one season after cover crops were grown. Although the techniques were not able to distinguish between the treatments, they were able to indicate a change in microbial activity. All three techniques give an indication of microbial activity and could indicate an increase in microbial activity from establishment in March to September (growing season of cover crops) but none could distinguish between treatments.

Both the Solvita CO_2-burst technique and the GC showed the same trend and recorded higher CO_2 levels in September, except for PC (Addendum B). However, the actual values varied between the techniques as well as reported by others, indicating the effect of the actual technique (sampling and analyses procedures), environmental conditions and crop selection. Therefore, the same technique should be selected to follow changes over time and not necessarily only the actual value and values should only be interpreted on the specific site, for optimum performance of these techniques. CO_2 was also confirmed to accurately describe the activity of the microbial communities in the soil over time, for various cover crop combinations after establishment of cover crops but could not distinguis between treatments, at this site.

A higher CO_2 level indicates a bigger C reservoir in the soil and is indirectly linked to nutrient mineralization. The Solvita CO_2-burst test can indicate the potential release of N and P that could be used in practice to reduce the application of these

elements in the orchard (This was not utilized in the trial, additional tests provided by Solvita). However, this would only be accurate for the topsoil profile, as the Soltiva test is only performed on the 0 – 10 cm soil profile, whereas the GC-method quantifies the first 30 cm of soil profile, allbeit without information on nutrient mineralization at present.

The molecular fingerprinting technique can provide additional information on the fungal and bacterial community of soil in terms of diversity and specie richness (results not discussed in paper because it focused on methodology), as well as the impact of specific treatments on soil microbial communities and whether a particular microbial community persists alongside the specific crop. This standardized technique (molecular fingerprinting) provides a holistic view of the microbial community in the soil, as well as the dynamics between seasons, which is ideal if the change in management practices needs to be quantified. This would give more insight into the soil microbiome of an orchard and whether microbiome is healthy and suited for a specific crop, but the practicality of this amount of information on producer level is questionable on large scale at present.

All three techniques will enable the producer to get an indication of the activity of the microbial communities, an indication of how well established these communities are and whether there was synergism between crops and groups of microorganisms which can be exploited/harmonised for better ecological management in future. However, a depth of knowledge regarding the microorganism diversity and interactions will only be available from the more intense molecular fingerprinting technique.

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Table 1: Cover crop treatment details for the apple orchard, on Glen Elgin farm, Elgin from 2019 to 2020.

Treatment	Cover crop	Treatment description
Phacelia	Phacelia (*Phacelia Tanacetifolia)* (0.1 kg/replicate).	Single species. No additional fertilizer.
Forage rye & Vetch	Forage rye (*Secale cereale*) (0.8 kg/replicate); Vetch (*Vicia villosa*) (0.2 kg/replicate).	Multiple species consisting of a legume and non-legume plant. No additional fertilizer.
Forage radish & White mustard	Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.14 kg/replicate).	Multiple species consisting of the *Brassicaceae* family. No additional fertilizer.
Forage barley & Peas	Forage Barley (*Hordeum vulgare*) (0.8 kg/replicate); Forage peas (*Pisum sativum*) (1 kg/replicate).	Multiple species consisting of a legume and non-legume plant. No additional fertilizer.
Forage rye	Forage rye (*Secale cereale*) (1.2 kg/replicate).	Single species. No additional fertilizer.

Table 2: Quantification and identification of soil fungal communities through molecular fingerprinting analysis (Shannon Index), during 2020, trial 3, at Glen Elgin farm. Values followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Shannon Index	
	March	September
Phacelia (PC)	2.13ns	1.89ns
Forage rye and Vetch (FRVC)	2.17	2.15
Forage radish and White mustard (FRWMC)	2.04	2.37
Forage barley and Peas (FBPC)	2.38	2.30
Forage rye (FRC)	2.57	2.36
P – Value	**0.5431**	**0.3581**

Table 3: Quantification and identification of soil bacterial communities through molecular fingerprinting analysis (Shannon Index), during 2020 season for trial three, at Glen Elgin farm. Values followed by a different letter are significantly different (P ≤ 0.05).

Treatment	Shannon Index	
	March	September
Phacelia (PC)	3.25ns	3.59ns
Forage rye and Vetch (FRVC)	3.41	3.45
Forage radish and White mustard (FRWMC)	3.46	3.50
Forage barley and Peas (FBPC)	3.37	3.36
Forage rye (FRC)	3.57	3.39
P – Value	**0.4673**	**0.3345**

Table 4: Results from the Solvita CO_2-Burst test, for Glen Elgin during 2020, indicating carbon release through microbial respiration. Values followed by a different letter are significantly different ($P \leq 0.05$).

Treatment	March		September	
	CO_2 (ppm)	Benchmark (ppm)	CO_2 (ppm)	Benchmark (ppm)
Phacelia (PC)	74.64ns	59.20ns	101.32ns	34.10ns
Forage rye and Vetch (FRVC)	79.90	64.01	102.16	36.61
Forage radish and White mustard (FRWMC)	73.70	58.70	96.32	32.32
Forage barley and Peas (FBPC)	87.16	57.70	87.02	35.61
Forage rye (FRC)	76.56	57.70	101.16	33.83
P-Value	**0.8689**	**0.4026**	**0.6579**	**0.6671**

Table 5: Quantification of carbon dioxide (CO_2) levels of soil determined by GC-analyses, during the 2020 season for Glen Elgin farm, as carbon released as CO_2 through microbial respiration. Values followed by a different letter are significantly different ($P \leq 0.05$).

Treatment	CO_2 levels			
	March		September	
	CO_2 (%)	CO_2 (ppm)	CO_2 (%)	CO_2 (ppm)
Phacelia (PC)	0.23ns	2366ns	0.17ns	1742ns
Forage rye and Vetch (FRVC)	0.20	2042	0.38	3866
Forage radish and White mustard (FRWMC)	0.19	1973	0.34	3363
Forage barley and Peas (FBPC)	0.19	1977	0.20	1972
Forage rye (FRC)	0.14	1468	0.42	4225
P – Value	**0.0809**		**0.5673**	

Paper 3: Quantifying the contribution of specific cover crop combinations towards biomass production for mulching in a perennial orchard.

Abstract

Biomass production of cover crops varies in quantity and quality among and within species and are also influenced by the type of soil, soil moisture, temperature, growing season (winter or summer) and management practices. A two-year field study (Trial 1 and 2) was conducted in Stellenbosch (33°56'51.27"S 18°52'19.29"E) and a three-year field study (Trial 3) in Elgin, (34°09'16.83" S, 19°02'28.01" E), Western Cape. The contribution of specific cover crop combinations towards biomass production for mulching and weed suppression was quantified with reference to the effect of climate, species selection and fertiliser application. Trial 1 (multi species: forage rye, radish and white mustard) and trial 2 (single species: forage rye) comprised four treatments each: i) Control (natural vegetation), ii) Cover crop with no fertilizer application (MNFA; SNFA), iii) Cover crop with a single fertilizer application (MSFA; SSFA) and iv), Cover crop with a double fertilizer application (MDFA; SDFA). Trial 3 comprised five annual winter cover crop treatments: i) Phacelia (PC) ii) Forage rye & vetch (FRVC) iii) Forage radish & white mustard (FRWMC) iv) Forage barley & peas (FBPC) and v), Forage rye (FRC). Cover crop density, biomass and weed suppression were determined at the end of the growing season. In trials 1 and 2, the control produced a significantly lower biomass (1 m^2) compared to the other treatments, and MSFA and SSFA produced the highest biomass. The highest biomass production was obtained in 2021 followed by 2019 and 2020 and was primarily due to the rainfall pattern. In trial 3, PC produced the lowest biomass, across all three seasons, indicating a poor adaptability of Phacelia at this site. Forage rye (single and multispecies) performed the best in terms of biomass production and consistency. Weed suppression efficiency was affected by cover crop biomass (both sites), species (Elgin), fertilisation (Elgin) and rainfall (both sites).

Keywords: Brassica, broadleaf crops, grasses, legumes, grasses, weed suppression

3.1. Introduction

Mulching the tree row, as well as the work row, has gained favour in the deciduous fruit industry of the Western Cape in recent years. The severe drought during 2016 to 2019 and the drive towards more sustainable agricultural practices emphasised the role of mulching in various ways (Hortgro, 2016; Steenkamp, 2017; Steenkamp, 2019). The contribution of mulching towards reducing evaporation, increasing water infiltration, minimizing soil temperature extremes, increasing soil organic material (SOM) and providing weed control are well known (Moebius-Clune, 2016). However, the availability of mulching material, transport thereof, cost, application target, as well as the hygiene status play a role in the selection of a mulch product.

One alternative to the cost of introducing a mulch from outside the orchard, would be to grow the mulch *in situ.* The production of mulching material *in situ* has the potential to reduce the cost of mulching directly via the cost of the mulch material and indirectly, via a reduction in transport cost of the mulching material to the orchard. In addition, incorporation of a living mulch (cover crops) into a farming system also creates the possibility to enhance multiple ecosystem services and increase biodiversity in a predominantly mono-cropping system. A sound understanding of different cover crop species is required to select suitable crops for biomass production as a mulch. The selection of species plays a critical role in the successful introduction of a cover crop. Various factors must be taken into account when designing a cover crop program for biomass production, i.e., climate, availability of water, soil type, soil conditions and slope of the orchard, the time and conditions of establishment (Larkin, 2019). There is a wide variation in the quantity and quality of cover crop selections for biomass production (Lu *et al*., 2000). In addition, the management of the cover crop for maximum biomass production furthermore influences the final choice of species. If the main aim is to produce an *in situ* mulch, cover crops can be mowed and applied to the tree row, or left in the work row, to serve as a mulch. Biomass production also differs amongst species, with cereals and diverse combinations producing a higher biomass than legume combinations. Therefore, the final selection of cover crop species depends on the specific circumstances. Growing cover crops without irrigation, it is recommended to plant cover crops during the rainfall season.

Mulches can be applied in the work row, or in the tree row, but success varies. Yang *et al.* (2020) evaluated the effect of long-term cover crop mulching management in the tree row of an apple orchard. They reported a significantly higher soil organic carbon (SOC) and dissolved organic carbon (DOC) (0 – 40 cm) for their cover crop treatments (vetch, orchard grass and white clover) compared to their control (bare plot). However, SOC and DOC levels were similar between treatments for the deeper soil profile (40 – 80 cm) analyses. Paušič *et al*. (2021) evaluated the effect of an in-row living mulch compared to glyphosate chemical weed control in a pear orchard. Their trial consisted of three cover crop mixes, as well as an herbicide and control plot (natural vegetation). There was no significant difference in pear yield ($kg.ha^{-1}$) between the cover crop mixes and herbicide treatment (2015 – 2019). However, the control plot had a significantly lower yield. Liu *et al*. (2021) evaluated the effect of optimizing mulching and irrigation management practices for mango production in a dry hot environment. Their mulch treatments were a sugarcane mulch (SM) and non-mulch (NM) plot. The irrigation treatments consisted of: i) full irrigation (FI) 100% mango evapotranspiration, ii) mild deficit irrigation (DI_{75}) and iii) severe deficit irrigation (DI_{50}). SM treatment together with the different irrigation treatments had a better yield compared to the NM treatment. SM with DI_{75} had a significantly higher mango yield compared to the other treatments.

Atucha *et al*. (2012) evaluated three different groundcover management practices in an avocado (*Persea americana* Mill) hillside orchard. Their treatments were as follow: i) Bare soil (BS), pre and post emergence herbicide; ii) Vegetation strip (VS), post emergence herbicide applied on the tree row strip and ground cover seeded between rows; iii) Groundcover (GC), cover crop covered entire plot surface and mowed and left as a mulch. The BS treatment had a higher yield as well as a significantly higher trunk cross sectional area in 2010 and 2011 compared to VS and GC treatment. However, VS and GC treatment had a significantly lower bulk density, and GC treatment had a higher total soil N and C content, as well as a higher availability of essential plant nutrients. BS treatment contaminated drinking water with high levels of Terbuthylazine (55.4 – 79.9 $ug.L^{-1}$). Their BS treatment promoted tree growth compared to the other treatments in the first few years of establishment, however VS and GC had a more positive effect on soil environment, which is important to consider if you want to conserve soil.

Mulching the tree row in a local apple orchard (Western Cape) indicated differences among the mulches. Kotze (2012), evaluated four different mulches (compost, wood chips, vermicompost/woodchips and geotextile fabric) and a control (no mulch) on a heavy silt loam soil (site 1) and a light sandy silt loam soil (site 2) in an apple orchard. The wood chips treatment was the most effective in regulating diurnal soil temperature in site 1, whereas all mulches were similar and more effective compared to the control in site 2. Vermicompost had the highest levels of K, Mg, Zn, B, and P for site 1 (0-10 cm analyses). Vermicompost and woodchips treatment had the highest level of K, whereas vermicompost, geotextile fabric and the control had the highest level of Mg for site 2 (0-10 cm analyses). The vermicompost and woodchips treatment significantly increased yield efficiency after two years, at site 1. The compost treatment reduced the yield efficiency at both sites compared to the control, whereas the woodchips resulted in the highest yield efficiency at both sites. Van der Merwe (2012) continued with this study and reported a significant difference for mineral analysis at both sites, but mulching had a more pronounced effect on the lighter soil. The wood chip mulch was recommended, as it was the only treatment that did not compromise fruit quality and would sustain or promote yield efficiency in established orchards, especially on heavier soils.

Kühn and Pedersen (2009) quantified the effect of cover crops and mulching on yield and fruit quality in unsprayed organic apple production. Three different cover crop mixtures and management practices were used to determine the optimal growing system for an organic apple orchard. Treatment 1 was a permanent grass mixture (Red fescue and Meadow grass) in the alley way. Treatment 2 was a permanent clover grass (White clover and perennial ryegrass) mixture in the alley way. Treatment 3 was an annual mixture of grass and clover (Italian ryegrass and Persian clover), which was sown every year and mulched down at the end of the season. They also conducted an additional study on treatments 1 and 2. After mowing the cover crops, the clippings were mulched in the tree row (treatment A) and clippings were also mulched in the work row (treatment B). The annual cover crop growing system produced vigorous tree growth, a high level of nitrogen (N) in leaves, high soil water content in work row, the highest yield of large fruits and a low percentage of skin colour. In their mulching trial, leaving mulch in the work row resulted in lower yield of coloured fruit, compared to mulching of clippings in the tree row.

Cover crops are efficient weed suppressors through shading, competing for space, nutrients and water, or producing allelopathic substances during weed germination and growth (Farooq *et al*., 2011; Bezuidenhout *et al*., 2012; Miglécz *et al*., 2015; MacLaren *et al*., 2019). Husrev and Ngouajio (2012) reported that their cover crop treatments had a significantly lower weed density compared to the control (bare plot) in a hazelnut orchard. Baraibar *et al*. (2018) evaluated the relationship between growing degree days and cover crop types with reference to biomass, in winter cover crops. A winter cover crop that provides biomass in spring, suppressed weeds efficiently. Grass and brassica, as monocultures or in combination, were more weed suppressive than legumes, whereas grass monocultures and combinations were the most suppressive.

Davenport (2019) investigated the use of living mulches in the tree row in citrus (Eastern Cape) and plums (Western Cape). They evaluated the effects of *Tradescantia fluminensis* Vell. (*Tradescantia*) in a 'Palmer' navel orange orchard and *Bromus diandrus* (*Ripgut*), in a 'Sunkiss' plum orchard, compared to a control (bare plot, chemical control). Both living mulches had a significantly higher soil water holding capacity (WHC), provided sufficient weed suppression and soil temperature fluctuated less compared to the control.

Winter cover crop selections for mulches to be planted in fall include the following: Forage peas (*Pisum sativum*), an annual leguminous crop with a taproot system that forms nodules on the surface of the roots and top growth can reach a height of 30 to 75 cm (Nda, 2021). Weaver and Bruner (1927) reported a root penetration of 15 to 50 cm. The crop establishes rapidly and grows in most soil types which are well drained (Barenbrug, 2020). Successful establishment is dependent on adequate soil moisture, and it grows well with most forage cereals like oats, triticale and barley.

Vetch (*Vicia villosa*) is an annual leguminous crop with a shallow weak taproot system. The plant has a vine-like growth habit and can reach a height of 30 to 90 cm (Clark, 2012b). Vetch can grow in marginal cropping areas and is resilient to variable weather patterns (Nguyen *et al*., 2020). It is an efficient nitrogen fixer, provides a dense canopy once established and more biomass than similar legumes, and it is suitable for grazing, hay and green manuring (Clark, 2012b; Barenbrug, 2020; Nguyen *et al*., 2020). Vetch grows well with small grains, field peas, bell peas, crimson clover and buckwheat (Clark, 2012b).

Forage rye (*Secale cereale*) is a cool season, annual cereal grain, characterised by an upright growth habit, reaching 90 to 180 cm, with fibrous root system which can grow to over 100 cm deep (Clark, 2012a; GRDC growth note, 2018). It outperformed all cover crops on infertile, sandy or acidic soil (Strivers *et al*., 1999; Clark, 2012a).

Forage barley (*Hordeum vulgare*) is a cool season, annual cereal grain (Clark, 2012a) with an upright growth habit that reaches 89 to 98 cm (Barley growth guide, 2018). It possesses a fibrous root system, which can reach a depth of 30 to 60 cm (St. John *et al*., 2017; Jia *et al*., 2019). Barley does not tolerate wet soils, a low Ph or low fertility (Strivers *et al*., 1999). It is recommended to combine forage barley with annual legumes, ryegrass or other small grains in a cover crop mixture (Clark, 2012a).

Forage radish (*Raphanus sativus*) is a cool season broadleaf crop, with a taproot system (Weil *et al*., 2009; Jacobs, 2012). The plant reaches a height of approximately 60 cm (Jacobs, 2012). It is quick to establish, grows fast during cool weather conditions and produces abundant biomass in a relatively short period (Ngouajio and Mutch, 2004). Radishes scavenge nutrients, alleviate soil compaction, and allow for deeper rooting (Magdoff and van Ess, 2009; Clark, 2012a).

White mustard (*Sinapus alba* or *Brassica hirta*) is an annual broadleaf crop with a taproot system that forms fibrous roots near the soil surface (De Baets *et al*., 2011; St. John *et al*., 2017). Plant height is between 25 and 38 cm (Bone *et al*., 2009). It establishes quickly, show good growth in fall which results in a high biomass production (Clark, 2012a; St. John *et al*., 2017).

Phacelia (*Phacelia tanacetifolia*) is an annual broadleaf plant with an erect growth habit and height varying between 30 and 90 cm (Smither-Kopperl, 2018). It has a small shallow taproot system with many lateral fibrous roots, forming an intensive network of roots (De Baets *et al*., 2011). Phacelia prefers a loam to clay type of soil with a Ph range of 6.0 – 7.5 (Barenbrug, 2020). They produce abundant biomass under favourable conditions.

The aim of this study was to quantify the contribution of different cover crops towards *in situ* cover crop biomass production. The mulching material was removed from the work row and applied in the tree row of the apple orchard, whereas cover crops were left in the work row, in the plum orchard. The selection of species was based on local commercial recommendations. Forage rye, forage barley, forage peas, forage radish, vetch, white mustard, and phacelia were selected for the apple trial and

forage rye, forage radish and white mustard, for the plum trial. The crops were grown during two consecutive seasons and the foliage and root biomass were recorded as fresh and dry weight at the beginning of spring, which coincided with the harvest of the cover crops for mulching.

3.2. Materials and Methods

3.2.1. Trial Site Description

Site description or Welgevallen Research Farm (Trial 1 and 2) in Stellenbosch and Glen Elgin farm (Trial 3) in Elgin. More detail can be found in paper 1.

3.2.2. Treatment Details

Details of the crop selections, combinations and planting information is summarised in Tables 1, 2 and 3.

3.2.3. Monitoring Cover Crops and their Environment

3.2.3.1. Climate Data

Climate data (temperature and precipitation) was recorded with automatic weather stations in close proximity to the trial plots on an hourly, daily (Trial 1 and 2) and monthly (Trial 3) basis for the relevant periods.

3.2.3.2. Destructive Study of Cover Crops

Cover crop density was determined in a 1 m^2 area per replicate, at the time of destruction. A 1 m x 1 m grid was placed on top of the cover crops, in the work row, in an area that represented a true reflection of the treatment, to identify the area for excavation of the cover crops (Figure 1). Excavation was performed on 21 August 2020 and 25 August 2021 for Glen Elgin, and on 17 September 2020 and 6 September 2021 for Welgevallen Research Farm. Cover crops in this dedicated area were carefully removed, with shoot and roots intact, and transported to the laboratory for further destructive analyses. Soil was carefully removed from each plant and roots were washed to remove excess soil. Each plant (foliage and roots) was weighed and then separated into foliage and roots, and weights recorded again. Thereafter, plant

tissues were oven dried at 65 °C to a constant weight to determine the dry weight. Average root length of cover crops was also determined.

3.2.3.3. Weed Suppression

Weed suppression was quantified at the end of August 2020 and 2021, allowing sufficient time for winter/spring weeds to emerge (Welgevallen Research Farm: 20 August 2020 and 16 August 2021; Glen Elgin: 19 August 2020 and 20 August 2021). Weed density was quantified using the same 1 m x 1 m grid, placed at random in each plot, in the work row. The weed density was expressed as percentage cover per m^2. In addition, the primary weed species were also identified.

Statistical Analyses

The layout of the trials was a randomised complete block design (RCBD) comprising out of four treatments repeated four times for trial 1 and 2 and five treatments repeated five times for trial 3. The general linear models (GLM) procedure of Enterprise Guide 7.1 (SAS Institute Incorporation, Cary, North Carolina, USA) was used to perform a one-way Analysis of variance (ANOVA). Means were separated at a 5 % significance level using Fisher's LSD test. Principal component analysis (PCA), employing the correlation matrix, was performed using XLStat (Version 2016, Addinsoft; New York, USA) to elucidate the associations amongst treatments over two seasons and observed variables, including plant biomass and soil physical data. Pearson correlation analysis was performed to confirm correlations between variables.

3.3. Results

3.3.1. Monitoring Cover Crops and their Environment

3.3.1.1. Climate Data

3.3.1.1.1. Welgevallen Research Farm

Daily rainfall and hourly temperatures for Welgevallen Research Farm are presented from March to October 2020 and 2021 (Figure 2 and 3). During the study period, the average rainfall from March to October generally increased from 328.2 mm (2020) to 471 mm (2021). The total rainfall for April 2020 was higher than 2021, however the

last rain event for 2020 was on 21 April, whereas in 2021, rain was also recorded later, between 26 and 29 April. In 2020, precipitation consistently increased from March to August and declined from September. In 2021, precipitation showed a more irregular pattern. Total precipitation from May to July was higher for 2021 than 2020, whereas total precipitation from August to September was higher in 2020 than 2021. April 2020 had a relatively lower maximum temperature (± 24.62 °C) compared to April 2021 (± 26.64 °C) and a lower minimum temperature (± 11.21 °C) compared to April 2021 (± 12.25 °C). The minimum and maximum temperature for August 2020 and 2021 was similar. However, September 2021 had a relatively higher maximum temperature (± 20.14 °C) compared to September 2020 (± 17.96 °C).

3.3.1.1.2. Glen Elgin Farm

Monthly rainfall and the average maximum and minimum temperatures for Glen Elgin are presented from March to October 2019 to 2021 (Figure 4 and 5). During the study period, the precipitation from March to October increased from 495.58 mm (2019) to 596.09 mm (2020) and then declined to 579.59 mm (2021). However, March 2019 received a higher rainfall (64.90 mm) compared to March 2020 (19.08 mm) and 2021 (35.75 mm). In 2019, precipitation varied between months, with no clear trend. In 2020, precipitation increased consistently from March to August and then declined from September onwards. In 2021, precipitation returned to an irregular pattern. March 2019 had a relative lower maximum temperature (± 23.5 °C) compared to March 2020 (± 25 °C) and 2021 (± 24 °C), whereas August 2019 had a relative higher maximum temperature (± 17.8 °C) compared to August 2020 (± 15.8 °C) and 2021 (± 16.5 °C). The minimum temperatures from March to June were similar for all three seasons (2019/2021). However, July 2019 had the highest minimum temperature, whereas July 2021 had the lowest minimum temperature.

3.3.1.2. Destructive Study of Cover Crops

3.3.1.2.1. Welgevallen Research Farm

For trial 1, there was a significant difference between treatments for all biomass parameters, in 2020 and 2021 (Table 4). In 2020, the control had a significantly lower foliage fresh weight (FW) and dry weight (DW) compared to the other three treatments. No fertilizer (MNFA), single fertilizer (MSFA) and the double fertilizer (MDFA)

treatments did not differ significantly from each other for either parameter. The control also had a significantly lower root FW and DW compared to the other three treatments. The MNFA, MSFA and MDFA treatments did not differ significantly from each other for either parameter. In 2021, the control had a significantly lower foliage FW and DW compared to the other three treatments. MNFA had a significantly lower foliage FW and DW compared to MSFA and MDFA treatment. MSFA and MDFA did not differ significantly from each other. The control had a significantly lower root FW and DW compared to the other three treatments. The MNFA, MSFA and MDFA treatments did not differ significantly from each other.

Typical crop root length of the different species is presented in Figure 6. The average root length of the specific cover crops was higher in 2020 than in 2021. In spite of the different crop combinations in the treatments, forage rye had the longest average root length for both years (2020/2021) compared to forage radish and white mustard. Forage radish had a higher root length than white mustard in 2020, however, their lengths were similar In 2021.

In trial 2, there was a significant difference between treatments for all biomass parameters in 2020 and 2021 (Table 5). In 2020, the control had a significantly lower foliage FW and DW compared to the other three treatments. The no fertilizer (SNFA), single fertilizer (SSFA) and double fertilizer (SDFA) treatments did not differ significantly from each other. The control had a significantly lower root FW and DW compared to the other three treatments. The SNFA, SSFA and SDFA treatments did not differ significantly from each other. In 2021, the control had a significantly lower foliage FW and DW compared to the other three treatments. The SNFA, SSFA and SDFA treatments did not differ significantly from each other with regard to foliage FW. However, SSFA had a significantly higher foliage DW compared to SNFA. SDFA treatment did not differ significantly from SNFA and SSFA treatment. The control had a significantly lower root FW compared to SSFA and SDFA treatment. The control and SNFA treatment did not differ significantly from each other. The SNFA, SSFA and SDFA treatments did not differ significantly from each other. The control had a significantly lower root DW compared to the other three treatments. SNFA, SSFA and SDFA did not differ significantly from each other.

Typical crop root length of treatments for trial 2 are represented in Figure 7. The control, which consisted of weeds, had the smallest average root length, for both years, whereas SDFA had the highest average root length, for both years. SNFA and

SSFA had similar average root lengths in 2020, however SNFA had a higher average root length in 2021.

PCA analyses were conducted for both trials to evaluate the association between cover crop biomass, pH, maximum depth of penetration, WHC and carbon (C) for 2020 and 2021 (Figure 9 and 10) on crop performance (biomass). In trial 1, the first two principal components (PC1 and PC2) explained 39.20% and 27.51% of the variation in the data, respectively, which was dominated by cover crop biomass for both years (squared cosine values above 0.7). Other variables that associated with PC1 were pH, correlating significantly with C and maximum depth of penetration for April and September. There was a significant correlation between pH, C and the maximum depth of penetration for April and September. The WHC and C did not correlate significantly for the combined two years, however WHC and C correlated significantly in 2021. The PCA for trial 2, including both years, revealed the same trends as for trial 1, with the PC1 and PC3 explaining 41.74 % and 11.24 % of the variation in the data respectively and was again dominated by cover crop biomass. For the two years' data combined, pH correlated significantly with C and maximum depth of penetration at all three months.

3.3.1.2.2. Glen Elgin Farm

There was a significant difference between treatments for all parameters, except for foliage FW in 2019 (Table 6). Phacelia (PC), forage rye & vetch (FRVC) had a significantly lower foliage DW compared to the other three treatments. Forage radish & white mustard (FRWMC), forage barley & peas (FBPC) and forage rye (FRC) did not differ significantly from each other. PC, FRWMC and FBPC had a significantly lower root FW and DW compared to FRVC and FRC treatment.

There was a significant difference between treatments for all of the parameters for both 2020 and 2021 (Table 6). In 2020, PC had a significantly lower foliage FW and DW compared to the other four treatments. FBPC had a significantly lower FW compared to FRWMC treatment. FBPC, FRVC and FRC did not differ significantly from each other for foliage FW. FRWMC, FRVC and FRC did not differ significantly from each other for foliage FW. FRVC, FRWMC, FBPC and FRC did not differ significantly from each other for foliage DW. PC had a significantly lower root FW compared to the other four treatments. FRWMC and FBPC had a significantly lower

root FW compared to FRVC and FRC. FRVC and FRC treatment did not differ significantly from each other. PC had a significantly lower root DW compared to FRVC, FBPC and FRC. PC and FRWMC did not differ significantly from each other. FRWMC had a significantly lower root DW compared to FRVC and FRC. FRWMC and FBPC did not differ significantly from each other. FBPC had a significantly lower root DW compared to FRC. However, it did not differ significantly from FRVC. FRVC and FRC did not differ significantly from each other.

In 2021, PC had a significantly lower foliage FW and DW compared to FRVC, FRWMC and FRC. PC and FBPC did not differ significantly from each other. FRWMC had a significantly lower foliage FW and DW compared to FRVC and FRC. FRWMC and FBPC did not differ significantly from each other. FRVC had a significantly lower foliage FW compared to FRC. FRVC and FRC did not differ significantly from each other for foliage DW. PC had a significantly lower root FW compared to FRVC, FRWMC and FRC. PC and FBPC treatment did not differ significantly from each other. FRWMC and FBPC had a significantly lower root FW compared to FRVC and FRC. FRVC and FRC did not differ significantly from each other. PC and FBPC had a significantly lower root DW compared to the other three treatments. FRVC, FRWMC and FRC did not differ significantly from each other.

Typical crop root length specifics are presented in Figure 8. In spite of different treatments, the average root length of vetch, forage rye, radish and peas were all higher in 2021 compared to 2020. In contrast, average root length of forage barley, white mustard and phacelia were higher in 2020 compared to 2021. The difference in root length was more prominent in forage peas, barley and rye from 2020 to 2021. Forage rye was the crop with the most consistent root length, followed by forage radish.

PCA analyses were conducted for trial 3 to evaluate the contribution and correlation of association between cover crop biomass, pH, maximum depth of penetration, WHC and C for 2020 and 2021 (Figure 11) on crop performance (biomass). The first two principal components (PC1 and PC2) explained 42.40 % and 19.96 % of the variation in the data, respectively, and were dominated by cover crop biomass for both years (squared cosine values above 0.7) as in trials 1 and 2. Other variables that associated with PC1 (biomass) were pH and WHC for the combined two years. In 2021, WHC had a more positive correlation with biomass. There was a

significant correlation between pH and WHC as well as with maximum depth of penetration for June (mid winter, saturated soil profile).

3.3.1.3. Weed Suppression

3.3.1.3.1. Welgevallen Research Farm

In trial 1, there was a significant difference in weed density between the treatments for both seasons (Table 7). In 2020, the control had a significantly higher weed density compared to the other three treatments. MNFA, MSFA and MDFA did not differ significantly from each other. In 2021, the control had a significantly higher weed density compared to the other three treatments. MNFA had a significantly higher weed density compared to MSFA and MDFA treatment. MSFA and MDFA did not differ significantly from each other. For trial 2, there was a significant difference between the treatments for both seasons (Table 8). In 2020, the control had a significantly higher weed density compared to SDFA. The control, SNFA and SSFA did not differ significantly from each other. SNFA, SSFA and SDFA treatment did not differ significantly from each other. In 2021, the control had a significant higher weed density compared to the other three treatments. SNFA had a significantly higher weed density compared to SSFA and SDFA treatments. The SSFA and SDFA treatments did not differ significantly from each other.

Primary weeds for both trial 1 and 2 are presented in Table 10. In trial 1, the weed species differed during the study, however bermuda buttercup (*Oxalis pescarprae*) and kikuyu (*Pennisetum clandestinum*) was present at both sampling dates. In 2021, bur clover (*Medicago polymorpha*) was one of the dominant weeds. In trial 2, the weed species also differed during the study, however bermuda buttercup, kikuyu, musky storkbill (*Erodium moschatum*) and wild radish (*Raphanus raphanistrum*) were present at both sampling dates. In 2021, bur clover was one of the dominant weeds.

3.3.1.3.2. Glen Elgin Farm

In trial 3, there was a significant difference between the treatments for both seasons (Table 9). In 2020, PC had a significantly higher weed density compared to FRVC, FBPC and FRC. PC and FRWMC did not differ significantly from each other. FRWMC had a significantly higher weed density compared to FBPC. FRWMC, FRVC and FRC

did not differ significantly from each other. FBPC, FRVC and FRC did not differ significantly from each other. In 2021, PC had a significantly higher weed density compared to the other four treatments. FRWMC and FBPC had a significantly higher weed density compared to FRVC and FRC treatment. FRVC and FRC did not differ significantly from each other.

Primary weeds for trial 3 are presented in Table 10. Weed species differed during the study, however birdseye speedwell (*Veronica persica*), common mallow (*Malva neglecta*), goosefoot (*Chenopodiastrum murale*), musky storkbill and wild radish were present at both sampling dates. In 2020, burning nettle (*Urtica dioica*) and wild radish were dominant species in the orchard. In 2021, rescue grass (*Bromus catharticus*) and bur clover were dominant species in the orchard.

3.4. Discussion

3.4.1. Monitoring Cover Crops and their Environment

3.4.1.1. Climate Data

3.4.1.1.1. Welgevallen Research Farm

Rainfall is the primary climate parameter that affects cover crop establishment and growth, followed by temperature. Adequate rainfall at planting and a constant pattern of rainfall throughout the season is required for a good cover crop establishment and growth. The second season (2021) received a higher total rainfall than the first season (2020), but precipitation was higher in April 2020 than in 2021. In addition, rainfall was condensed in April 2021, just after planting of cover crops, which was favourable for the establishment of cover crops. April, august and September 2021 had a higher maximum temperature and May 2021 a lower maximum temperature compared to 2020. Temperatures were favourable for the growth of cover crops and the higher temperatures in April did not influence the growth of winter cover crops negatively, because it was accompanied with high precipitation. Biomass production was lower for both trials in 2020 than 2021. After sowing cover crops in 2020, 1.2 mm precipitation was recorded, which was followed by a dry-spell for about two weeks, before another 2.2 mm precipitation was recorded, followed by another dry spell. It was only at the end of May when consistent rainfall was recorded. This indicated the

vital role of adequate rainfall after sowing cover crops under dry land production and the effect thereof on successful establishment and biomass production of the species.

3.4.1.1.2. Glen Elgin Farm

The second season (2020) received a higher total rainfall compared to the other two seasons (2019 and 2021) from March to October. However, March 2020 received the lowest rainfall and had a higher maximum temperature. Biomass production was lower in 2020 compared to 2019 and 2021 which can be ascribed to planting conditions being more favourable in terms of total precipitation and maximum temperature in 2019 and 2021, compared to 2020. Although 2021 received the lowest rainfall in April, there was a follow-up event with sufficient rain in May (± 160 mm) which coincided with the ideal time promote the growth of these cover crops. PC seemed to be more susceptible to unfavourable environmental conditions, because establishment and biomass production was poor in 2020 and 2021 and this was in contrast with the characteristics of the species which stipulated that it is drought tolerate.

3.4.1.2. Destructive Study of Cover Crops

3.4.1.2.1. Welgevallen Research Farm

Biomass production in trials 1 and 2 was higher in 2021 than in 2020. In trial 1, treatments that received fertiliser (MSFA and MDFA) produced a higher biomass than the other two treatments. In trial 2, cover crops produced a higher FW and DW biomass compared to the control, except for root FW of SNFA in 2021. The treatments that received a fertiliser application (SSFA and SDFA) produced a higher biomass, irrespective of season. Therefore, we can conclude that the increase in fertiliser dosage, followed by high rainfall events (2021), increased biomass production across treatments. Organic fertiliser did not have an effect on the below ground biomass of cover crops for either trial 1 or 2. Foliage biomass for all treatments (trial 1 and 2) increased, whereas the biomass of roots decreased, for all treatments, except for FW roots of MDFA, from 2020 to 2021. This increase in foliar biomass in 2021, may reflect the higher total rainfall for 2021, as well as timing of rain events before and after planting cover crops, as well as temperatures during 2021.The control of both trial 1 and 2, produced a significantly lower biomass during the study, thus cover crops provide a higher biomass during the winter as well as provides more mulching material

during spring. Forage rye had the deepest root penetration compared to the other two cover crops. Root length of forage rye was similar for both trial 1 and 2, but higher compared to trial 3. SDFA had the highest average root length for both years. However, root length was less than reported in literature (GRDC growth note, 2018). Average root length of forage radish and white mustard were similar to trial 3, but again less than reported before (Weil *et al*., 2009; De Baets *et al*., 2011; Jacobs, 2012; St. John *et al*., 2017).

The control plots for both trial 1 and 2 produced the lowest biomass for both years, which corresponded with Sánchez *et al*. (2007). For trial 1, biomass from the first season was lower, but higher in the second season. Other papers reported a biomass for radish, white mustard and cereal rye ranging from 2.03 to 6.30 $t.ha^{-1}$, 4.50 to 6.7 $t.ha^{-1}$ and 3.49 to 3.9 $t.ha^{-1}$, respectively (Wendling *et al*., 2016; Chahal and van Eerd, 2020; Elhakeem *et al*., 2021; Hansen *et al*., 2021). Our biomass data corresponded with theirs even though trial 1 comprised a cover crop mixture (forage rye, forage radish and white mustard), whereas they reported on monoculture biomass.

In trial 2, foliar DW was 2.88 to 3.98 $t.ha^{-1}$ and 5.23 to 7.68 $t.ha^{-1}$ for 2020 and 2021, respectively. This was much higher compared to 2.10 and 1.50 $t.ha^{-1}$ for cereal grains (Chahal and van Eerd, 2020) or ryegrass of 2.49 to 2.90 $t.ha^{-1}$ (Mennan *et al*., 2005; Hansen *et al*., 2021). The difference in cereal rye biomass may be attributed to the difference in climate, type of soil and planting conditions.

PCA analyses for both trial 1 and 2 confirmed that crop species was the primary factor determining plant performance. These results implied that crop species were the primary factor in plant performance and any additional factors contributed less towards plant performance. The significant correlation between pH and maximum depth of penetration as well as C justifies for further investigation. The WHC and C correlated significantly in 2021 for trial 1, however there was no correlation in trial 2. It is possible that the species present in a cover crop mixture played a role. The PCA's from trial 1 and 2 were similar, although their treatments differed.

The best selections for maximum biomass production at this site were MSFA and SSFA, indicating the species selection was less important in this trial, where the additional application of fertiliser and rainfall during winter were the primary factors influencing biomass production.

3.4.1.2.2. Glen Elgin Farm

Biomass production for trial 3 was also higher in 2021, followed by 2019 and 2020. FRC performed the best, followed by FRVC and FRWMC.The difference in biomass production between seasons seemed to be primarily attributed to seasonal changes/climate. In terms of climate data, temperatures in March 2019 and 2021 were lower and temperatures in August higher, than in 2020. Although 2020 received more rain during the season, conditions for planting in terms of soil moisture were more favourable in 2019 and 2021.

A decline in plant performance were noticed, which may be an early indicator of the need to rotate treatments due to the introduction of another monoculture system in the work row, after three years of planting the same crops. This was evident in the multi-species mixtures, especially white mustard and forage peas, whereas they did not contribute that much to biomass production in 2021. Previous applications of fertilisers on this trial (visual observations) indicated no effect of inorganic fertilisers on biomass.

Phacelia did not perform well in the study as a single crop, except for 2019, but performance with other species was not evaluated. Biomass production declined from the start of the trial and establishment was poor. However, other papers reported that Phacelia performed well as a cover crop and reported biomass values of 3.44 and 6.30 $t.ha^{-1}$ (Wendling *et al*., 2016; Elhakeem *et al*., 2021). These trials were conducted in Europe, in sandy-clay soils. In addition, cover crops were fertilised with N and these factors would have influenced biomass production.

Foliar DW for FRWMC was 4.09, 2.20 and 2.98 $t.ha^{-1}$ for 2019, 2020 and 2021, respectively and was similar to previous papers, however the brassica mixtures differed (Wendling *et al*., 2016; Chahal and van Eerd, 2020; Elhakeem *et al*., 2021). Brennan and Smith (2005) reported an average of ± 3.80 to 11 $t.ha^{-1}$ for a brassica mixture (White mustard and brown mustard) for the first year and ± 1.50 to 6.10 $t.ha^{-1}$ for the second year. They also saw a decline in mustard performance.

The legume-grass mixture (FRVC and FBPC) ranged from 1.96 to 7.42 $t.ha^{-1}$, which was similar to other papers who reported biomass ranging from 1.85 to 8 $t.ha^{-1}$ for their legume-grass mixtures (Poffenbarger *et al*., 2015; Lavergne *et al*., 2021). However, biomass in our legume-grass mixture was much lower compared to Demir and Isik (2020), for a different grass-legume mixture (*Trifolium repens*, *Festuca rubra*

and *F. arundinacea*) (57.79 t.ha^{-1}and 33.19 t.ha^{-1}) and clearly illustrated the role of species selection. Biomass for vetch and peas ranged from 0.59 to 5.29 t.ha^{-1} and 2.64 to 3.30 t.ha^{-1}, respectively, in previous reports (Mennan *et al*., 2005; Poffenbarger *et al*., 2015; Wendling *et al*., 2016; Demir and Isik, 2020; Lavergne *et al*., 2021; Hansen *et al*., 2021). A higher biomass from our results could be attributed to the grass being a stronger grower than the legume. Poffenbarger *et al*. (2015) reported similar biomass levels between cereal rye monoculture and mixtures, with cereal rye being the dominant species in the mixture – which was also evident in our legume-grass mixture.

Biomass for FRC (5.25, 2.22 and 8.64 t.ha^{-1} for 2019, 2020 and 2021) corresponded with biomass of trial 2 and other reports between 5.25 to 10.95 t.ha^{-1} (Poffenbarger *et al*., 2015; Blesh, 2017). In terms of dry biomass production and consistency, FRC performed the best during the study.

Biomass DW may serve as an indication of decomposition rate potential. FRC followed by FRVC and FRWMC (highest DW) therefore have the potential of providing a mulch much longer than other treatments. In terms of root length, forage rye and radish performed the best in 2020 and 2021, rendering them the preferred crops when high root penetration is required. Forage rye did not perform as well as in trial 1 and 2, whereas the performance of brassica crops were comparable to the trial 1. Root lengths of forage rye, barley, peas and radish, white mustard and phacelia were still less compared to literature (Weaver and Bruner, 1927; Weil *et al*., 2009; De Baets *et al*., 2011; Clark, 2012b; Jacobs, 2012; Kilian, 2016; St. John *et al*., 2017; GRDC growth note, 2018). This may be due to the soil type and environmental conditions which differed from theirs.

PCA analyses for trial 3 also confirmed that crop species was the primary factor determining plant performance. The Ph correlated with maximum depth of penetration as in trial 1 and 2 and could not be explained. The more positive correlation between WHC and biomass in 2021 indicated a plant effect, because total precipitation for 2020 and 2021 were similar. Thus, for maximum biomass production, plant species is the most important factor to take into consideration.

Forage rye (single or combination) performed the best in terms of biomass production, average root length, weed suppression and consistency from one year to the next, indicating the strong impact of species.

3.4.1.3. Weed Suppression

3.4.1.3.1. Welgevallen Research Farm

Weed suppression efficiency was affected by cover crop biomass and species. In both seasons, the control had a significantly higher weed density compared to the other treatments in trial 1. Weed density (high to low) ranged from C > MNFA > MSFA > MDFA in 2020 and from C > MNFA > MDFA > MSFA. The significantly higher weed density for the control (both seasons) compared to cover crop treatments is most likely attributed to biomass production. However, after two years we found an additional difference between the different types of cover crop treatments (MNFA, MSFA and MDFA). MNFA had a significantly higher weed density and a lower biomass compared to MSFA and MDFA. Our findings corresponded with Husrev and Ngouajio (2012). Weed suppression for trial 1 varied between from 69 % to 96 % (2020/2021) and confirmed findings from Teasdale (2003), who stated that cover crop residue can reduce weed density up to 90%.

In trial 2, there was a significant difference between treatments for both seasons (2020/2021). In 2020, weed density (high to low) ranged from C > SNFA > SSFA > SDFA and in 2021, from C > SNFA > SDFA > SSFA. Results from the second season were similar to the results of trial 1 and Husrev and Ngouajio (2012), with weed suppression for trial 2 ranging from 60 to 96 % (2020/2021).

Fertilization of cover crops primarily and significantly increased weed suppression via an increase in biomass, especially under the high rainfall scenario in 2021, followed by species. Cover crops competed for weeds in terms of area, light, nutrients and water.

3.4.1.3.2. Glen Elgin Farm

During both seasons, a significant difference was observed between the five treatments, however efficiency varied. In 2020, weed density (high to low) ranged from PC > FRWMC > FRVC > FRC > FBPC and in 2021, from PC > FRWMC > FBPC > FRVC > FRC. Weed suppression was related to biomass production, and the higher the biomass production, the lower the weed density, which agreed with Husrev and Ngouajio (2012). Although there was no significant difference in biomass between PC and FBPC, PC still had a higher weed density, indicating other factors also have an influence. Hayden *et al* (2012) reported that rye was the most efficient suppressor of

annual weeds, however rye-vetch mixtures are just as effective in suppressing weed populations. Although our study was conducted in a perennial cropping system (apple orchard), we found similar results, with both FRVC and FRC suppressing weeds efficiently. Weed suppression ranged from 2 to 98 % (assumption weed control is 100%) (2020/2021). Cover crops suppressed weeds satisfactorily and that biomass, as well as cover crop species, influence efficiency.

In all three trials, weed species composition varied between years and trial study, thus cover crops influenced the type of weed species present in the orchard probably through changing the soil conditions, i.e., mineral composition or Ph, but this needs to be confirmed.

3.5. Conclusion

In this paper we quantified the contribution of specific cover crop combinations (multi and single species) towards biomass production for mulching. The role of climate and species selection were evaluated, as well as the efficiency of the different treatments with regard to weed suppression. In terms of climate, rainfall was the primary component affecting cover crop establishment and growth, in the non-irrigated trial, followed by temperature. This was evident in the varying biomass production between seasons, across treatments and sites. Rain before planting was as important as a continuous rainfall pattern after establishment and this was most prominent when 2019 and 2020 were compared to 2021, with the highest biomass. Cooler temperatures from May to August with a constant rainfall pattern and no dry-spells promoted the growth of the specific selections of cover crops. Forage rye (single or combination) performed the best in terms of biomass production, average root length, weed suppression and consistency from one year to the next, indicating the strong impact of species.

Cover crops had a positive effect on weed suppression throughout the trial and may serve as an alternative to herbicides and tillage in the work row of orchards.

PCA analyses across trials confirmed that crop species was the primary factor determining plant performance. The significant correlation between Ph and maximum depth of penetration justifies further investigation.

Crop species selection was the primary factor in biomass production. As in standard crop rotation for annual crop production, a cover crop rotation in orchards should be followed in order to prevent *in situ* monoculture systems in the orchard rows.

A multi-species mixture with one or more types of grass (forage rye), a legume (vetch) and brassica (forage radish), is recommended for the highest biomass production in these sites.

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Table 1: Treatment details for trial 1 (Multi species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, during 2020 and 2021.

Treatment	Cover crop	Treatment description
Control	Fallow / Natural weeds	Natural weeds.
No fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop.
Single fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop with single fertilizer application. First season: 21 April 2020 @ 1 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg per plot (Commercial organic fertilizer, 5:1:2).
Double fertilizer application	Forage rye (*Secale cereale*) (1.2 kg/replicate); Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.03 kg/replicate).	Cover crop with two fertilizer applications. First season: 21 April 2020 @ 1 Kg and 19 May 2020 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg and 26 May 2021 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2).

Table 2: Treatment details for trial 2 (Single species), in the plum orchard, on Welgevallen Research Farm, Stellenbosch, during 2020 and 2021.

Treatment	Cover crop	Treatment description
Control	Fallow / Natural weeds.	Natural weeds.
No fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop.
Single fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop with single fertilizer application. First season: 21 April 2020 @ 1 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg per plot (Commercial organic fertilizer, 5:1:2).
Double fertilizer application	Forage rye (*Secale cereale*) (1.3 kg/replicate).	Cover crop with two fertilizer applications. First season: 21 April 2020 @ 1 Kg and 19 May 2020 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2). Second season: 28 April 2021 @ 4 Kg and 26 May 2021 @ 8 Kg per plot (Commercial organic fertilizer, 5:1:2).

Table 3: Treatment details for trial 3, an apple orchard, on Glen Elgin farm, Elgin, from 2019 to 2021.

Treatment	Cover crop	Treatment description
Phacelia	Phacelia (*Phacelia Tanacetifolia)* (0.1 kg/replicate).	Single specie treatment. No additional fertilizer.
Forage rye & Vetch	Forage rye (*Secale cereale*) (0.8 kg/replicate); Vetch (*Vicia villosa*) (0.2 kg/replicate).	Multiple specie treatment consisting of a legume and non-legume plant. No additional fertilizer.
Radish & White mustard	Radish (*Raphinus sativus*) (0.06 kg/replicate); White mustard (*Sinapus alba*) (0.14 kg/replicate).	Multiple specie treatment consisting only out of Brassicaceae family. No additional fertilizer.
Forage barley & Peas	Forage Barley (*Hordeum vulgare*) (0.8 kg/replicate); Forage peas (*Pisum sativum*) (1 kg/replicate).	Multiple specie treatment consisting of a legume and non-legume plant. No additional fertilizer.
Forage rye	Forage rye (*Secale cereale*) (1.2 kg/replicate).	Single specie treatment. No additional fertilizer.

Table 4: Cover crop density (foliage and roots) for trial 1 (multi species). Values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	2020				
	Fresh foliage weight (g)	**Dry foliage weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Root:Shoot ratio**
Control	468b	104b	125b	35b	0.49
No fertilizer (MNFA)	1163a	345a	914a	320a	0.96
Single fertilizer (MSFA)	1213a	288a	828a	276a	0.97
Double fertilizer (MDFA)	1330a	328a	755a	263a	0.82
P-Value	**0.0347**	**0.0060**	**0.0230**	**0.0486**	
	2021				
	Fresh foliage weight (g)	**Dry foliage weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Root:Shoot ratio**
Control	652c	101c	94b	32b	0.98
No fertilizer (MNFA)	2200b	520b	653a	190a	0.36
Single fertilizer (MSFA)	3203a	858a	737a	223a	0.26
Double fertilizer (MDFA)	3098a	788a	858a	252a	0.34
P-Value	**0.0002**	**0.0001**	**0.0005**	**0.0023**	

Table 5: Cover crop density (foliage and roots) for trial 2 (single species). Values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	2020				
	Fresh foliage weight (g)	**Dry foliage weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Root:Shoot ratio**
Control	326b	74b	174b	66b	0.86
No fertilizer (SNFA)	1105a	295a	1101a	382a	1.45
Single fertilizer (SSFA)	1025a	288a	1110a	416a	1.41
Double fertilizer (SDFA)	1495a	398a	1074a	323a	0.85
P-Value	**0.0024**	**0.0012**	**0.0002**	**0.0139**	
	2021				
	Fresh foliage weight (g)	**Dry foliage weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Root:Shoot ratio**
Control	477b	101c	104b	45b	0.46
No fertilizer (SNFA)	1980a	523b	634ab	238a	0.46
Single fertilizer (SSFA)	2915a	768a	902a	243a	0.31
Double fertilizer (SDFA)	3045a	713ab	913a	238a	0.33
P-Value	**0.0018**	**0.0004**	**0.0273**	**0.0474**	

Table 6: Cover crop density (foliage and roots) for trial 3. Values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	2019				
	Foliage fresh weight (g)	**Foliage dry weight (g)**	**Fresh root weight (g)**	**Dry root weight (g)**	**Root:Shoot ratio**
Phacelia (PC)	1139ns	182c	95b	37b	0.22
Forage rye and Vetch (FRVC)	720	335c	735a	535a	1.54
Forage radish and White mustard (FRWMC)	750	409a	217b	112b	0.28
Forage barley and Peas (FBPC)	649	381a	382b	195b	0.60
Forage rye (FRC)	1005	525a	824a	687a	1.35
P-Value	**0.5980**	**0.0108**	**0.0006**	**< 0.0001**	
	2020				
	Foliage fresh weight (g)	**Foliage dry weight (g)**	**Fresh root weight (g)**	**Dry root weight (g)**	**Root:Shoot ratio**
Phacelia (PC)	114c	24b	15c	7d	0.14
Forage rye and Vetch (FRVC)	828ab	214a	412a	143ab	0.65
Forage radish and White mustard (FRWMC)	1094a	220a	201b	66cd	0.31
Forage barley and Peas (FBPC)	616b	196a	226b	85bc	0.42
Forage rye (FRC)	840ab	222a	502a	162a	0.84
P-Value	**0.0001**	**0.0001**	**< 0.0001**	**0.0015**	
	2021				
	Foliage fresh weight (g)	**Foliage dry weight (g)**	**Fresh root weight (g)**	**Dry root weight (g)**	**Root:Shoot ratio**
Phacelia (PC)	188d	42c	164c	10b	0.32
Forage rye and Vetch (FRVC)	2540b	742a	517a	153a	0.22
Forage radish and White mustard (FRWMC)	1336c	296b	279b	133a	0.43
Forage barley and Peas (FBPC)	682cd	204bc	204bc	30b	0.19
Forage rye (FRC)	3484a	864a	864a	196a	0.22
P-Value	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0002**	

Table 7: Weed density for all four treatments for trial 1 (multi species), the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	Weed Density (%)	
	August 2020	August 2021
Control	92.50a	95.75a
No fertiliser (MNFA)	27.50b	29.50b
Single fertiliser (MSFA)	17.75b	6.50c
Double fertiliser (MDFA)	3.00b	7.00c
P-Value	**0.0069**	**< 0.0001**

Table 8: Weed density for all four treatments for trial 2 (single species), the plum orchard, on Welgevallen Research Farm, Stellenbosch for 2020 and 2021. Mean values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	Weed Density (%)	
	August 2020	August 2021
Control	73.25a	94.75a
No fertiliser (SNFA)	29.00ab	21.50b
Single fertiliser (SSFA)	27.75ab	4.00c
Double fertiliser (SDFA)	2.25b	5.00c
P-Value	**0.0480**	**< 0.0001**

Table 9: Weed density for all five treatments for trial 3, in an apple orchard, on Glen Elgin farm, Elgin for 2020 and 2021. Mean values in a column followed by a different letter are significantly different (P ≤ 0.05), (LSD-FISHER).

Treatment	Weed Density (%)	
	August 2020	August 2021
Phacelia (PC)	57.80a	98.00a
Forage rye and Vetch (FRVC)	25.20bc	3.80c
Forage radish and White mustard (FRWMC)	47.60ab	70.00b
Forage barley and Peas (FBPC)	14.00c	60.00b
Forage rye (FRC)	22.20bc	1.60c
P-value	**0.0270**	**< 0.0001**

Table 10: Primary weeds present during the study (2020/2021).

Trial	Primary weeds	
	2020	2021
Trial 1	Bermuda buttercup (*Oxalis pescaprae*); Kikuyu (*Pennisetum clandestinum*); Ribwort plantain (*Plantago lanceolata*); Scarlet pimpernel (*Anagallis arvensis*); Wild radish (*Raphanus raphanistrum*).	Bermuda buttercup; Bur clover (*Medicago polymorpha*); Kikuyu; Musky storkbill; Pink sorrel.
Trial 2	Bermuda buttercup; Kikuyu; Musky storkbill (*Erodium moschatum*); Pink sorrel (*Oxalis articulate*); Scarlet pimpernel; Wild radish.	Bermuda buttercup; Bur clover; Creeping wood sorrel; Hemlock (*Conium maculatum*); Kikuyu; Musky storkbill; Pink sorrel; Wild radish.
Trial 3	Birdseye speedwell (*Veronica persica*); Burning nettle (*Urtica dioica*); Common mallow (*Malva neglecta*); Creeping wood sorrel (*Oxalis corniculata*); Goosefoot (*Chenopodiastrum murale*); Wild radish.	Birdseye speedwell; Bur clover; Cape dandelion (*Arctotheca* calendula); Common mallow; Goosefoot; Musky storkbill; Rescue grass (*Bromus catharticus*); Wild radish.

Figure 1. Visual representation of the 1 m x1 m grid used during the excavation of cover crops.

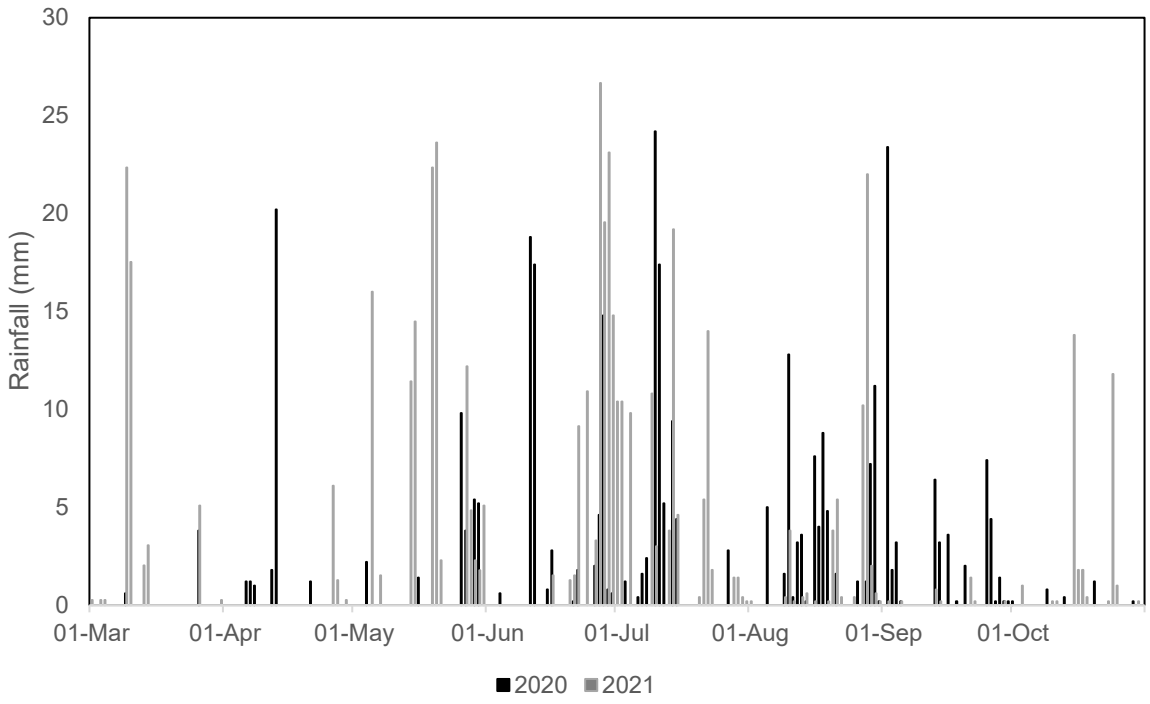

Figure 2. Daily rainfall (mm) for Welgevallen Research Farm for 2020 and 2021.

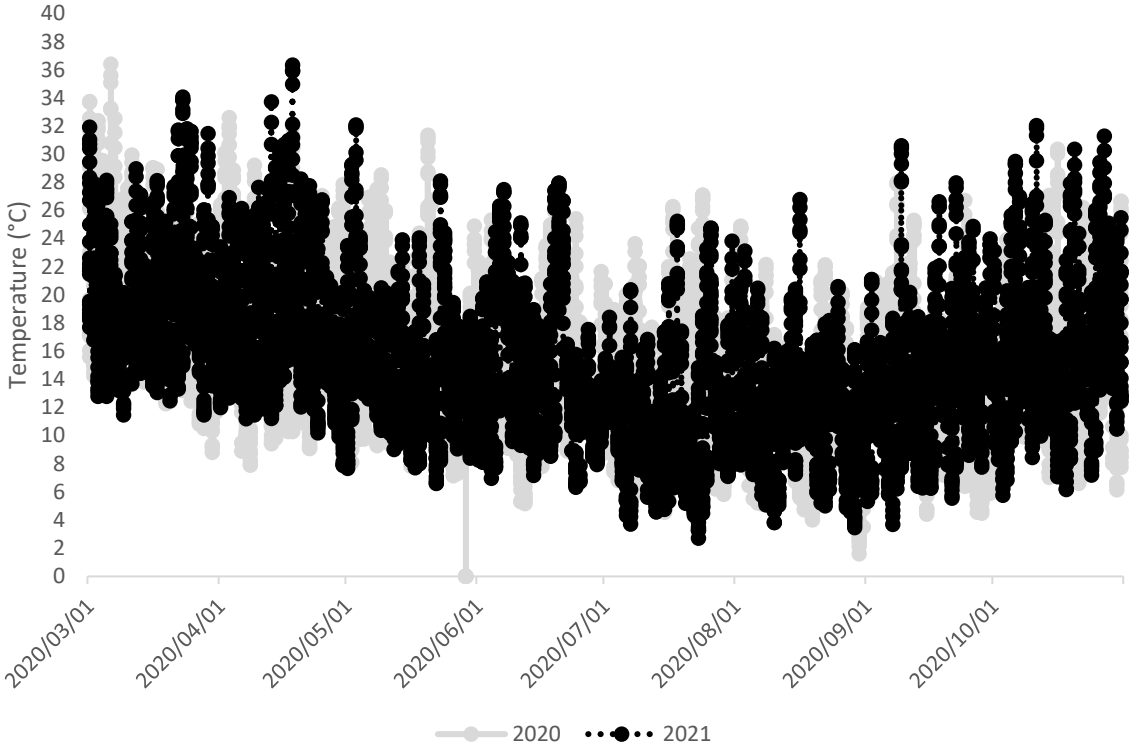

Figure 3. Hourly temperature (°C) from March to October (2020/2021) for Welgevallen Research Farm, Stellenbosch.

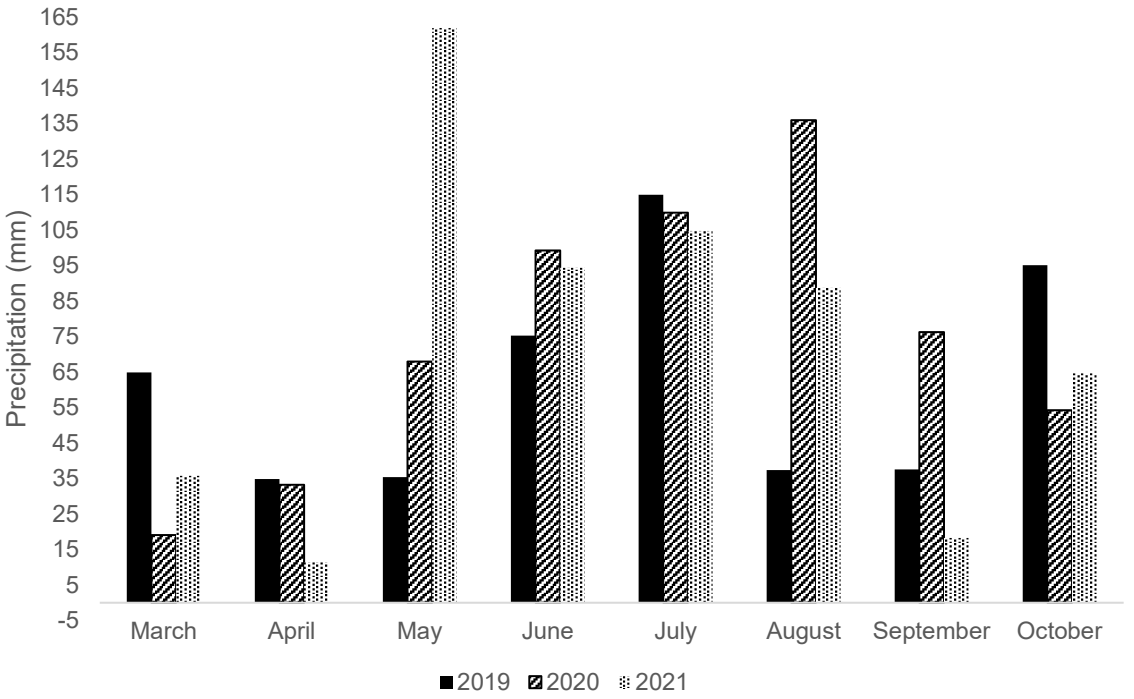

Figure 4. Monthly rainfall (mm) from March to October (2019-2021) for Elgin Western Cape.

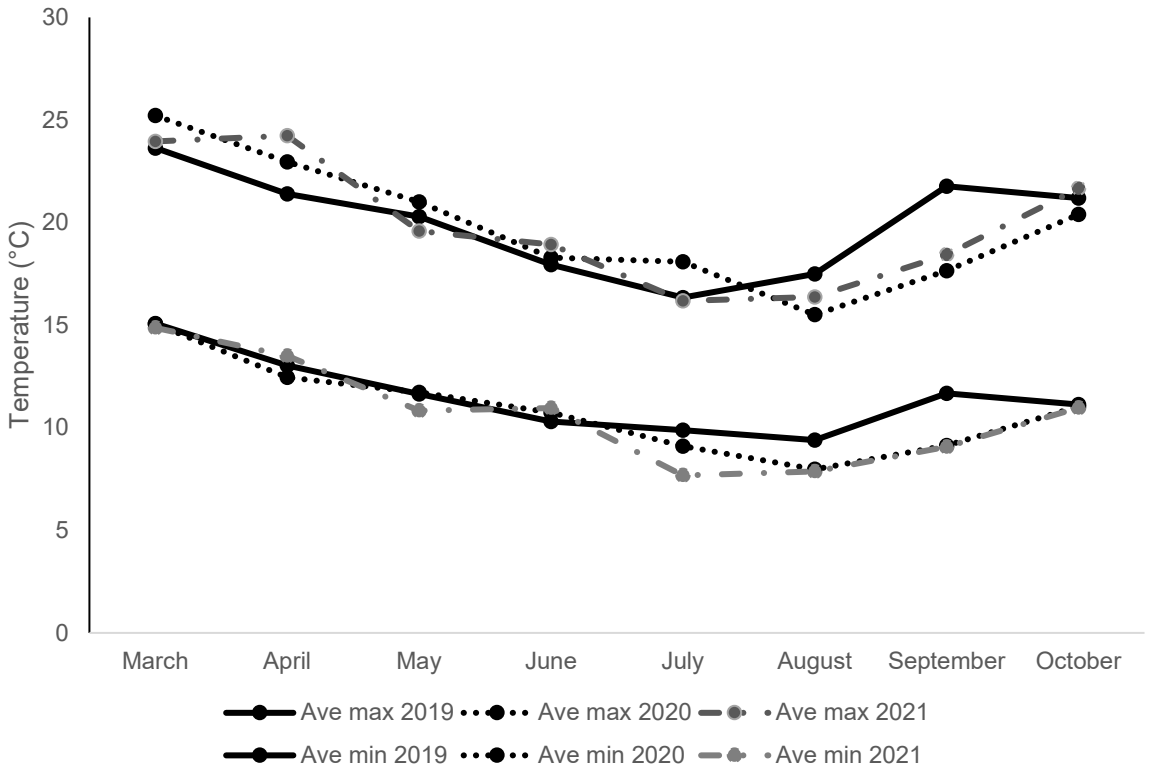

Figure 5. Average maximum and minimum temperature on a monthly basis from March to October (2019/2021) for Elgin, Western Cape.

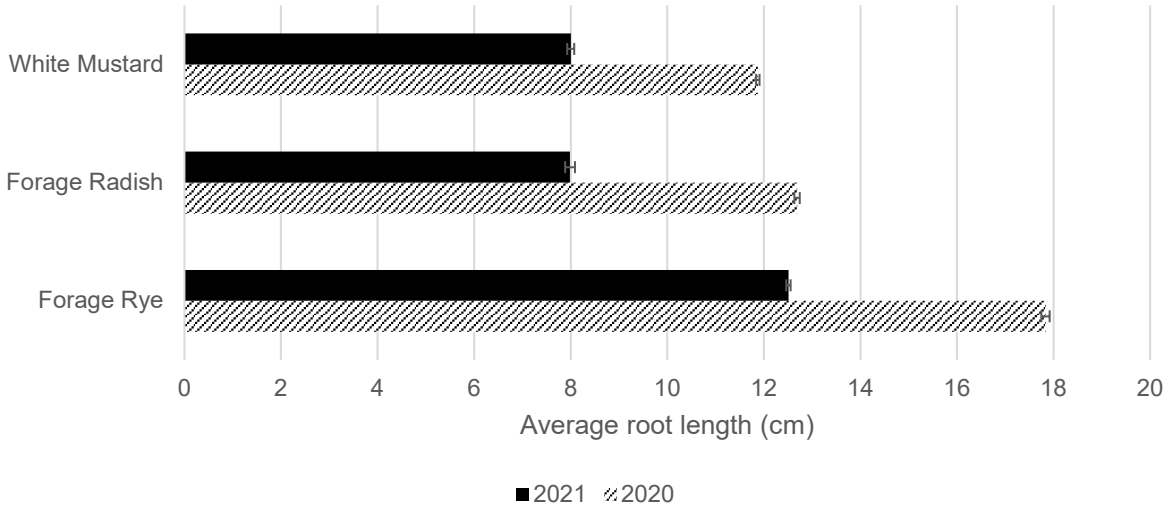

Figure 6. Average root length (cm) of cover crops for trial 1, Welgevallen Research Farm, 2020 and 2021.

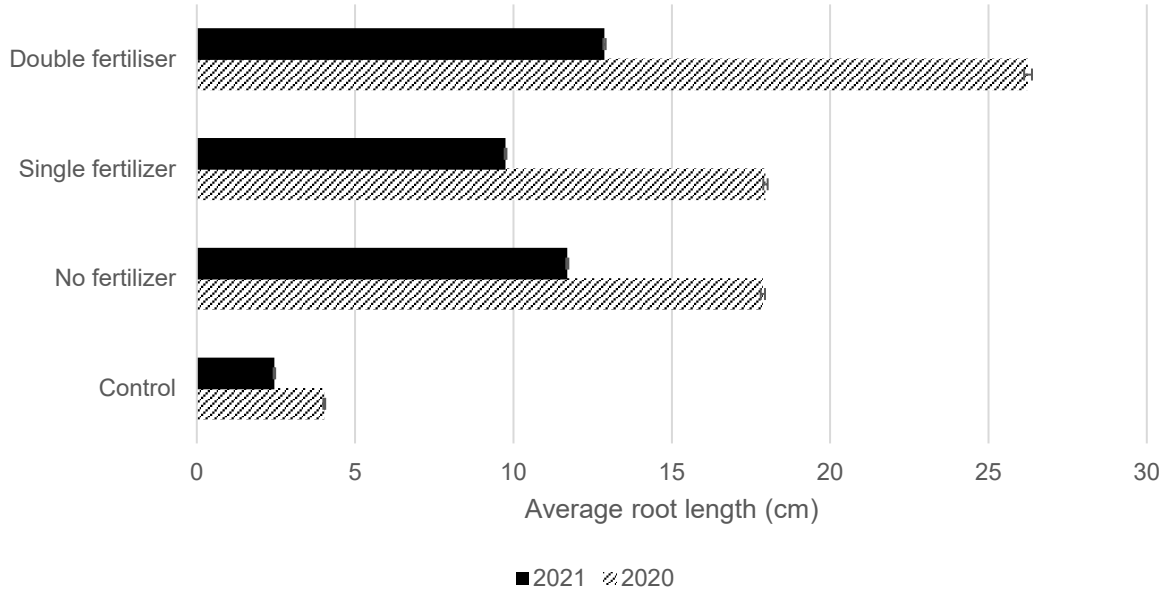

Figure 7. Average root length (cm) of treatments for trial 2, Welgevallen Research Farm, 2020 and 2021.

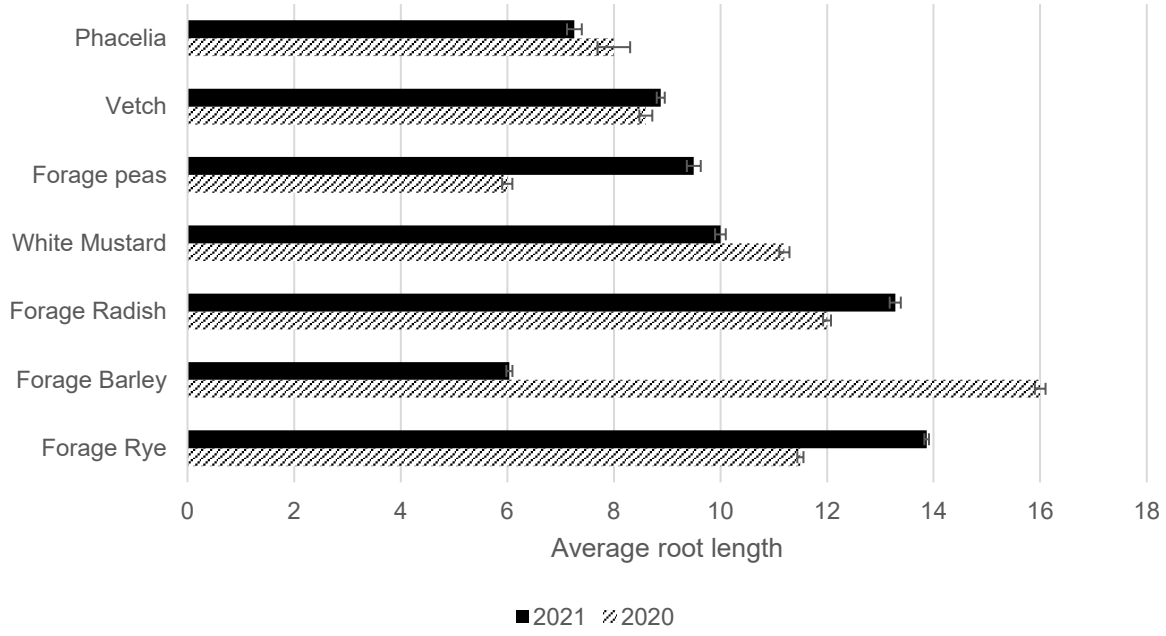

Figure 8. Average root length (cm) of cover crops for trial 3, Glen Elgin, 2020 and 2021.

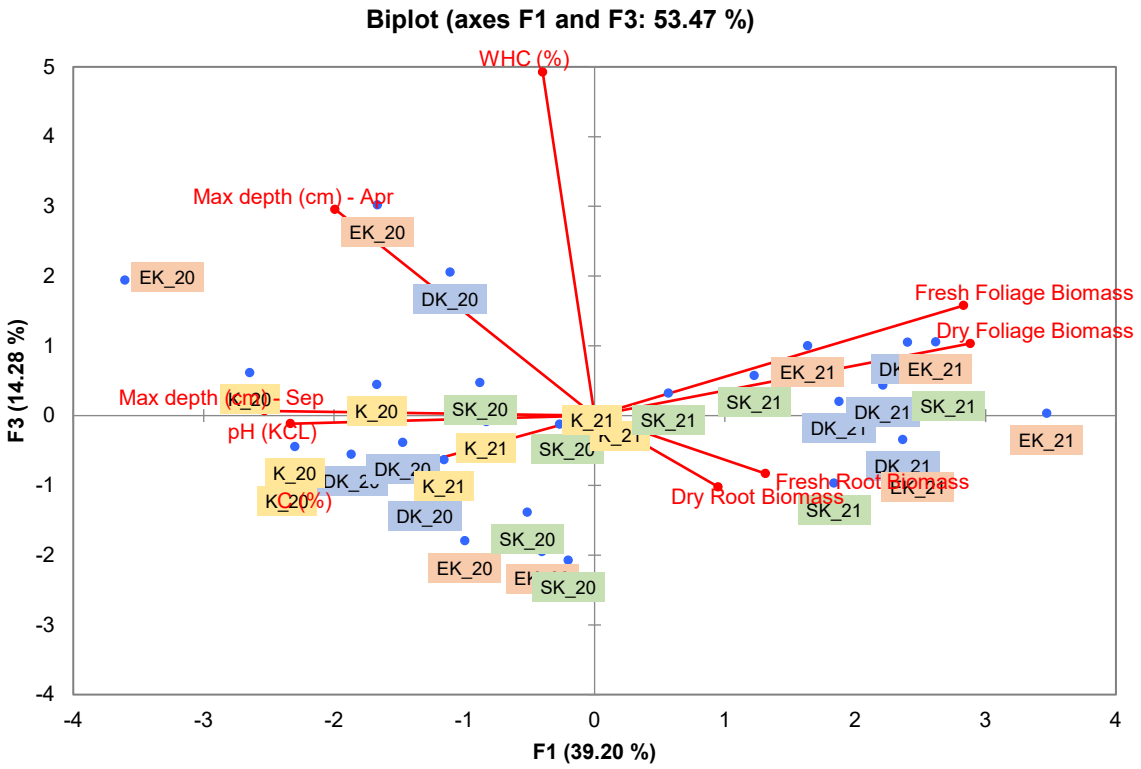

Figure 9. Principal component analyses for trial 1 (multi species), Welgevallen Research Farm for 2020 and 2021.

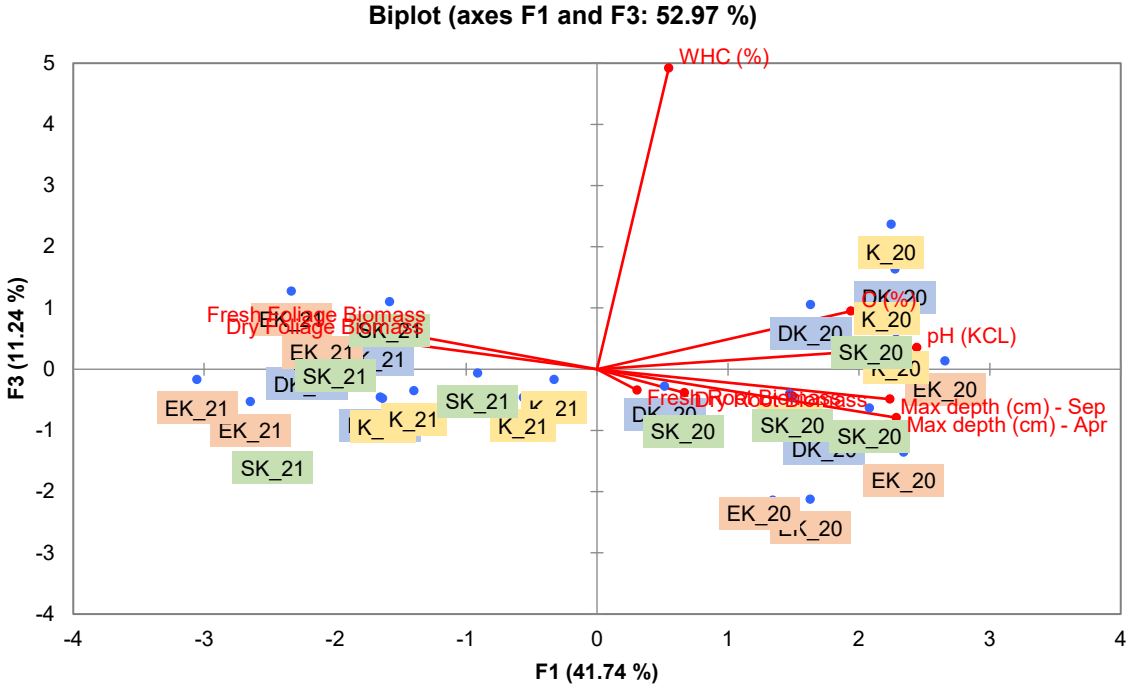

Figure 10. Principal component analyses for trial 2 (single species), Welgevallen Research Farm for 2020 and 2021.

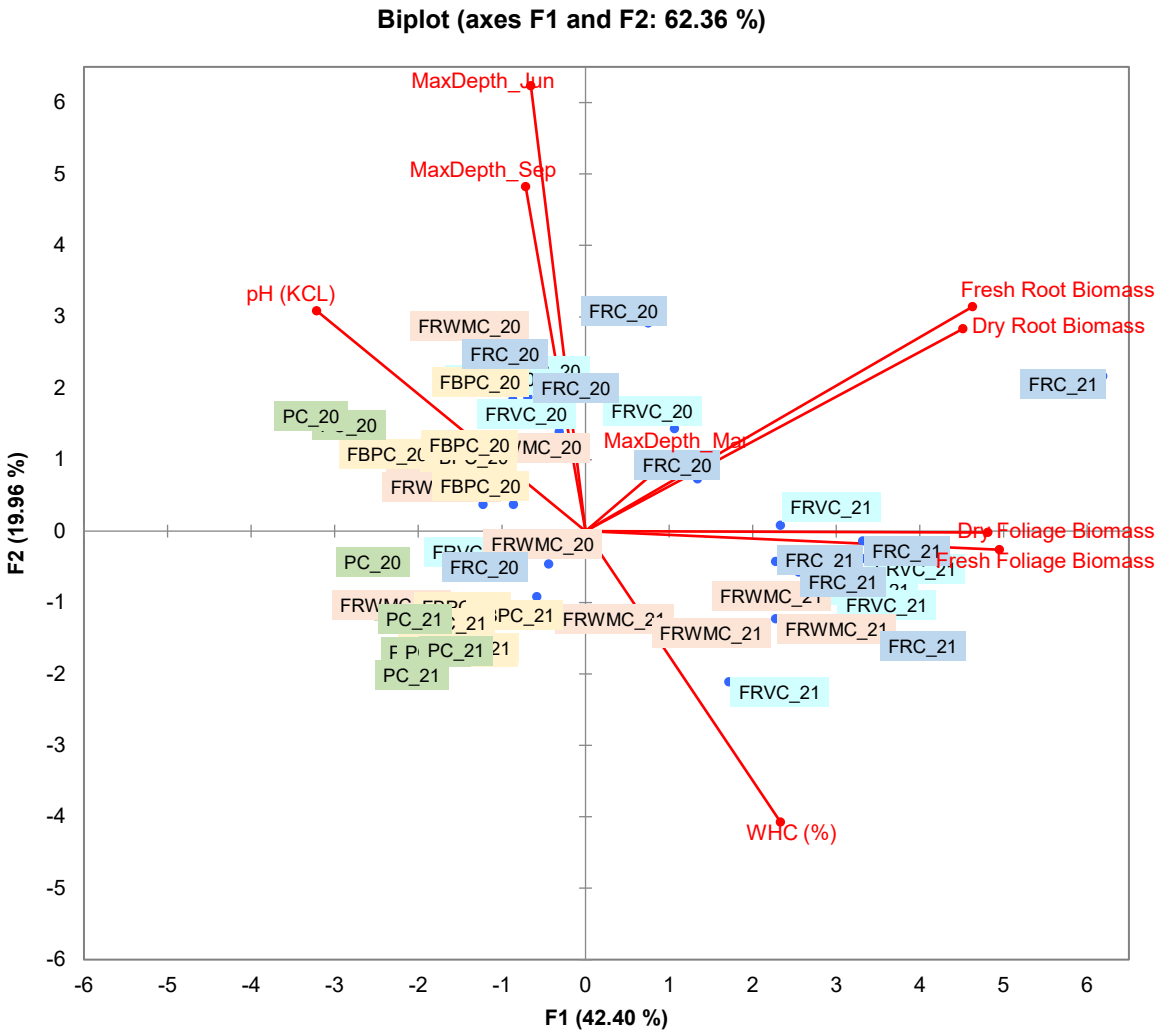

Figure 11. Principal component analyses for trial 3, Glen Elgin Farm for 2020 and 2021.

General Conclusion

Three field studies were conducted to evaluate the contribution of cover crops towards soil health/fertility with reference to the interaction between cover crop species and the physical, chemical and biological components of soil. Cover crops did not have a negative effect on the chemical composition of soil, however the primary weed species were different between the seasons, implying a change in mineral composition and availability. A poor establishment of white mustard (brassica) was noticed, this might be due to the sulphur-level being below optimal range in 2021. It was noted that soil calcium-levels should be monitored when planting forage rye. Forage radish and white mustard mixture had a higher nutrient content in their foliage, implying that they were more effective in scavenging nutrients. Legume-grass mixtures did not have the highest nitrogen (N) content in their foliage and thus it is recommended to inoculate legumes with the correct strains, enhancing symbiotic relationship and N-fixation.

Cover crops provided a significantly higher above- and below ground biomass compared to natural vegetation during the trial, in addition, biomass also differed significantly among cover crop treatments. It was concluded that crop species are the primary factor in plant performance and any additional factors contributed less. A movement towards monoculture was observed in the orchard after planting the same crop for two/three years in a row, however some cover crops are more sensitive to this phenomena. A multi-species mixture is recommended in order for the stronger cover crop (less affected by monoculture) to carry a weaker cover crop (more sensitive to monoculture) and still provide sufficient biomass for mulching purposes. From our study I would recommend including forage rye into any mixture due to its growth consistency and contributions it delivers.

Cover crops provided a canopy and additional growing roots during the winter (rainfall season), enabling the orchard to capture rainfall more efficiently by increasing the retention time of water on soil, improving infiltration rate (visual observation and according to literature). The additional canopy protects soil by reducing the impact of rain droplets.

After mulching cover crops, they conserved soil moisture much better than the control (natural vegetation), however contradictions were found between cover crop biomass of treatments and soil moisture conservation, which might be due to a

difference in plant architecture, however further research is required to explain this phenomenon.

Cover crops created a favorable environment for the microbial community in terms of conserving soil moisture, regulating diurnal changes in soil temperature (visual observation and according to literature) and providing a food source through rhizodeposition during the season and material to break down after cover crop growth cycle. This was evident from our results which indicated a high microbial activity for cover crops. There was a trend between the cover crop biomass and diversity with microbial activity (CO_2). This was evident in our results where the control and PC treatment of trial 1 and 3, respectively had a significantly lower biomass production in 2020 followed by a significantly lower CO_2 production in March/April of 2021. There was no difference in CO_2 production between treatments for trial 2 (single species), although their biomass differed. Mycorrhizal fungi were observed in the cover crop treatments after two years for both trials in Stellenbosch, this might be linked to the P-levels being below optimal range. All three techniques indicated a high microbial activity, whereas molecular fingerprinting technique gave additional information of the fungal and bacterial composition. However, there is a need for the development of an easy and robust soil health benchmark assessment in perennial orchards under South African conditions. Our data can aid in the development of such a benchmark.

We were able to see the change in soil compaction over depth (≤ 3000 kPa) for the different cover crop treatments, however cover crops were not the primary factor influencing soil compaction. Soil pH and maximum depth of penetration correlated with each other. Soil WHC measurements are season bound, due to the difference in a saturated and unsaturated soil water profile. Although we did not find the significantly higher WHC we anticipated, our cover crop treatments for trial 1 and 2 had a higher WHC in September 2021 compared to their control, whereas trial 3 WHC levels improved from July 2020 to 2021, both indicated that this should be monitored in future as it may require a longer time span for final conclusions. According to literature WHC is influenced by soil texture and OM. A significantly higher biomass did not deliver a significantly higher SOC in our study, however C-levels of trial 1 significantly correlated with WHC in 2021, whereas WHC correlated positively with the biomass of cover crops from trial 3.

Cover crops suppressed weeds efficiently and may be used as an alternative to herbicides and tillage in the work row. There was a shift in primary weeds from one

season to the next and the biomass and species of cover crop were the main factors responsible for a reduction in weeds. A future study should evaluate the duration of weed suppression offered by cover crops, after mulching them in the work row. Cover crops did not have a negative effect on plant performance; however, it is necessary to monitor it over the long term.

Cover crops either had a direct or indirect effect on the parameters quantified in the study. Cover crops directly contributed to stimulating microbial community and conserving soil moisture during spring, when fruit trees start to grow actively, however from our results it was evident that soil is a dynamic system and that it should be managed holistically in terms of soil physical, chemical and biological components. A long-term study should be initiated to evaluate the effect of using different cover crop rotations in a perennial orchard to improve soil biological components (microbial community) which will contribute to an alternative system such as farming with nature, minimizing external inputs, building soil structure and providing nutrient dense food.

Addendum A

Table 24: Leaf analyses for plum orchard (Trial 1 and 2) for 2020 and 2021, Welgevallen Research farm, Stellenbosch.

Year	N	P	K	Ca	Mg	S	Na	Zn	Cu	Mn	Fe	B	Mo
	(%)							(mg/kg)					
2020	2.62	0.14	1.65	2.11	0.51	0.15	0.03	26.00	7.00	125.00	213.00	42.00	0.17
2021	1.80	0.21	2.05	1.94	0.43	0.15	0.02	29.00	7.00	50.00	211.00	38.00	284.00

Table 25: Leaf analyses for apple orchard (Trial 3) for 2021, Glen Elgin farm, Elgin.

Year	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	(%)						(mg/kg)				
2021	2.93	0.19	1.77	1.34	0.27	262.00	183.00	141.00	18.20	43.20	47.10

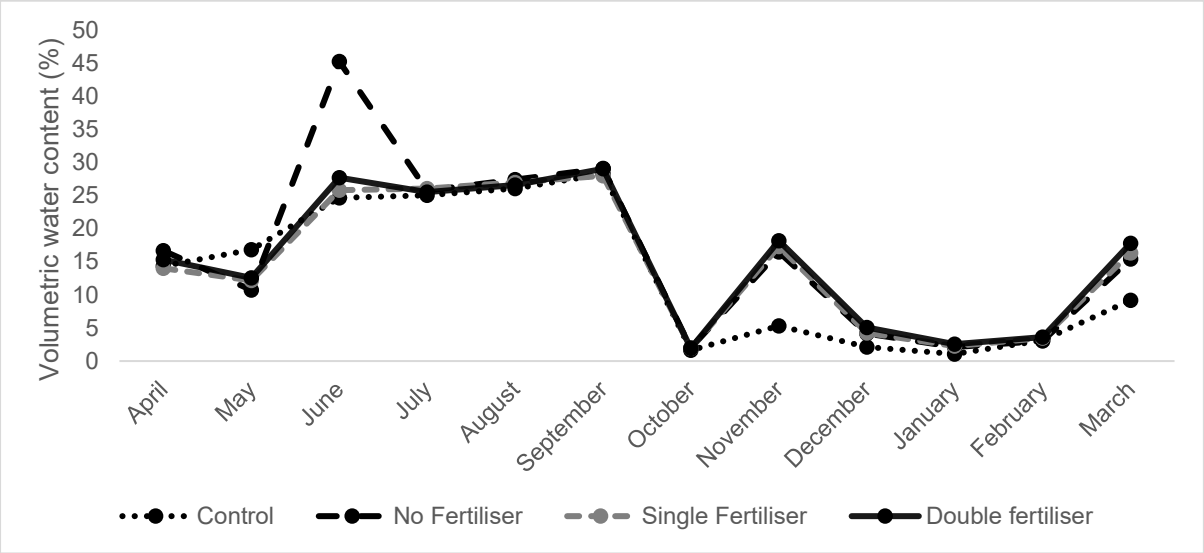

Figure 17. Monthly volumetric water content for trial 1 (Multi specie) from April 2020 to March 2021.

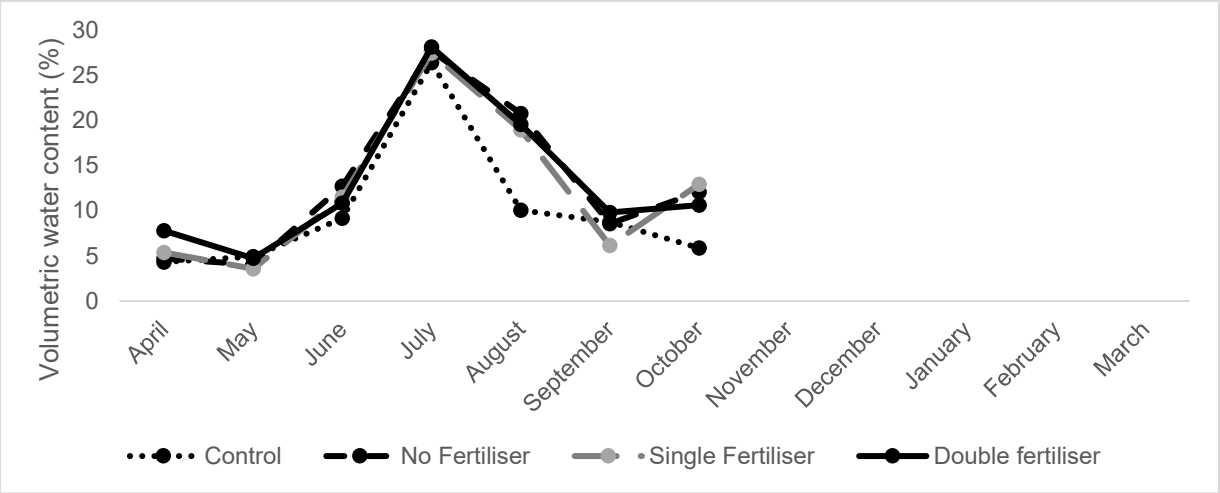

Figure 18. Monthly volumetric water content for trial 1 (Multi specie) from April 2021 – October 2021.

Figure 19. Monthly soil water deficit for trial 1 (Multi specie) from April 2020 to March 2021.

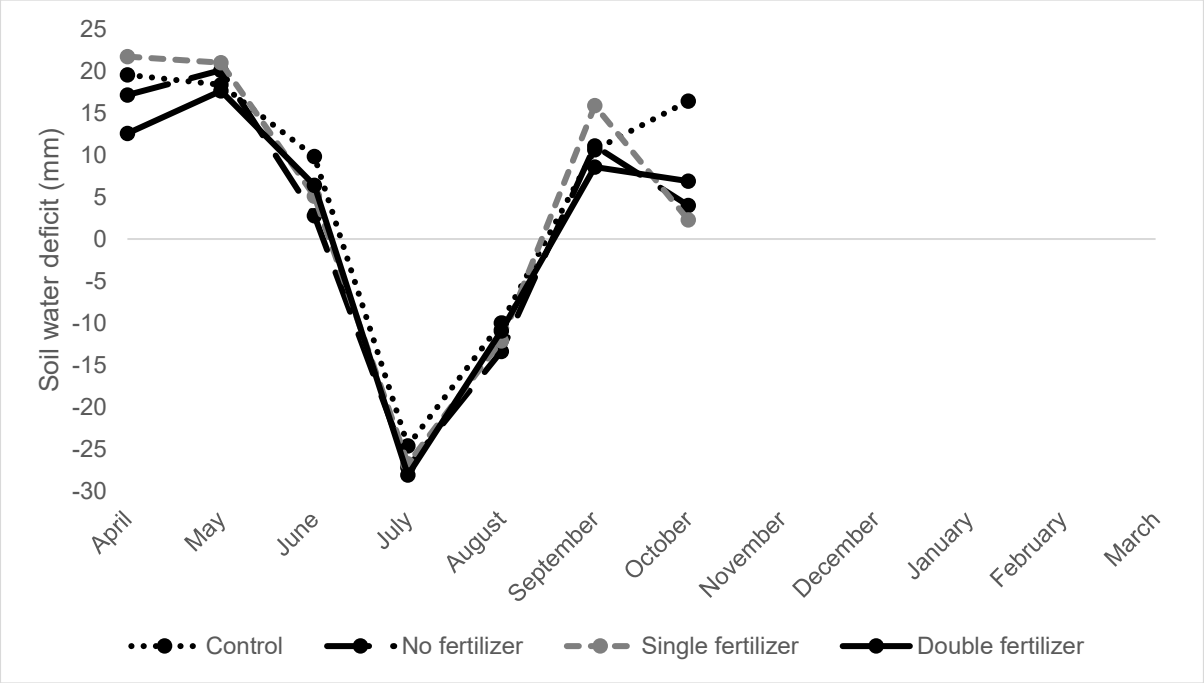

Figure 20. Monthly soil water deficit for trial 1 (Multi specie) from April 2021 to October 2021.

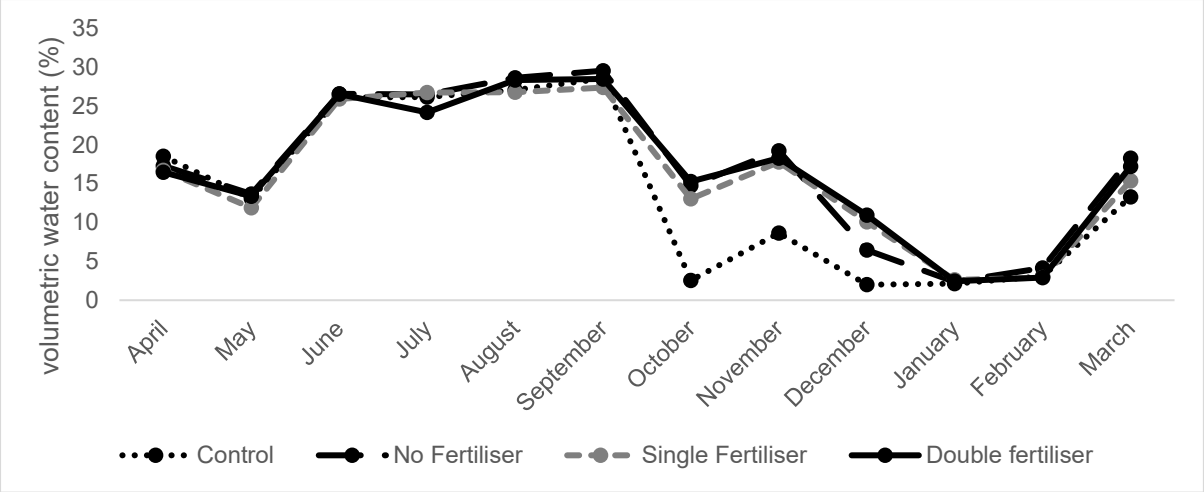

Figure 21. Monthly volumetric water content for trial 2 (Single specie) from April 2020 to March 2021.

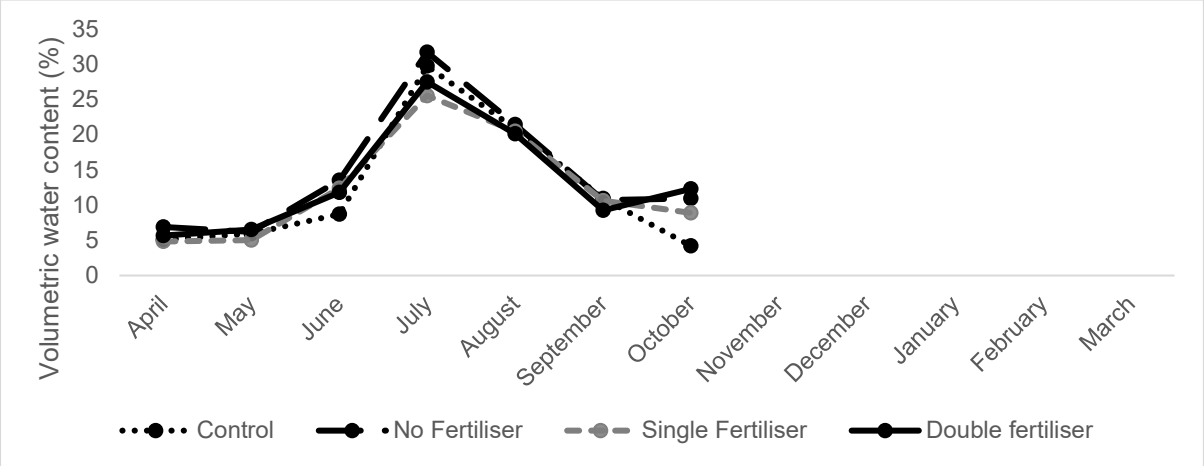

Figure 22. Monthly volumetric water content for trial 2 (Single specie) from April 2021 to October 2021.

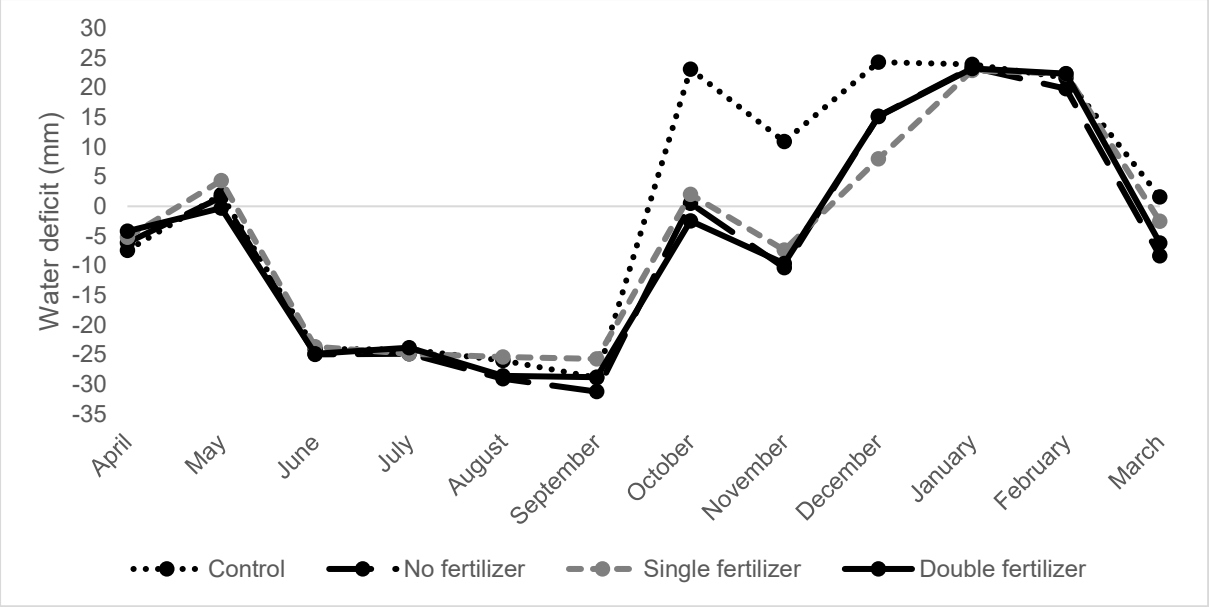

Figure 23. Monthly soil water deficit for trial 2 (Single specie) from April 2020 to March 2021.

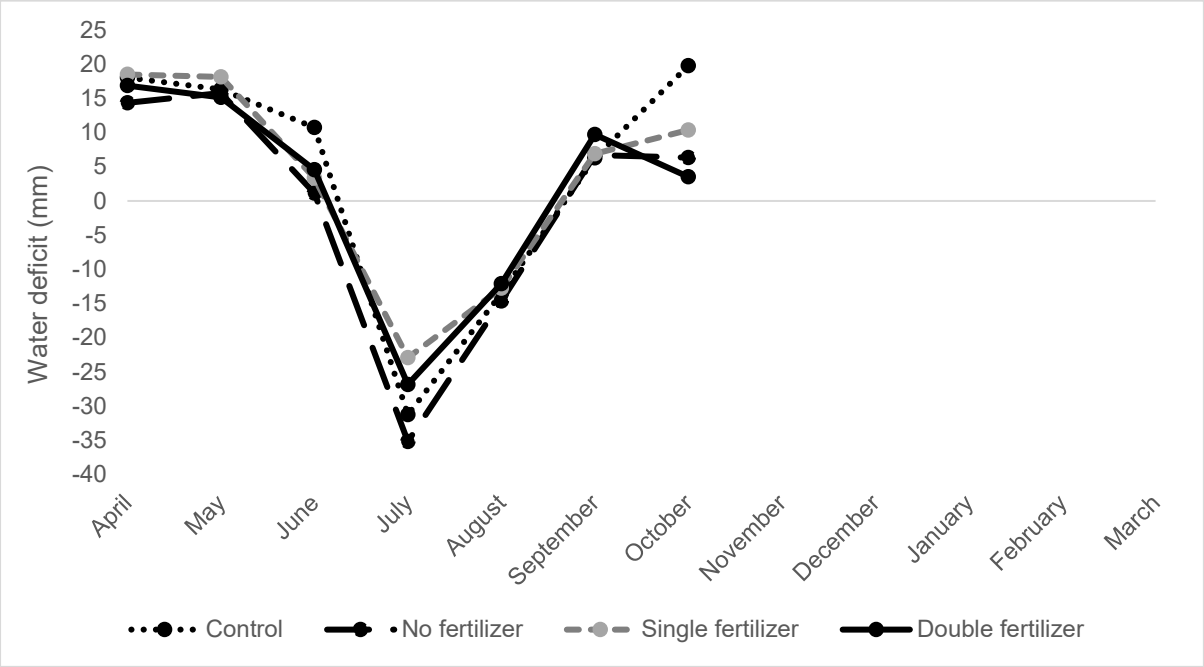

Figure 24. Monthly soil water deficit for trial 2 (Single specie) from April 2021 to October 2021.

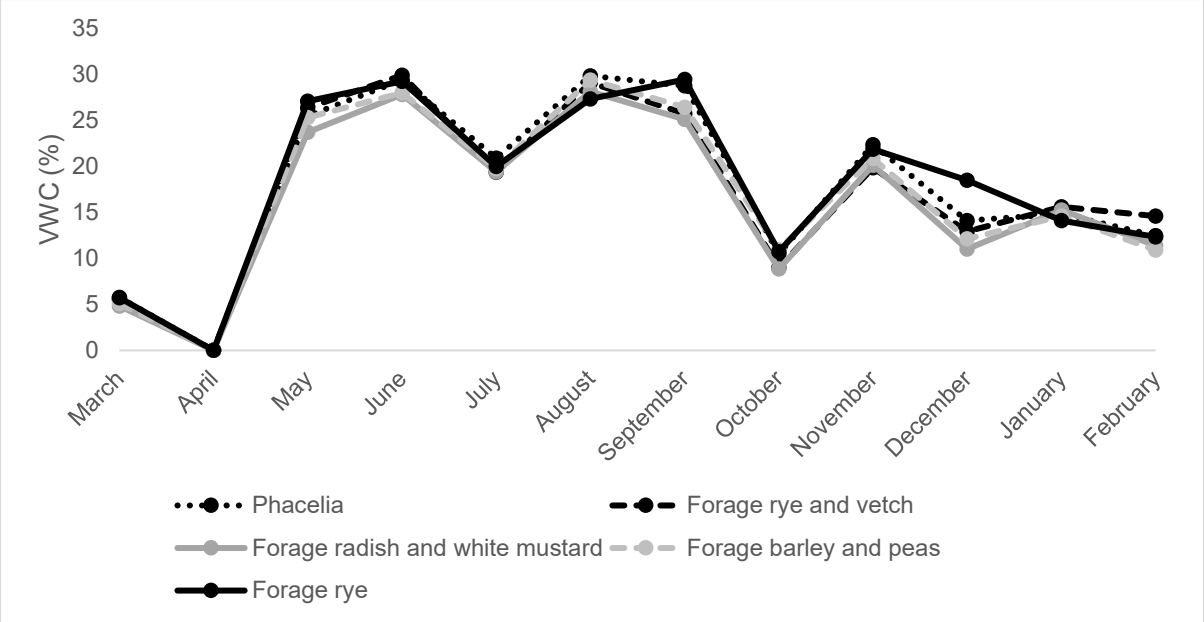

Figure 25. Monthly volumetric water content for trial 3, from March 2020 to February 2021, Glen Elgin farm.

Figure 26. Monthly volumetric water content for trial 3, from March 2021 to October 2021, Glen Elgin farm.

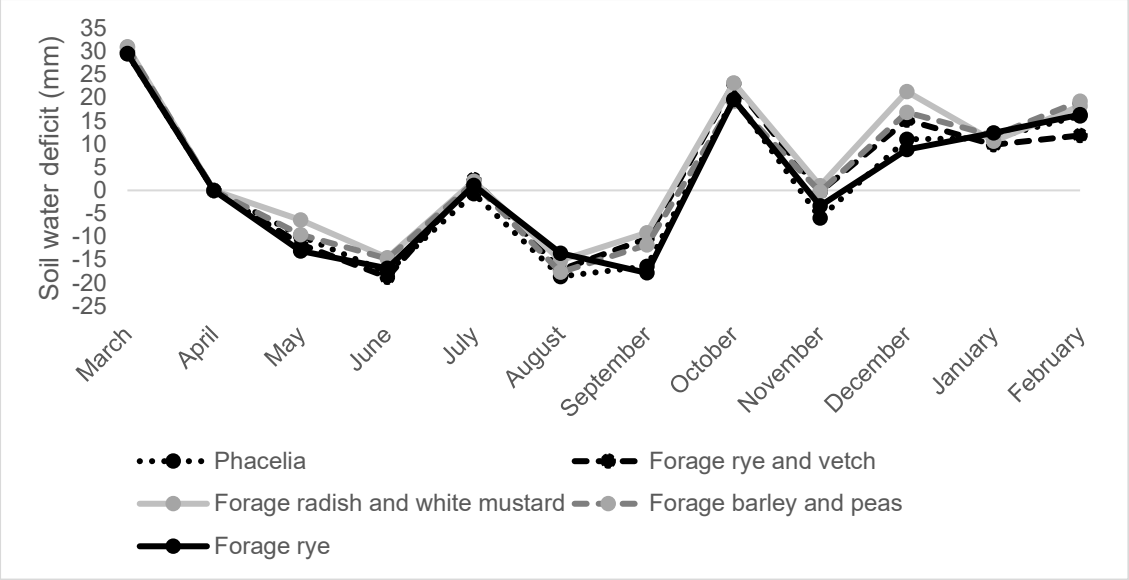

Figure 27. Monthly soil water deficit for trial 3, from March 2020 to February 2021, Glen Elgin farm.

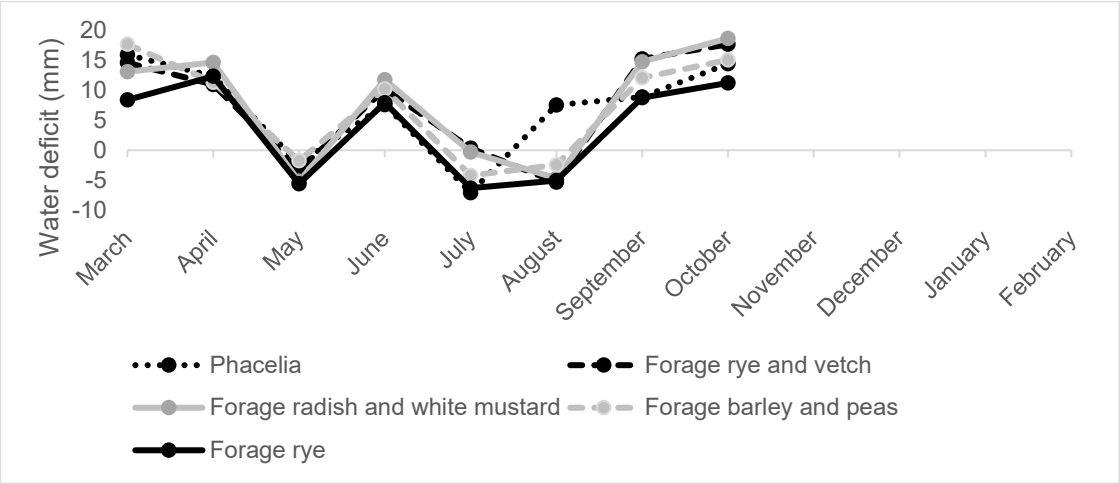

Figure 28. Monthly soil water deficit for trial 3, from March 2021 to October 2021, Glen Elgin farm.

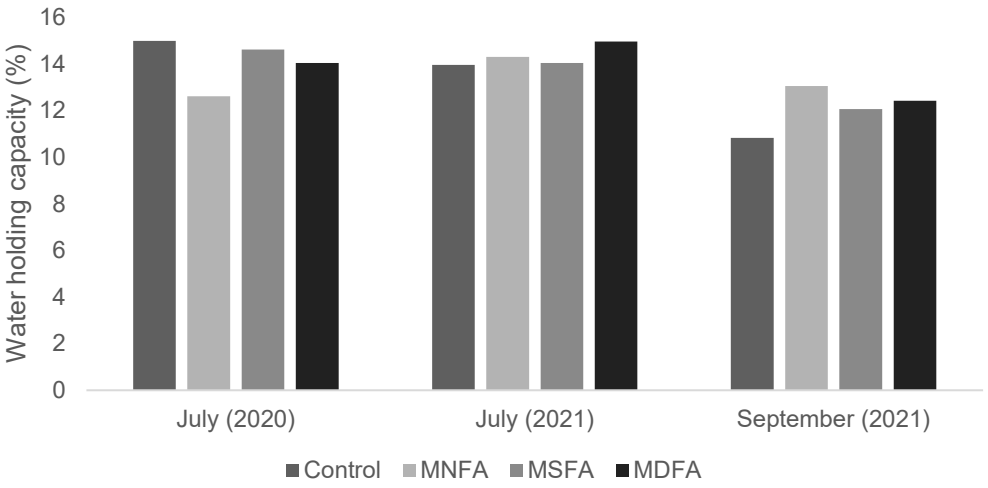

Figure 29. Water holding capacity for trial 1, Welgevallen Research farm.

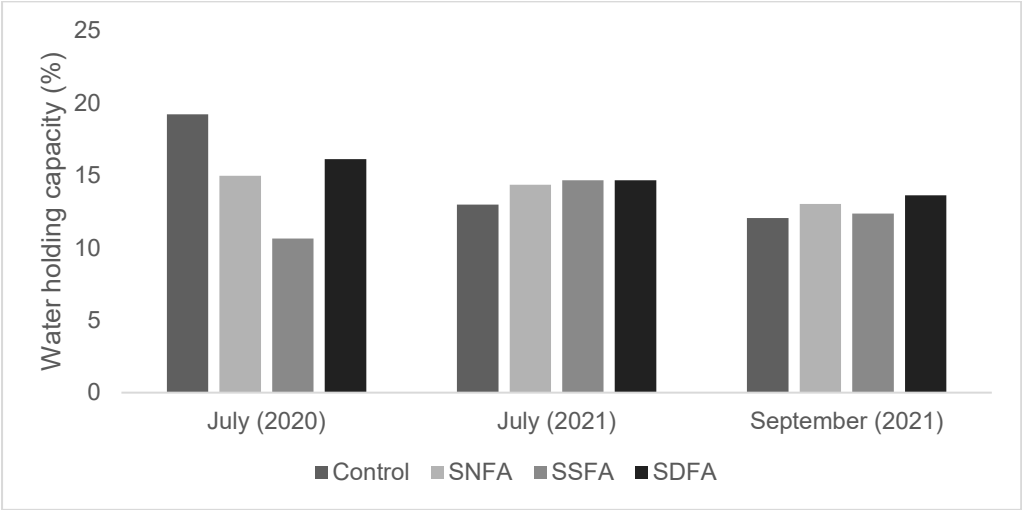

Figure 30. Water holding capacity for trial 2, Welgevallen Research farm.

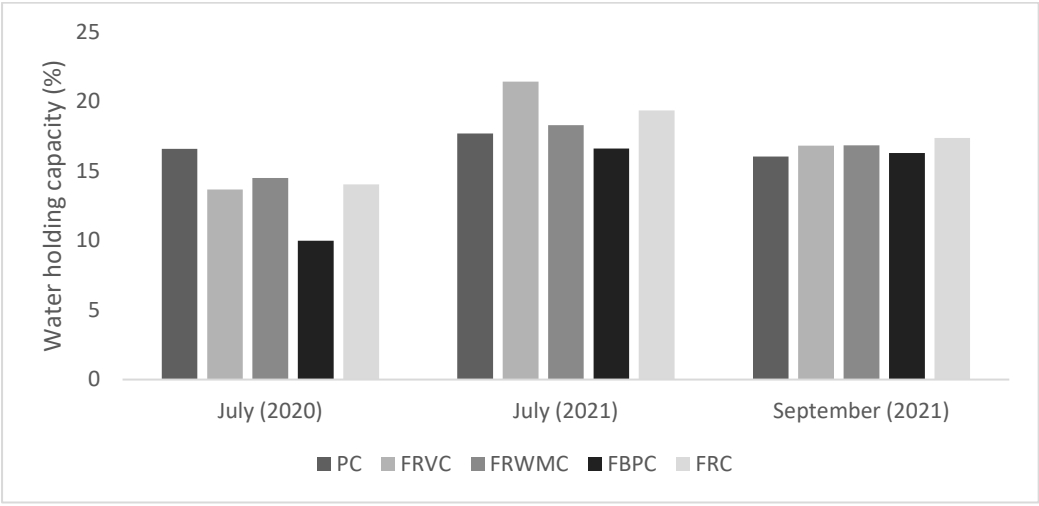

Figure 31. Water holding capacity for trial 3, Glen Elgin farm.

Addendum B

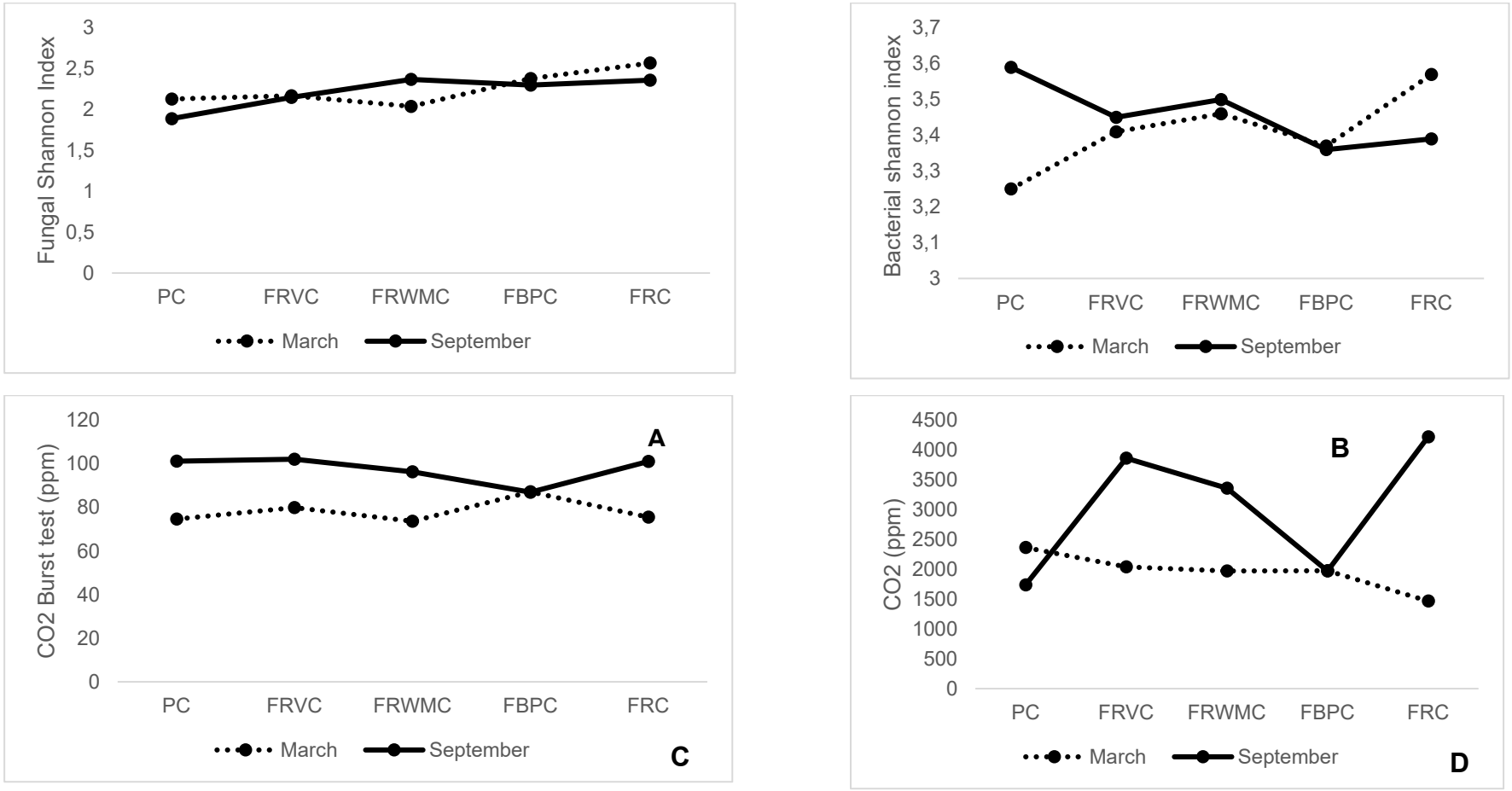

Figure 1. Different methods used to quantify microbial community. A) Molecular fingerprinting technique (fungal community). B) Molecular fingerprinting technique (Bacterial community). C) Solvita CO_2 – Burst test D) Gas chromatography.