Soil health and the soil biota

JJ Bezuidenhout

E-mail: jurgbez@gmail.com

ABSTRACT

Agricultural practices are reviewed in relation to soil health. Classical approaches to improve soil health, i.e. biological control of *Phytophthora cinnamomi* and soil solarization, did not meet the expectations. Although appropriate mulching and cover crops is usually beneficial to avocado plants, few studies paid attention to the physical, chemical and biological properties of the soil. Tremendous developments are touched upon, which include soil microbes, fungi and fauna. The magnificent advances in gene technology, biochemistry and a better knowledge into the soil biota, pave the way to improve soil health and yield.

INTRODUCTION

With success of *Phytophthora* control in the early 1980s, meaningful disease/pest control and horticultural practices could be implemented. The third development phase in avocado production, with the improved yields and lower price, was that cost management became crucial. Simultaneously consumers became more concerned about safe, healthy food and caring about environment where the commodities were produced. Farm practices accordingly shifted to sustainable agriculture with the conservation of non-renewable resources such as soil, water and minerals, and the maintenance or restoration of the surrounding ecology.

The Soil Health fit comfortable in with sustainability, a concept introduced to South African avocado farmers by Pieter Pieterse in 2011 (Nzanza & Pieterse, 2011; 2012 & 2013). The idea of soil health was developed by a team at the Cornell University (Gugino *et al.*, 2009; Moebius-Clune *et al.*, 2017). Soil health refers to the biological, chemical and physical features of soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. Thus, soil health provides an overall picture of soil functionality. Although it cannot be measured directly, soil health can be inferred by measuring specific soil properties (e.g. organic matter content) and by observing soil status, e.g. fertility.

The aims of this paper are:

- Summarise the soil health (SH) concept.
- Review research been done on avocado related to SH.
- Summarise recent advancements in soil ecology.

The Soil Health concept

Soil health (SH) is established through the interactions of soil's physical, chemical and biological properties. The state of SH are defined by the overlap of these three components for a specific plant (Fig. 1) while Table 1 summarise the main features of the SH concept.

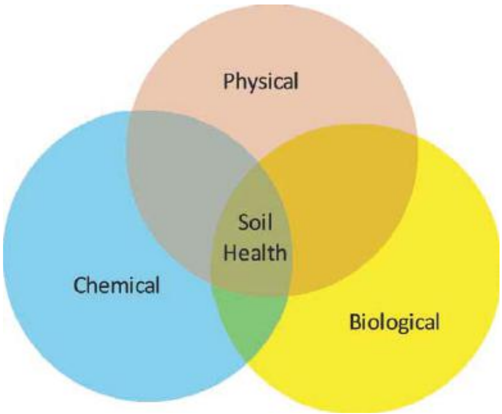

Figure 1. Diagrammatical representation of the physical, chemical and biological parameters that define soil health.

Avocado research related to soil health

Although the physical and chemical properties of ideal avocado soils are well defined, biological factors of soil, with a few exceptions, are poorly understood. Due to its devastating effect of *Phytophthora cinnamomi* on avocado health, almost all the research was directed to the control of this pathogen, while soil health *per se* was not the purpose of the studies. Majority of the investigations concentrated on the nature of suppressive soils, antagonists towards *P. cinnamomi*, mulching and other soil amendments to improve avocado health or yield.

Avocado root distribution

Avocado roots are not static. Soil type, root-rot and phenology of the plant, irrigation and cover crops influence root distribution through the soil profile.

A healthy avocado tree in a homogenous sandy soil with a low bulk density has roots that are uniformly distributed, vertically and horizontally. However, where *Phytophthora* infected the roots, the distribution became discontinuous horizontally and

Table 1. Description of the main abiotic and biotic parameters which define Soil Health.

Physical parameters	Biological parameters	Chemical parameters
Available water capacity	Total amount of organic matter	Standard soil test analysis of pH and plant nutrients
Surface hardness at a depth between 0 and 15 cm	Amount of organic matter easily metabolized by microbes	Salinity and sodicity: Salinity is a measure of the soluble salt concentration in soil. Sodicity is the sodium absorption ratio calculated from Na^+, Ca^{2+}, and Mg^{2+} concentration
Subsurface hardness at a depth between 15 and 45 cm	Amount of protein	Levels of metals detrimental to human or plant health
Aggregate stability - how well soil aggregates resist disintegration when hit by rain drops	Soil respiration rate	
	Potentially mineralizable nitrogen: It is a combined measure of soil biological activity and substrate to mineralize nitrogen to make it available to plants.	

with depth. In soils where the bulk density is above 1,7 g/cm^3, avocado roots did not effectively penetrate from one soil texture and structure into another and the root mass was very low compared to sandy homogenous soils (Durand & Claassens, 1987).

Salgado and Toro (1995) observed the number of roots found in fall is more than double the number found in the other seasons, when the roots are in their first phase of rapid growth. Heavy soils obtained 25% more roots than light ones, and 30% more roots were counted in trees under drip irrigation than those irrigated by micro-sprinkler. In all cases more than 70% of the active roots were found deep (50-75 cm) and in the intermediate soil layer (25-50 cm), 170-220 cm apart from the trunk. Maximum total density of the active roots (TDAR) was found in fall, in the superficial soil layer of heavy soil. This was reduced to a sixth in spring, and later in summer increased to a half of TDAR in fall. A similar variation pattern was observed for light soil, even though fall TDAR presented a half as many roots as were found in heavy soil. With micro-sprinklers, the superficial soil layer hold 15% of total roots as a maximum, with the exception of light soil in fall (44%) and spring (31%). Additionally, the observed TDAR is 80% greater in light soils. These facts allows to suggest that the micro-sprinkler irrigation system restricts root growth in heavy soils, possibly as a consequence of an unfavourable air/water balance in soil, which limits the interchange of gases and the accumulation of inadequate substances derived from anaerobic reactions. No roots were found in the first, most humid soil layer of the sectors closest to the micro-sprinkler in heavy soils.

Similar to the type of irrigation, soil properties and *Phytophthora* infections, cover crops influence root distribution. Atucha *et al*. (2012) reported on the effect the mixed cover crops, *Medicago polymorpha* and ryegrass (*Lolium rigidum* var. *wimmera*), had on roots. The authors observed that avocado trees in the bare plots (i.e., no cover) had more roots of a bigger diameter (>0.2 mm) in the top 30 cm of soil, while trees in temporary and permanent ground cover plots had more roots in the 30-60 cm depth of a smaller diameter. Root production was higher in a non-bearing year than in the bearing year. Lifespan of spring-produced roots was greater than those produced during autumn and summer, and soil depth and root diameters were positively correlated with root longevity. Lifespan of thinner roots (<0.2 mm) in the bare plots and temporary ground cover plots were greater than those in the permanent ground cover plots. The authors concluded that avocado trees grown in contrasting conditions compared with their native habitat, show high morphological root plasticity, in response to resource and non-resource competition when grown in mixed stands.

Note: *Medicago polymorpha* forms a symbiotic relationship with the bacterium *Sinorhizobium,* which is capable of nitrogen fixation. Velvet bean (*Mucuna pruriens*), often used as a cover crop by avocado growers, has the potential to contribute to soil N. It has often been assumed that Mucuna will freely nodulate, fix N2 and therefore contribute to soil N, however, it is not the case and inoculation with compatible symbiotic *Rhizobium* is required for N fixation (Houngnandan *et al*., 2000).

Soils suppressive to P. cinnamomi and antagonists of Phytophthora

The impact of microbes on plant health is evident, most clearly in disease-suppressive soils. The microflora of most soils is starved. As a result, there is a fierce battle in the rhizosphere between the microorganisms that compete for plant-derived nutrients (Raaijmakers, 2009). Most soil-borne pathogens need to grow saprophytically in the rhizosphere to reach their host or to achieve sufficient numbers on their host before they can infect host tissue and

effectively escape the rhizosphere battle zone. The success of a pathogen is influenced by the microbial community of the soil in which the infection takes place. Every natural soil has the ability to suppress a pathogen to a certain extent. This can be deduced from the disease severity following pathogen inoculation in pasteurized soils compared with non-pasteurized soils. Avocado soils are no exception, as the following research illustrate. The same is true for antagonists toward *P. cinnamomi* where many species were isolated. However, with in vivo tests the antagonist either did not control the pathogen or only for a limited period of time.

The phenomena known as *P. cinnamomi* suppressive soil was first reported by Broadbent *et al*. in an avocado orchard at Tamborine Mountain on the east coast of Australia in the early 1970s (Broadbent, Baker & Waterworth, 1971). Later Baker & Cook (1974) defined a disease suppressive soil in which either the pathogen cannot establish, becomes established but fails to produce disease, or becomes established and causes disease at first but diminishes with continued cultivation of the crop.

The discovery of soils that suppress disease, even in the presence of *P. cinnamomi,* led to an increased interest in soil ecology (Malajczuk, 1983). Malajczuk & McComb (1979) and Weste & Vithange (1977) found microbial populations to differ qualitatively and quantitatively, with greater numbers occurring in suppressive soil. Contradictory to this, Maas & Kotzé (1989) found greater number of microbes to occur in the diseased soils. However, each soil harbored specific dominant genera. *Trichoderma* spp and *Gliocladium roseum* occurred in greater numbers in each of the diseased soils, whereas *Penicillium* species were predominantly associated with the three healthy soils.

Greater numbers of pseudomonads were also found to occur in the *P. cinnamomi* diseased soil and the soil collected from the stunted tree. Pseudomonads have been reported to stimulate sporangium formation of *P. cinnamomi* (Marx & Haasis, 1965). Malajczuk (1983) showed that there was a correlation between the number of antagonistic bacteria and actinomycetes and the suppressive potential of soils. The results of Maas & Kotzé (1989) study corroborate this tendency, as a significantly greater number of antagonistic bacteria and actinomycetes were found to occur in the healthy soils.

Maas & Kotzé (1989) reported that fungi isolated from avocado soil in general were more antagonistic in vitro to *P. cinnamomi* than bacteria and actinomycetes.

Botha *et al*. (1989) compared different in vitro screening techniques to evaluate four avocado rootstocks for susceptibility to *P. cinnamomi*. Attraction of *P. cinnamomi* zoospores by exudates from excised avocado roots, diffusing through a dialysis membrane, accurately reflected tolerance or susceptibility and eliminated the need for laborious processing of roots for microscopy. Similarly, lesion development on detached roots and leaves, after inoculation with the pathogen, correlated well with field performance of the various rootstocks, yet was simple to perform.

From soil where avocados showed exceptional growth in presence of *P. cinnamomi*, ninety five bacteria were isolated, and of those, nine precluded the pathogen to invade detached avocado roots in vitro but none in artificial infected soil (Maas & Kotzé, 1990).

Duvenage *et al*. (1991) found, of the 48 soils evaluated in Tzaneen area, 12 significantly reduced root rot of blue lupine seedlings in comparison to the control treatments. When sampled again, together with soil from diseased trees from each orchard, evaluation of the 12 soils showed eight to consistently and significantly reduce root rot. Further investigations into the nature of these soils were abandoned or not published.

A strain of *Myrothecium roridum* proved to be the most active antagonist in controlling *P. cinnamomi* in repeated greenhouse pot tests with highly susceptible seedlings of *Persea indica* inoculated with *P. cinnamomi. M. roridum* was grown on a wheat-bran medium and introduced into a peat-perlite mixture at 2.5% (w/v) two weeks before inoculation with *P. cinnamomi*. In a medium with *P. indica* inoculated with zoospores of *P. cinnamomi*, *M. roridum* suppressed root infection by 50 to 94% compared with uninoculated controls. In the same experiments there was no significant difference in the level of control achieved by either *M. roridum* or the fungicide potassium phosphonate (2.5 mg/pot). In three naturally-infested field soils, root infection ranged from 12 to 54% in the presence of *M. roridum*, compared with 58 to 93% for controls over the same 4-week period. On a selective medium containing carbendazim, a fungicide-resistant mutant of the of *M. roridum* strain was isolated consistently from the root tips of *P. indica* growing in infested soil four weeks after transfer, demonstrating the apparent rhizosphere competence of this strain in the three soils (Coffey & Gees, 1992).

The use of alginate pellets imbedded with skim milk powder and antagonistic bacteria proved much more effective than a seed dip of 'Edranol' seed into the same alginate, skim milk powder and bacteria suspension (DV Van der Merwe, 1992).

Duvenage & Köhne (1995) found three fungal antagonists, *Paecilomyces filacinus*, *Aspergillus candidus* and *Trichoderma hamatum*, isolated from suppressive soil were effective in suppressing root rot. These antagonists have been evaluated since 1992 for control of root rot in avocado trees in the field. Populations of the antagonists have been found to increase in the root zone of newly planted trees (after antagonist treatment in the nursery and in the orchard) and in the root zone of 15-year old 'Hass' and 'Fuerte' trees after antagonist treatment in the orchard. These findings were not substantiated in long term studies.

Eight *Trichoderma* isolates that overgrew and halted growth of *P. cinnamomi* in vitro were evaluated in the green house for biological control of *Phytophthora* root rot of avocado seedlings grown in pre-sterilized pine bark medium. Millet seed inoculum of *Trichoderma harzianum* (C4 and BB5) and *T. hamatum* (F56) significantly reduced root rot and stimulated root

regeneration of seedlings. *T. harzianum* (BB5) isolated from pine bark, caused an increase in root mass of avocado seedlings grown in the absence of *P. cinnamomi*. Pc populations were significantly reduced by C4 and F56, but not by BB5 (McLeod, Labuschagne & Kotzé, 1995).

Of the four carrier substrates (millet seed, peat, composted citrus waste and composted pine bark) tested for sustaining antagonist for controlling *P. cinnamomi*, the highest colonization was obtained with millet seed at 60% water content (Landman, Labuschagne & Kotzé, 1996).

Multiple applications of *T. harzianum* eliminated the effect of *Phytophthora* in the greenhouse, whereas *B. megaterium* was ineffective. In the field, the best effect on plant growth was obtained with *T. harzianum* in combination with solarization, followed by application of 4 kg composted citrus waste per tree (Landman *et al*., 1997).

Avocado plants that received antagonistic endophytes prior to *P. cinnamomi*-infection showed a significant decrease in disease incidence with ratings from 2-40%, compared to 94-100% for the control plants (Hakizimana *et al*., 2011).

In a study at the north coast of Peru, treatments with *Trichoderma* sp., followed by *Trichoderma harzianum*, have proven to have the highest percentage of healthy roots 60 days after inoculation with *P. cinnamomi.* These two isolations were taken from healthy avocado trees of Chavimochic Irrigation area. In general, local strains have shown the best control while strains introduced from other areas have responded poorly (Apaza *et al.*, 2015).

Alternative approach to treat the plants with antagonists to *P. cinnamomi* exists, e.g. apply antagonistic substances or inoculate nursery seedlings excised with mycorrhiza colonized avocado roots. An example of the first approach is where volatiles produced by rhizobacteria (bacteria in the region of root) were found to inhibit *P. cinnamomi* in vitro (Me´ndez-Bravo *et al*., 2018). The latter approach is much more challenging (Shu *et al*., 2016) though promising results were obtained paving the way for future developments.

Soil solarization

Although solar solarization reduce the incidence of soil-borne pathogens, inoculum levels of other microbes also decrease, therefore reinfestion is often more severe than prior to solarization.

Gallo-Llobet & Siverio (1995) conducted two field trials to evaluate soil solarization effectiveness in controlling avocado root rot. Results showed 88% of avocado and 92% of *P. indica* survived in the solarized plot (covered with transparent polyethylene for 4-6 weeks); control showed 21% for avocado and 8% for *P. indica* survival.

López *et al*. (1995) found solarization of 5-8 weeks achieved good control of *P. cinnamomi* infecting rootlets in the 10-20 cm upper soil layer, but the pathogen remained viable in deeper layers from which a recolonization was initiated. That accounts for the reinfestation of the upper layer of soil noticed after ca. 1 yr since the beginning of solarization.

Cover crops

Avocado farmers are well aware of the benefits of cover crops on soil structure and texture. Less known is the use of an oat strain that produce avenacine as a cover crop for avocados which showed promising results (Bezuidenhout, unpublished).

In laboratory experiments, roots of oats (*Avena sativa*) and the grass *Arrhenatherum elatius* caused attraction and subsequent lysis of zoospores of *Allomyces arbuscula, Aphanomyces* sp.*, Phytophthora cinnamomi, Pythium aphanidermatum, P. arrhenomanes, P. graminicola, P. intermedium, P. ultimum* var. *sporangiferum* and *Saprolegnia litoralis*. The lytic compound, thought to be the saponin avenacin, was released from apparently undamaged oat roots. It prevented cyst wall formation by zoospores, but it was only weakly active or inactive against pre-encysted zoospores and vegetative mycelia of *Pythium* spp. Its effects on zoospores were paralleled by the saponin ß-aescin. In preliminary tests, these compounds retained activity after passage through, or incubation in, soil (Deacon & Mitchell, 1985).

Mulching and soil amendments

Extensive literature exists on the mulching of avocado trees. In general, mulching had a beneficial effect on the tree condition if combined with chemical *P. cinnamomi* control. One research group reported that soluble silicon inhibit *P. cinnamomi* to the same extend than phospite/phoshonate. However, these findings have yet to be commercially verified.

On a silt-clay-loam soil (the soils are usually alkaline with a high potassium content) in an avocado growing region north of Los Angeles, *Eucalyptus* mulch and gypsum did not affect 'Hass' avocado and the mulched trees had increased diseased symptoms while gypsum had no effect on disease ratings (Faber *et al*., 1995).

Duvenage *et al*. (1993) reported on avocado trees (Tzaneen region) which had recovered from root rot that received different types of organic amendments, while chemical treatment was discontinued. Condition of trees from all treatments declined significantly during the third and fourth year of the study, possibly as a result of drought stress. The yield of trees receiving no treatment at all, or only cattle manure, declined significantly after three or four years. However, legume cover crop (*Dolichos lablab*) or lucerne straw mulch (on its own or combined with cattle manure) seemed to inhibit yield decline. However, the researchers cautioned the results and it must be seen in the light of the drought during the study and may differ in years of normal rainfall.

'Hass' root health improved by the application of coarse pine-bark mulch and calcium acetate crystals, 500 g every month, with trials in KwaZulu-Natal Midlands. Rate of fruit growth and total growth were significantly greater on the mulch treatment. At harvest, the mulch treatment resulted in a significant increase in mean fruit mass, mean fruit number per tree and total yield (Moore-Gordon *et al*., 1995).

In a subsequent report, Moore-Gordon *et al*. (1997) found over three seasons, mulching elevated average fruit yields by 22,6%, and increased mean fruit mass by 6,6%. The number of fruit that were considered highly suitable, and acceptable for export, were increased by 45% and 20% respectively. Initial costs of the pine bark were off-set within two seasons, thus providing growers with a practical means of boosting financial returns, especially since pine bark is considered to have a half life of approximately five year.

Wolstenholme *et al*. (1996) concluded that the most suitable mulches are mulches with C : N ratio of more than 25 : 1, but less than 100 : 1; fibrous, stalk, straw materials with a moderate rate of breakdown; and composted, chunky pine barks.

Mavuso & Willis (2007) evaluated in pot and Tzaneen field trials the effect of different mulch materials, compost and organic products on tree condition and root health. In the pot trial, ungrafted Duke 7 seedling plants were transplanted into a soil mixture and inoculated with *P. cinnamomi*, then treated with different mulches. The trial was evaluated after eight months. Root health was significantly improved by the application of gypsum, pine bark / antagonist mix, coarse eucalyptus wood chips and Braak's pine bark medium, when compared to the control. In the field trial, tree health ratings were done at the start of the trial in August 2005 and mulches were applied in October 2005. Small differences between treatments have been noted in the tree health ratings done in August 2006. Root health and density were assessed by means of digital photographs. Soil moisture fluctuations underneath the mulches were monitored with tensiometers. Initial results indicated that under wet conditions the sawdust mulch was saturated and this lead to higher soil moisture readings when compared to other mulches. Under dry conditions water did not easily reach the soil beneath the mulches when compared to the control. *P. cinnamomi* infestation in the soil underneath the mulches was determined and the lowest inoculum of *P. cinnamomi* was found associated with avocado wood chips. Leaf mineral analysis results indicated that partially composted Westfalia compost and cattle manure resulted in below normal nitrogen levels in the leaves.

In the following year, Mavuso (2008) reported on a field trial that was established in August 2005 with 'Lamb Hass' to evaluate the effect of different mulch materials, compost and organic products on tree condition and root health: Wood shavings, Compost tea / Cattle manure Foliar, 50% mature Westfalia compost, Fully mature Westfalia compost, *Eucalyptus* wood chips, Westfalia compost / *Eucalyptus* wood chips (1:1), Saw dust, Cattle manure, Avocado wood chips, Organic gypsum + Avocado wood chips, and untreated control (natural mulch removed). Between 2005 and 2007 a general decline in tree health rating was observed in all treatments except in the treatment with avocado wood chips. Under wet conditions the sawdust mulch was saturated and lead to higher soil moisture readings. Under dry conditions, water did not easily reach the soil beneath the mulches when compared to the control. In 2006 the lowest level of *P. cinnamomi* inoculum was found associated with avocado wood chips, which was continued in 2007, as well as where avocado wood chips were applied as mulch in combination with gypsum. The use of avocado wood chips, alone or in combination with gypsum, might reduce the levels of *P. cinnamomi* present in the soil.

Three applications of soluble potassium silicate per season resulted in significantly higher concentrations of crude phenolic compounds in the roots, compared to the untreated control and potassium phosphonate (Avoguard) according to Bekker *et al*. (2007).

THE SOIL BIOTA

The five distinct habita of trees are: branches, canopy (leaves – phyllosphere), mulch layer, rhizosphere (region of the roots) and soil without roots. Each of these habita harbor a specific community of organisms which include prokaryotes (without a nuclear membrane – bacteria and archaea) and eukaryotes (with a nuclear membrane – fungi, plants e.g.

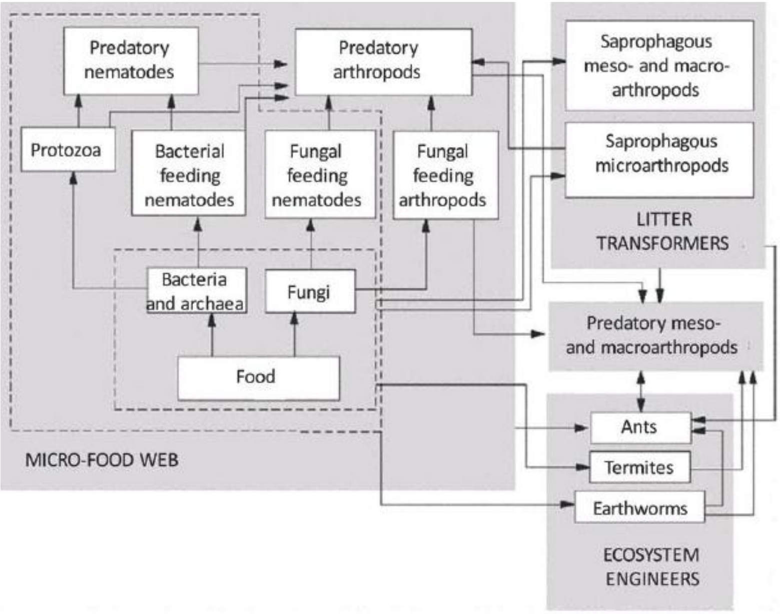

Figure 2. Organization of the soil food web into three categories – ecosystem engineers, litter transformers and micro-food webs. (After Wardle, 2002, and Lavelle *et al*., 1995.)

algae and microfauna – insects, spiders, nematodes etc.). Soil biota consists of the organisms which occupy mulch, rhizosphere and the surrounding soil.

Complex Interactions exist between the communities in a habitat with other habita. Modifying the community structure in the rhizosphere results in change in the phyllosphere organisms, and vice versa. Ecological studies and related fields of research between plants and the associated biota are therefore extremely challenging, however most rewarding.

The food web of soil biota illustrates activities and interplay between the various groups (Fig. 2).

Prokaryotes – Bacteria and Archaea

Significant breakthroughs in molecular biology the last thirty years, with advances in automated-gene sequencing, allow the characterization of a greater proportion of the soil biota. Less than 1-5% of the soil microbes can be cultured. Though advances in analysis of enzymes provide information regarding the microbial diversity, physiology and biochemistry led to the conclusion that soil is the greatest repository of genes in nature.

Studies based on sequences of 16S rRNA and 18S rRNA distinguishes three groups of life - bacteria, archaea and eukaryotes (fungi, plants and animals). 16S rRNA is present in the small subunit of bacteria and archaea ribosomes as well as mitochondrial ribosomes in eukaryotes. 18S is the homologous small subunit rRNA of eukaryotes. In terms of phylogenetic distance and biochemistry, archaea and bacteria are as distinct from each other as they are from eukaryotes (Woese *et al*., 1990). Despite the huge difference between bacteria and archaea, they are conveniently called prokaryotes.

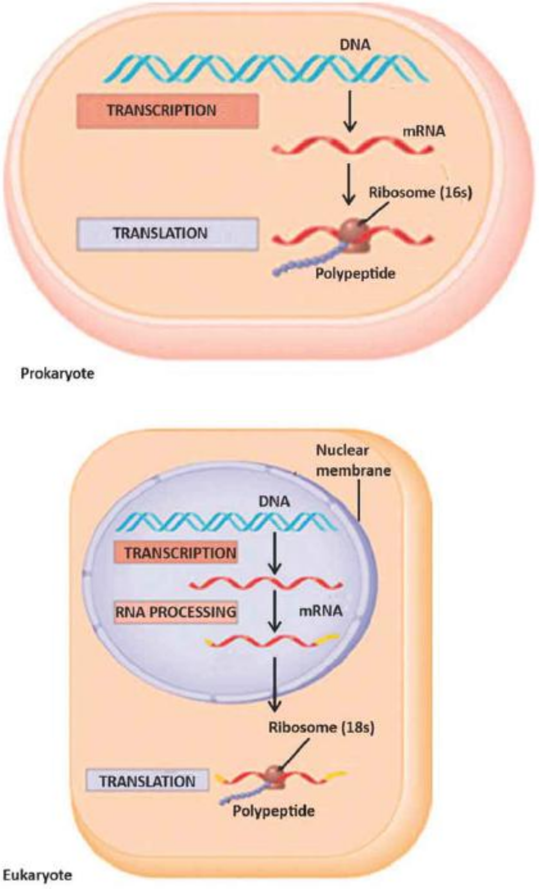

Figure 3. Difference in structure and mechanism of polypeptide synthesis between a prokaryote and eukaryote.

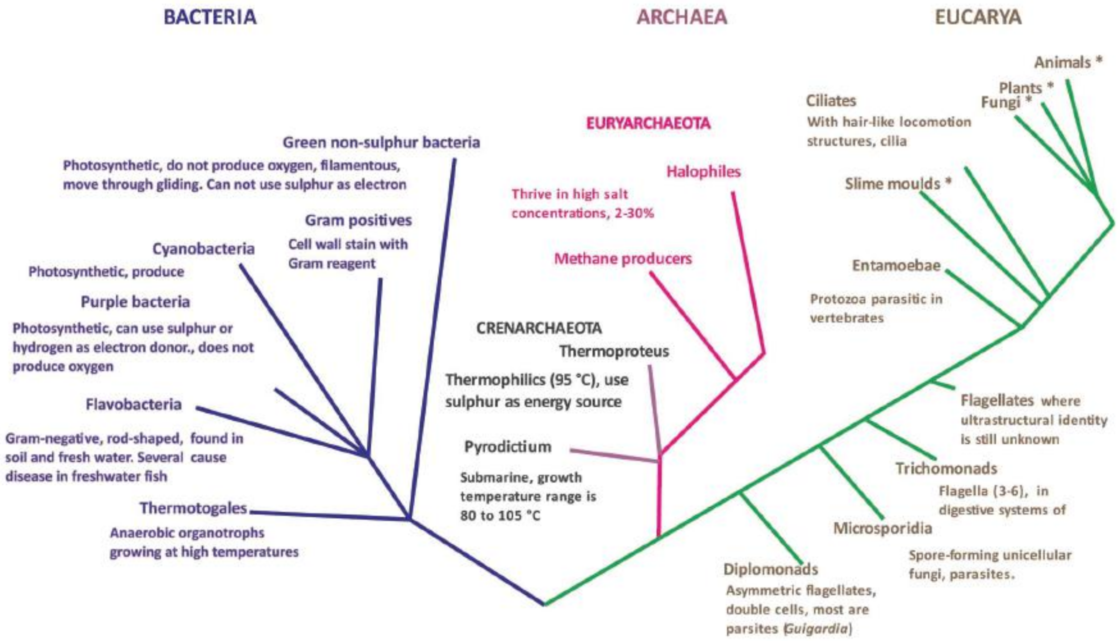

Figure 4. The universal tree of life constructed by sequence analysis of single subunit rRNA genes, redrawn and adapted from Wheelis *et al.* 1992. Groups with an asterisk (*) do have some macroscopic members.

Runner™ 240 SC

INSECTICIDE

FALSE CODLING MOTH • BOLLWORM • CODLING MOTH

Avocados, Apples, Citrus, Cruciferae, Cucurbits, Lettuce, Litchis, Pears, Peas, Peppers, Spinach, Tree Nuts

- Target-specific control with unique insect growth regulating mode of action
- Acts effectively against eggs and larvae with its ovicidal and larvacidal action
- Larvae stop feeding within 4-8 hours after ingestion
- Not photo sensitive; does not degrade under direct sunlight
- Recommended rates will provide long lasting residual control
- Low risk for bees and brood
- Low risk to beneficial insects and mites
- Suitable for use in IPM systems

Presidential Green Chemistry Challenge Award
EPA classification as a "Reduced Risk Pesticide" in U.S.A

CortevaZA on Facebook
CortevaAME on Twitter
@Corteva on Instagram

For more information contact the registration holder: Dow AgroSciences Southern Africa (Pty) Ltd Reg. No. 1967/007147/07 • Paarl (021) 860 3620 • Centurion (012) 683 5700 • Emergency No. 082 887 8079 • Maxwell Office Park, Magwa Building, Ground Floor, Magwa Crescent, Waterfall City, MIDRAND, 1686 South Africa • corteva.co.za

ALWAYS USE AS PER LABEL RECOMMONDATION • Runner™ 240 SC contains methoxyfenozide (Caution) Reg. No. L7779 | Act No. 36 of 1947 • Runner™ is a registered trademarks of Dow AgroSciences LLC

Dow AgroSciences

Solutions for the Growing World

® ™Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow

Soil bacterial and archaeal communities are now characterized by analysis of 16S rRNA. The results showed that bacteria that were previously considered to be typical soil inhabitants, such as pseudomonads and actinobacteria, are often rare, whereas many novel, yet to be cultured organisms are allmost everywhere and dominant (Rappe & Giovannoni, 2003). Similarly, archaea were considered to be extremophiles, adapted to conditions atypical of most soils (high temperature, acidity, salt concentration and anaerobic), but one group, the crenarchaea in general, represent 1-2% of soil prokaryote communities (Buckley & Smith, 2003) and even colonize plant roots (Simon *et al*., 2005).

It is now possible to isolate a single bacterial or archaeal cell to amplify and sequence its DNA, and to reconstruct its genome without cultivation. This approach enables investigation of metabolic potential of uncultivated organisms.

Diversity is critical to the maintenance of soil health and productivity, driving many functions that determine productivity. The prokaryotes carry out all the metabolic processes that occur in eukaryotes, but they also generate energy in several other unique ways (Table 2).

Proteobacteria is a major phylum of gram-negative bacteria, i.e. their cell walls do not stain when treated with the Gram-solution; they include a wide variety of animal pathogens. Others are symbionts of plants, while others are free-living.

The Firmicutes are bacteria, most of which have gram-positive cell wall structure. A few, however, have a porous pseudo-outer membrane that causes them to stain gram-negative. Scientists once classified the Firmicutes to include all gram-positive bacteria, but have recently defined them to be of a core group of related forms called the low-Guanien+Cytidine (building blocks of DNA) group, in contrast to the Actinobacteria. They have round cells, called cocci (singular coccus), or rod-like forms (bacillus). Many Firmicutes produce endospores, which are resistant to desiccation and can survive extreme conditions. They are found in various environments, and the group includes some notable pathogens. Those in one family, the heliobacteria, produce energy through anoxygenic photosynthesis.

Actinobacteria is Gram-positive bacteria. They are of great economic importance to humans because agriculture and forests depend on their contributions to soil systems. In soil, they behave much like fungi, helping to decompose the organic matter of dead organisms so the molecules can be taken up anew by plants. In this role the colonies often grow extensive mycelia, like a fungus would, and the name of an important order of the phylum, Actinomycetales. Some soil Actinobacteria lives symbiotically with the plants whose roots pervade the soil, fixing nitrogen for the plants in exchange for access to some of the plant's sugars. Other species, such as many members of *Mycobacterium* (cause tuberculosis) are important pathogens. Actinobacteria is one of the dominant bacterial phyla and contains one of the largest of bacterial genera, *Streptomyces*. *Streptomyces* and other Actinobacteria are major contributors to biological buffering of soils. They are also the source of many antibiotics. Some of the largest and most complex bacterial cells belong to the Actinobacteria.

Soil prokaryotes are extremely versatile in the utilization of a variety of substrates. A few selected examples of unusual substrates are: cellulose, lignin, chitin (exoscelet of insects), xylan (organic solvent similar to benzine), petroleum oil and kerosene.

These substrates, and the more easily metabolized nutrients, can be sequestered at extremely low concentrations due to the small size of prokaryotes. The smaller the size, the higher is the surface area to volume ratio (A/V). Most prokaryotes are smaller than eukaryotes, ranging from one to ten microns. Although the internal solute concentrations and pH of prokaryotes are governed by homeostatic principles, prokaryotes respond rapidly and are influenced more by external fluctuations than eukaryotes due their high A/V. Therefore studies must take into account the micro-environment on the micron scale under which prokaryotes operate.

Table 2. Unique forms of prokaryote energy production and related metabolism.

Metabolic system	Basis of metabolism
Anaerobic respiration	Respiration that use alternatives to oxygen as electron acceptors, such as sulphate and nitrate
Prokaryotic fermentation	Produce pyrovate from glucose whereas eukaryotic yeast produce ethanol
Lithotrophy	Use inorganic sources of energy, such as iron, sulfur or ammonium
Photoheterotrophy	Photosynthesis where organic compounds are used as carbon sources
Anoxygenic photosynthesis	Photosynthesis without the production of oxygen
Archaean methanogenesis and light driven formation of ATP (Note: ATP is the universal energy "currency" for all life).	Unique archaean metabolism, using H_2 as an energy source in methane production and conversion of light into chemical energy
Alternative autotrophic CO_2 fixation	Fixation of carbon dioxide through a different metabolic pathway than plants or algae

The small size of prokaryotes bring along its technical and conceptual challenges. Individual prokaryotes are difficult to study, thus populations are used. On the other hand, the spatial heterogenity of soil with the limited transport of nutrients and prokaryotes through soil, require investigation of biotic-abiotic interactions on individual basis.

Fungi

A thousand million years ago fungi were instrumental in the colonization of land by the ancestors of terrestrial plants and the termination of organic carbon into geological reserves, i.e, fossil fuels. These illustrate why fungi play such important roles in soils. Most fungi actively interact with living and dead organisms, especially plants. The mycorrhizal symbiosis with plant roots has permitted aquatic plants to transition into the terrestrial habitat. Fungal interaction with living plants may be highly specific or of more general nature.

The growth habitat, external food digestion and the wide range secondary metabolites of fungi have profound influences on biogeochemical cycles. The evolution of the white-rot fungi with their polyphenolic-degrading enzymes has halted the accumulation of undecayed plant materials during the carboniferous period. The filamentous growth of soil-dwelling fungi permits them to bridge gaps between pockets of soil water and nutrients; force their way into substrates such as decaying wood; and distribute carbon, minerals and water through the soil. The filamentous growth nature may underlie the abilities of some fungi to withstand soil water deficits and cold temperature beyond the tolerance of prokaryotes. Fungi constitute large fractions of living and dead soil biomass. The growth and production of cell wall lead to the creation and stabilization of soil aggregates, which are key elements of soil structure. Rates of turnover of fungal biomass have important consequences for carbon cycling and long-term sequestration in soil.

Fungi are the main agents responsible for respiration in the soil, and competition between them plays an important role in the structuring fungal communities. For example, studies showed the arrival order of ectomycorrhizal species can shift dominance in colonization of seedling root systems (Kennedy *et al*., 2009). In general, synergetic species co-occur more often than antagonistic species.

Soil fauna

Animals, heterotrophs in soils, facilitate bacterial and fungal activity and diversity, regulate nutrient cycling by feeding directly on plant materials and organic substrates. The fragmentation materials enhance their decomposition (Garcia-Palacios *et al*., 2013). Translocation of nitrogen (N) from the soil to the substrate in the form of fecal material and through fungal hyphae. Grazing by invertebrates disseminates microbes from one organic source to another as many microbes adhere to invertebrate exoskeletons and cuticles and survive passage through their digestive tracts (Coleman *et al*., 2012).

Animal members of the soil biota are numerous and diverse and include representatives of all terrestrial phyla. Many groups of species are not described taxonomically.

The free-living protozoa (single cell eukaryotes) of litter and soils belong to four ecological groups: the flagellates, naked amoebae, testacea (outer casing) and ciliates (Lousier & Bamforth, 1990).

Only about 10% of microarthropod species have been described (Andre *et al*., 2002). While about 2 600 arthropods are known in South Africa, it represent probably less than 5% of the species in the region.

Among the microarthropods, collembolans are examples of permanent soil residents. The morphology of collembolans reveals their adaptations for life in different soil strata. Species that dwell on the soil surface or in the litter layer may be large, pigmented, and equipped with long antennae and a well developed jumping apparatus (furcula). Collembolans living within mineral soil tend to be smaller.

More than 6 500 species of Collembola are known from throughout the world and these are only a small part of the still undescribed species. Collembola have well differentiated ecomorphological life-forms and feeding guilds which enable the functional role that Collembola play in ecosystems to be recognised in some degree. Collembola play an important role in plant litter decomposition processes and in forming soil microstructure. They are hosts of many parasitic Protozoa, Nematoda, Trematoda and pathogenic bacteria and in turn are attacked by different predators. They utilize Protozoa, Nematoda, Rotatoria, Enchytraeidae, invertebrate carrion, bacteria, fungi, algae, plant litter, live plant tissues as food, and some plant pathogens. Soil acidification, nitrogen supply, global climate change and intensive farming have greatly impacted collembolan diversity.

Plant-soil biota interactions

Whereas the soil ecosystem includes both the biotic and abiotic components, "soil biota" refers solely to biotic part. Soil biota is the complete community within a specific type of soil which vary from soil to soil and with a plant species. The most dominant groups, both in numbers, variety and biomass, is represented by the microbes, i.e. bacteria, archaea and fungi. These groups exploit different nutrient strategies and lifestyles such as saprotrophs, pathogens or symbionts. The soil surface and litter layer, except microbes, also harbor a multitude fauna species, mainly insects, spiders, beetles, millipedes, centipedes and snails.

Because soil function depends on the activity of soil biota and their diversity, these have often proposed as indicators of soil health.

The rhizosphere

Life is unevenly distributed in soil, many soil microbes tend to live in aggregates to form spots of activity. One of the most important hot spots of activity and diversity is the rhizosphere (Jones & Hinsinger, 2008). Although the rhizosphere only extends a few millimeters from the root surface, it can contain up to 1011 microbial cell per gram of roots (compared to 106 in soil), with the collective microbial community being referred to as the root microbiome.

Some plants allocate 20-50% of photosynthate to roots and release other organic compounds. This provides a valuable nutrient resource for root-associated organisms. By using metagenomics techniques in the study of the rhizosphere microbiome, more than 33 000 bacterial and archaeal species were detected, with Proteobacteria, Firmicutes and Actinobactera (described under the heading, Prokaryotes) consistently associated with disease suppression (Mendes *et al*., 2011).

Metagenomics is the study of genetic material recovered directly from environmental samples where modern genomic techniques are used to study microbial communities under natural conditions.

The root biome composition change significantly with infection. Bacterial communities from healthy roots were represented by simple DNA banding profiles, suggestive of colonization by a few predominant species, and were approximately 80% similar in structure. In contrast, roots that were infected with *Phytophthora*, but which did not yet show visible symptoms of disease, were colonized by much more variable bacterial communities that had significantly different community structures from those of healthy roots. Root samples from trees receiving repeated applications of the disease suppressive bacterium *Pseudomonas fluorescens* were free of *Phytophthora* infection, and had bacterial community structures that were similar to those of nontreated healthy roots. Profiles for healthy and infected roots suggest that rhizosphere bacterial community structure may serve as an integrative indicator of changes in chemical and biological conditions in the plant rhizosphere during the infection process (Yang *et al*., 2001).

Metagenomic approaches on rhizosphere microbial studies have had a major impact on two of the main questions in microbial ecology. Which organisms are present and what is their function? On the former, relative abundances of previously reported rhizosphere bacteria have been reassessed, as well as the presence of novel bacterial groups, or previously reported as rhizosphere inhabitants. Regarding the second, functional and genetic screening of metagenomic libraries has led to several reports on new active molecules and unreported genes. The use of this approach has proven effective for the discovery of novel enzymes with unexpected activities unpredictable from their amino acid sequences.

However, there are several gaps to be filled. Can the presence of a bacterium in an environment be linked to specific activities?

The significance of arbuscular mycorrhiza

Arbuscular mycorrhiza (AM) is a classical example of a terrestrial plant symbionts with a huge impact on most terrestrial ecosystems. It is formed by ~80% of land plants and by obligate symbiotic fungi of the phylum Glomeromycota. The glomeromycotan fungi usually are called 'arbuscular mycorrhizal (AM) fungi', or 'AMF', and obviously play an enormous ecological (and economical) role. Most land plants and glomeromycotan fungi are 'joint systems', forming the intimate AM. European Bank of Glomeromycota stated: "The study of plants without their mycorrhizas is the study of artefacts; the majority of plants, strictly speaking, do not have roots – they have mycorrhizas".

AM fungi supply the vast majority of land plants with inorganic nutrients, mainly phosphorous, but also nitrogen, trace elements, and water. In return, they obtain up to >20% of the photosynthetically fixed CO_2 as carbohydrates from the plants. It was calculated that, each year, 5 milliard tonne of carbon are transferred from plants to fungi (and therefore partly get deposited in the soil) via the AM symbiosis. AM fungi therefore represent a large sink for atmospheric CO_2 on our planet and play a role in C-deposition in the soil.

Mulch/Litter layer

The interface between above ground habitat and the soil, the mulch and litter layer, influence abiotic parameters (solar irradiation, water infiltration, etc) and the microbial biomass, pathogen and fauna community structure. Soil desiccation enhanced carbon immobilization into microbial cells especially on mulched and cut/harvested sites. While due to mulching accumulation of soil, organic matter significantly increased. (Růžková, 2008).

CONCLUDING REMARKS

Classical approaches to biological control of *P. cinnamomi*, and thus soil health, did not yet to date meet the expectations. However, with phosphite/phosphonate application, nursery and horticultural practices, the pathogen is contained. Resources should be directed to improve soil health (and yield) and towards a better understanding of the soil/rhizosphere/avocado root relation.

The *arbuscular mycorrhiza* on avocados presents an exciting venue to introduce beneficial genes, while appropriate mulching is at this stage the most practical means to increase the soil organic matter and biological activity and therefore soil health.

REFERENCES

APAZA, W., VILLAVICENCIO, Y., MORENO, R. & HUALLANCA, C. 2015. Control the avocado root rot caused by *Phytophthora cinnamomi* with different isolates of *Trichoderma* in the Chavimochic irrigation project in Peru.

APPE, M.S. & GIOVANNONI, S.J. 2003. The uncultured microbial majority. *Ann. Rev. Microbiol.* 57: 269-274.

ATUCH AMAYA, MERWIN IAN A., BROWN MICHAEL G., GARDIAZABAL FRANCISCO, MENA FRANCISCO, ADRIAZOLA CECILIA, GOEBEL MARC & BAUERLE TARYN. 2012. Root distribution and demography in an avocado (*Persea americana*) orchard under groundcover management systems. CSIRO PUBLISHING: Functional Plant Biology. http://dx.doi.org/10.1071/FP12317.

BAKER, K. & COOK, R. 1974. Biological Control of Plant Pathogens. San Francisco: WH Freeman.

BÁRCENAS, A., ALMARAZ, C., REYES, L., VARELA, L., LARA, B., GUILLÉN, A., CARREÓN, Y., AGUIRRE, S. & CHÁVEZ, A. 2007. Diversity of arbuscular mycorrhizal fungi on avocado orchards from Michoacan. Proceedings VI World Avocado Congress. ISBN No 978-956-17-0413-8.

BEKKER, T.F., LABUSCHAGNE, N., AVELING, T., KAISER, C. & REGNIER T. 2007. Accumulation of total phenolics due to silicon application in roots of avocado trees infected with *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 30: 57-64.

BOTHA, T., WEHNER, F.C. & KOTZÉ, J.M. 1989. An evaluation of in vitro screening techniques for determining tolerance of avocado rootstocks to *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 12: 60-63.

BROADBENT, P., BAKER, K. & WATERWORTH, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australia. *Australian Journal of Biological Sciences* 24: 925-944.

BUCKLEY, D.H. & SCHMIDT, T.M. 2003. Diversity and dynamics of microbila communities in soils from agro-ecosystems. *Environ. Microbiol*. 5: 441-452.

COFFEY, M.D. & GEES, R. 1992. Integrated Control of Phytophthora Root Rot using Strain TW of *Myrothecium roridum*. Proc. of Second World Avocado Congress p. 142.

DEACON, J.W. & MITCHELL, R.T. 1985. Toxicity of oat roots, oat root extracts, and saponins to zoospores of *Pythium* spp. and other fungi. Transactions of the British Mycological Society. Volume 84. Issue 3: Pp 479-487.

DURAND, B.J. & CLAASSENS, N.J.F. 1987. Root distribution of avocado trees in different soil types. *South African Avocado Growers' Association Yearbook* 10: 15-19.

DUVENHAGE, J.A., KÖHNE, J.S. & KIRKMAN, B. 1993. The Influence of Organic Amendments and Discontinuation of Chemical Root Rot Control on Tree Condition and Yield of Avocado. *South African Avocado Growers' Association Yearbook* 1993. 16: 75-76.

DUVENHAGE, J.A., KOTZÉ, J.M. & MAAS ERNA, M.C. 1991. Suppressive Soils and Biological Control of *Phytophthora* Root Rot. *South African Avocado Growers' Association Yearbook* 1991 14: 6-11.

DUVENHAGE, J.A. & KÖHNE, J.S. 1995. Biocontrol of *Phytophthora cinnamomi* on Avocado: Identification and Field Testing of Local Natural Antagonists, and Evaluation of Rootstocks for Resistance. Proceedings of The World Avocado Congress III. pp. 392-395.

FABER BEN, MENGE JOHN, DOWNER JAMES & OHR HOWARD. 1995. Mulch Effects on Avocado Root Rot. Proceedings of The World Avocado Congress III, 1995 pp. 412-416.

GALLO-LLOBET, L. & SIVERIO, F. 1995. Influence of Soil Solarization on *Phytophthora cinnamomi* Rands in Avocado (*Persea Americana* Mill.). Proceedings of The World Avocado Congress III. pp. 400-403.

GINSBURG, O. & AVIZOHAR-HERSHENSON, Z. 1965. Observations on vesicular-arbuscular mycorrhiza associated with avocado roots in Israel. T*rans. Brit. Mycol. Soc*. 48:101-104.

HAKIZIMANA, J.D., GRYZENHOUT, M., COUTINHO, T.A. & VAN DEN BERG, N. 2011. Endophytic diversity in *Persea americana* (avocado) trees and their ability to display biocontrol activity against *Phytophthora cinnamomi*. Proceedings VII World Avocado Congress, Australia.

HOUNGNANDAN, P., SANGINGA, N., WOOMER, P., VANLAUWE BERNARD, VAN CLEEMPUT OSWALD. 2000. Response of *Mucuna pruriens* to symbiotic nitrogen fixation by rhizobia following inoculation in farmers' fields in the derived savanna of Benin. *Biology and Fertility of Soils* 30(5): 558-565.

JONES, D.L & HINSINGER, P. 2008. The rhizosphere: complex by design. *Plant Soil*. 312: 1-6.

KENNEDY, P.G., PRAY, K.G. & BRUNS, T.D. 2009. Root tip competition among ectomycorrhizal fung: are priority effect a rule or an exception? *Ecology* 90: 2098-2107.

LANDMAN, W.P., LABUSCHAGNE, N. & KOTZÉ, J.M. 1996. Optimaization of Biological Control of Phytophthora Root Rot of Avocados. *South African Avocado Growers' Association Yearbook* 19: 52-53.

LANDMAN, W.P., VAN HEERDEN, I., KOTZÉ, J.M., LABUSCHAGNE, N. & WEHNER, F.C. 1997. Evaluation of *Trichoderma harzianum, Bacillus megaterium*, Composted Citrus Waste and Soil Solarization on Avocados with and without *Phytophthora cinnamomi*. Preliminary Results. *South African Avocado Growers' Association Yearbook* 20: 113-115.

LÓPEZ, C.J., BASALLOTE, M.J., PÉREZ, R.M., JIMÉNEZ & MELERO, J.M. 1995. Control of *Deamtophora necatrix* and *Phytophthora cinnamomi* in Established Avocado Orchards by Soil Solarization. Proceedings of The World Avocado Congress III. pp. 404-407.

LAVELLE, P., PASHANASI, B., CHARPENTIER, F., GILOT, C., ROSSI, J., DEROUARD, L., ANDRE, J., PONGE, J. & BERNIER, N. 1998. Influence of earthworms on soil organic matter dynamics, nutrient dynamics and microbiological ecology. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, pp. 103-122.

MAAS ERNA, M.C. & KOTZÉ, J.M. 1989. Evaluating micro-organisms from avocado soils for antagonism to *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearboo*k 12: 56-57.

MAAS ERNA, M.C. & KOTZÉ, J.M. 1990. The effect of bacteria on root rot severity caused by *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 13: 65-66.

MALAJCZUK, N. & McCOMB, A.J. 1979. The microflora of unsuberized roots of Eucalyptus calophylla R Br and E margínata Donn ex Sm seedlings grown in soils suppressive and conducive to *Phytophthora cinnamomi* Rands. I. Rhizosphere bacteria, actinomycetes and fungi. *Aust. J. Bot*. 27: 235-254.

MALAJCZUK, N. 1983. Microbial antagonism to Phytophthora pp 197-217 in Phytophthora its biology, taxonomy, ecology and pathology. D.C. Erwin, S. BartnickiGarcia & P.H. Tsao (eds). American Phytopathological Society, St Paul.

MARX, D.H. & HAASIS, F.A. 1965. Induction of aseptic sporangial formation in *Phytophthora cinnamomi* by metabolic diffusates of soil microorganisms. *Nature* 206: 673-674.

MAVUSO, Z. 2008. Effect of different mulch materials, composts and organic treatments on tree condition and root health. *South African Avocado Growers' Association Yearbook* 31: 32-35.

MAVUSO, Z. 2009. Effect of different mulch materials, composts and organic treatments on tree condition and root health. *South African Avocado Growers' Association Yearbook* 32: 6-9.

MAVUSO, Z. & WILLIS, A. 2007. Effect of different mulch materials, composts and organic treatments on tree condition and root health. *South African Avocado Growers' Association Yearbook* 30: 21-25.

MENDES, R., KRUIJT, M., DE BRUIJN, I., DEKKERS, E., VAN DER VOORT, M., SCHNEIDER, J.H., PICENO, Y.M., DESANTIS, T.Z., ANDERSEN, G.L., BAKKER, P.A. & RAAIJMAKERS, J.M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332(6033): 1097-1100.

MÉNDEZ-BRAVO, A., CORTAZAR-MURILLO, E.M., GUEVARA-AVENDAÑO, E., CEBALLOS-LUNA, O., RODRÍGUEZ-HAAS, B., *et al*. 2018. Plant growth-promoting rhizobacteria associated with avocado display antagonistic activity against *Phytophthora cinnamomi* through volatile emissions. PLOS ONE 13(3): e0194665. https://doi.org/10.1371/journal.pone.0194665.

MENGE, JOHN A., MICHAEL, R., EDWARD, DAVIS, JOHNSON, L.V. & ZENTMYER, GEORGE, A. 1978. Mycorrhizal fungi increase growth and reduce transplant injury in avocado. *California Agriculture* April 1978: 6-7.

MOORE-GORDON, C., WOLSTENHOLME, B.N. & LEVIN, J. 1995. Effect of Mulching on Hass Avocado Fruit Growth and Yield in the KwaZulu-Natal Midlands. *South African Avocado Growers' Association Yearbook* 18: 62-65.

MOORE-GORDON, C., COWAN, A.K. & WOLSTENHOLME, B.N. 1997. Mulching of Avocado Orchards to Increase Hass Yield and Fruit Size and Boost Financial Rewards. A Three Season Summary of Research Findings. *South African Avocado Growers' Association Yearbook* 20: 46-49.

PAUL, ELDOR. 2015. Soil Microbiology, Ecology, and Biochemistry: An Exciting Present and Great Future Built on Basic Knowledge and Unifying Concepts. In: Eldor Paul (Editor). 2015, 4th Edition. Soil Microbiology, Ecology, and Biochemistry. Elsevier. Amsterdam. Pp 604.

RAAIJMAKERS, J.M. *et al*. 2009. The rhizosphere: a playground and battlefield for soil-borne pathogens and beneficial microorganisms. *Plant Soil* 321: 341-361.

RŮŽKOVÁ, M., RŮŽEK, L. & VOŘÍŠEK, K. 2008. Soil biological activity of mulching and cut/harvested land set aside. *Plant Soil Environ*. 54, 2008 (5): 204-211.

SALGADO, E.A. & TORO, M.A. 1995. Spatial Distribution of Avocado (*Persea Americana* Mill) Roots Under Drip And Micro-sprinkler Irrigation. Proceedings of The World Avocado Congress III. 206- 208.

SHU BO, LIU LIQIN, JUE DENGWEI, WANG YICHENG, WEI YONGZAN & SHI SHENGYOU. 2016. Effects of avocado (*Persea americana* Mill.) scion on arbuscular mycorrhizal and root hair development in rootstock. Journal Archives of Agronomy and Soil Science. Volume 63, 2017 - Issue 14: 1951-1962.

SIMON, HOLLY M. *et al*. 2005. Cultivation of Mesophilic Soil Crenarchaeotes in Enrichment Cultures from Plant Roots. Applied and Environmental Microbiology, Aug. 2005, p. Vol. 71, No. 8: 4751-760.

WARDLE, D.A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton.

WESTE, GRETNA & VITHANAGE, K. 1977. Microbial populations of three forest soils: seasonal variations and changes associated with *Phytophthora cinnamomi*. *Aust. J. Bot*. 25, 377-383.

WHEELIS, M.L., KANDLER, O. & WOESE, C.R. 1992. On the nature of global classification. Proc. Natl. Acad. Sci. U.S.A. 89: 2930-2934.

WOESE, C.R., KANDLER, O. & WHEELIS, M.L. 1990. Towards a natural system of organisms – proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. U.S.A. 87: 4576-4579.

WOLSTENHOLME, B.N, MOORE-GORDON, C. & ANSERMINO, S.D. 1996. Some Pros and Cons of Mulching Avocado Orchards. *South African Avocado Growers' Association Yearbook* 18: 87-91.

YANG, C-H., CROWLEY, D.E. & MENGE, J.A. 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiology Ecology. Volume 35, Issue 2: 129-136.