EFFECTS OF SOIL HEALTH MANAGEMENT PRACTICES ON PLANT ROOT DEVELOPMENT

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Declaration

The work contained in this thesis has not been previously submitted either in whole or in part for a degree at Central Queensland University or any other tertiary institution. To the best of my knowledge and belief, the material presented in this thesis is original except where due reference is made in text.

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Abstract

The plant root system is the connection between the aboveground biomass and the soil system, and it requires a healthy soil environment to support optimum root growth. A healthy and extensive root system allows the plant to explore a greater soil volume for water and nutrients, and thereby increase the likelihood of more productive crop.. Intensive vegetable production systems with little or no organic materials incorporated into soil combined with heavy farm traffic and use of chemical fertilizers often lead to detrimental impacts on soil health characteristics, reducing capacity to support extensive root system development. Crops in these systems may also be more susceptible to high incidence and severity of soil-borne root diseases, which further reduces the root system and yield capacity of the crop. Pathogen damage to crop root systems is a major threat to crop production, and while current management systems can mitigate against this threat, alternative strategies based on building soil health through building soil organic carbon content and strong plant root systems is more sustainable. This research investigated the effects of different organic materials to soil microbial communities, plant root system development and crop performance in cropping soils.

Field experiments were conducted to investigate the effect of various application rates of composted and uncomposted organic amendments incorporated in soil on soil biological activity and root system development in capsicum (Bell pepper) and chilli crops. While organic material addition was found to increase soil respiration rate, indicating increased microbial activity, the effects on root system development were more variable. In the field, soil respiration was measured by using closed respiration chamber (EGM3, PP system, UK) and root characteristics (root length, surface area, volume and average root diameter) were measured by using WinRhizo softwares. Uncomposted cane residue, and very high rate of compost application, resulted in a shorter capsicum root length and smaller surface area and root volume than in control treatments with no amendment to the soil. Experiments to ascertain whether the reduced root development response in the field experiments was due to high carbon to nitrogen (C:N) ratio were completed in pot trials utilising varying rates of organic carbon and nitrogen application.The root system development responses observed in these trials were not always corresponding with the proposed mechanism of reduced nitrogen availability at high C:N ratio due to microbial immobilisation of available nitrogen.

Chemical leachates from uncomposted organic material were responsible for reduced root development when soil was amended with these materials. Seed germination was also inhibited when seeds were imbibed in water in which uncomposed organic matter had been soaked. The inhibitory effect was reduced when leachate concentration was reduced, suggesting an allelochemical response. Similarly, root growth rate was reduced when the root system was exposed to the leachate and the effect was concentration dependant. Root tip necrosis symptoms were noted in field, pot and solution culture conditions when plants or seeds were exposed to either leachate or soil amended with uncomposted material, suggesting that reduced root system development under field conditions could be attributed to damage due to allelopathic. Application of beneficial bacteria cultures to seeds and seedling root systems was shown to reduce the detrimental effect of the allochemicals released from the uncomposted crop residue. It was concluded that use of microbial solution has commercial potential as a management strategy to improve crop establishment when vegetables are planted into soils amended with uncomposted cane residue.

When soil was amended with composted organic material, root development in chilli was decreased in soil containing a high rate of compost. The highest rate of compost applied in a commercial chilli field trial, 22.5 Mg ha^{-1} produced a shorter root length and lower fruit yield than in blocks amended with 7.5 Mg ha^{-1} and 15 Mg ha^{-1} compost. The 22.5 Mg ha^{-1} compost application rate produced lesser no of lateral roots (n=6.9) than in 15 Mg ha^{-1} (n=10.4). The shorter root length and reduced root branching at the high compost application rate was concluded to be the result of either increased root damage due to high nitrogen availability or lower number of lateral root development at high nitrate availability.

Capsicum plants grown in soil amended with uncomposted organic material produced significantly shorter root systems at vegetative, flowering and fruiting stage than in the control without organic matter applied to the soil. At fruiting stage the uncomposted treatment showed 52.9, 50.3 and 47.4% lower root length, surface area and root volume, respectively, compared to the control. The reduced total root system development in uncomposted organic matter was related with the higher C:N ratio causing nitrogen starvation. Total root length and number of root tips reduced by 61.8 and 72.7% at 31.3 C:N ratio compared to 24.5 C:N ratio. Very low C:N ratio (9.9) was also detrimental to plant root growth producing 47.3 and 61.9% shorter root length and number of lateral root, respectively, compared to 24.5 C:N ratio. Very high C:N ratio (31.3) produced shortest root system and shoot biomass than lower C:N ratio. The shorter root system at very high C:N ratio was associated with higher microbial activity. The soil microbial activity increased at higher C:N ratio. In Community Level Physiological Profiling (CLPP) tests, the average well colour development (AWCD), showed that the soil microbial activity after 120 hrs of incubation was 69.24% and 78.57% less for bacteria and fungi AWCD, respectively, compared to 31.34 C:N ratio. Microbial diversity did not vary with the C:N ratio but evenness was significantly lower at lower C:N ratio possibly the different microbial composition at different C:N ratio.

Root growth was also significantly reduced with the leachates from uncomposted organic matter application. A 100% leachate produced 92.1% shorter capsicum root length compared to the control with deionised water. In the field, the fresh cane residue incorporation produced 63.3, 71.5 and 77.8% shorter total root length, surface area and root volume, respectively, compared to the control without cane residue. The reduced root growth in uncomposted cane residue caused the root damage and the damage caused by cane residue was reduced with the microbial inoculation. The increased root growth in microbial solution applied treatment in the field grown capsicum suggest that microbes protect the root system against the deleterious allelopathic effect of uncomposted cane residue incorporated in soil. The positive effect of using microbial solution could be associated with the formation of microbial biofilm around the root surface, or by the degradation of the toxic chemicals present in the cane residue by the beneficial microbes therefore protecting root from the damage by the allelochemicals present in the cane residue leachate.

The effect of beneficial bacterial culture applications in protecting the plant root system from allelochemical exposure was examined under controlled environment conditions. Microbial inoculation reduced the capsicum seedling root length and surface area damage by 44 and 53% compared to the control without microbial inoculation but exposed to cane residue leachate. The reduced root damage in microbial inoculated seedlings was attributed to microbial protection of the roots against the toxic chemicals. Exposing capsicum seedlings to beneficial microbial solutions for longer duration (24 hrs) prior to exposing them to leachate from uncomposted cane residue resulted in less root damage compared to plants exposure to shorter microbial inoculation times and longer leachate exposure time. The microbial biofilm formation around the root surface could be responsible for protecting the root from the allelochemicals when exposed for longer time. In addition, root damage was lower when roots were inoculated at higher temperature ($35^{o}C$), presumably due to higher bacterial growth rate that supports forming a thicker microbial protective barrier. Further research to optimise beneficial bacteria application strategies to reduce the root damage by the allelopathic affect of crop residue is recommended in order to increase the rate of root system development during crop establishment in soils where allelochemical impacts occur.

This PhD research provided a better understanding of soil amendment incorporation that contributes to root system development in vegetable crops. This research has provided further research opportunities on how organic material applications affect soil microbial composition and root system development.

Table of Contents

Table of Tables

Table of Figures

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1 Introduction

1.1 Research context

Queensland occupy second largest and largest in fruit and vegetable production, respectively in term of value in Australia (ABS, 2016). Vegetable production in Queensland alone counts AUD a billion in production (ABS, 2016), and represents about one-third of the Australian vegetable production in term of gross value (ABS, 2016). The importance of this region in vegetable production highlight with the data that Queensland alone produce 75% and 50% of Australia's capsicum and tomato production, respectively (SQAR, 2014). Vegetable productions in Queensland are concentrated in the sub-tropical coastal region such as Wide Bay, Bowen, Gumlu, Whitesundays and Moreton areas. The main vegetable crop grown in these areas include tomato, capsicum and chilli, potato, lettuce and mushroom (CDI, 2004).

Bundaberg is one of the major agriculture areas in Wide Bay region in Queensland. Sugarcane is the main crop produce in Bundaberg, and other horticulture crops include tomato, capsicum, zucchini, sweet potato, bean, potato, chilli, snow pea, mushroom and melons (CDI, 2004). Of the total vegetable production, tomato, zucchini, capsicum and chilli are within the five important vegetable crops in Bundaberg, in term of gross value. Growing differnet types of vegetable in Bundaberg and in this region is made possible with suitable climate and different soil types. Bundaberg has 11 different types of soil and crops are grown based on their land use suitability (Donnollan et al., 1998), but the soil in vegetable growing areas in Bundaberg are mainly with Red Ferrosol and Hydrosol. In the recent years, in Bundaberg, the sugarcane production area is decreasing, but the production of fruit and vegetable is increasing (SQAR, 2014).

Despite the increasing contribution of horticulture in local and national economy, the vegetable production in Bundaberg is based on the intensive cropping system based on repeated soil cultivation, use of chemical fertilizer and pesticide. The use of intensive cropping system in vegetable production in Bundaberg is based on the removal of organic materials after crop harvest and preparing the land for transplanting vegetable seedlings with little or no addition of organic material.

Vegetable production based on those practices is increasingly facing problems related to the soil borne diseases that causes root damage to the crops by root rot diseases, causing massive economic loss to the Australian farmers. For example, 25% of capsicum plants dies in Bundaberg, queensland by sudden wilt disease, which is a root rot problem caused by a *Pythium* sp. (Stirling et al., 2004). Similarly root rot disease in tomato counts about 25% of total annual production (Fleet & Ashcroft, 1997), and in parsley the root rot disease, which is wide spread in Australian farms causes up to 100% yield loss (Minchinton et al., 2006). To control these and other soil-borne diseases cost more than AUD 100 million per year in Australian vegetable industry (Condon, 2016).

Considering the economic importance of managing soil borne diseases in vegetable production, vegetable industry uses different soil and nutrient management practices including the incorporation of organic materials in soil and utilizing the beneficial microbes. One of the organic materials that farmers in Bundaberg are using is the cane residue, residue left after the sugarcane harvest, which is abundant, cheaper and easily available in this area. Although the benefits of using organic materials such as crop residues to reduce the soil-borne pathogen and severity of disease are known, it is important to understand the effect of incorporating of fresh cane residue and its compost on the soil biology and root development in vegetable crops for the sustainable vegetable production in this region. Capsicum being one of the major vegetable crops in Bundaberg, with their shallow root system and vulnerable to several soil borne pathogens, was chosen as model crop in this research.

1.1 Overview

The relationship between soil health and plant growth has gained research interest to assist growers in maintaining sustainable crop yields (Abawi & Widmer, 2000; Lehman et al., 2015; Pankhurst et al., 2003). Intensive crop production systems based on the heavy machine and frequent soil disturbance with no or little organic material retention often lead to detrimental impacts on soil health. Those crop production practices are characterised with the problems associated with soil compaction, increase in disease causing pathogen populations and reducing the capacity of the plant to support root system development (Braunack, 1991; Tu & Tan, 1991.). Crops in the intensive cropping systems are susceptible to increased incidence and severity of soil borne root diseases, which further reduce root development and yield potential. Therefore, understanding root development in relation to soil health is important for sustainable crop production.

Crop production with mechanised, high input production system based on intensive tillage and high rates of chemical fertilizer and pesticide use in Australia and elsewhere face problems associated with soil health and environment impacts (Hamza & Anderson, 2005; Steiner et al., 2007; Wood et al., 2006). Soil health in crop production encompasses a combined effect of soil physical, chemical and biological properties. The consequence of decreased soil health in high input systems includes losses in soil structure and aggregate quality, reduced nutrient availability (Bulluck et al., 2002; Marinari et al., 2006), increasing pathogen population, root disease severity and potential for reduced plant biomass and therefore reduced crop yield (Forge and Kempler, 2009; Stirling & Eden, 2008; Stirling et al., 2005).

The root damage caused by soil borne pathogens increased in soil with low soil organic matter (Stirling & Eden, 2008). To improve the soil organic matter, alternate production practices including: organic matter amendments, crop residue retention, and no or minimum tillage are encouraged (Chan, 2008). Use of organic matter amendment, including crop residue, in soil is more common in the broadacre cropping system than in intensive vegetable production. Vegetable production in mechanised cropping such as in Australia generally involves little or no crop residue retention in order to facilitate the land preparation and reduce the insect pest and disease source from the previous crops, thus resulting in declining soil organic carbon stock.

In the part of the world where intensive cropping is common, low soil organic carbon is considered one of the indicators of reduced soil health. To improve the soil health condition in vegetable production systems, organic material is incorporated in soil before planting. Organic materials improve soil properties (Bulluck et al., 2002; Marinari et al., 2006). The effects of organic materials on soil physical and chemical properties, and their impact on plant growth have been widely considered in soil health studies (Barzegar et al., 2002; Celik et al., 2004). Addition of organic materials also improves soil biological characteristics such as increase in microbial activity and biomass (Araújo et al., 2009). Use of organic materials such as cane residue reduces the soil borne plant pathogen population (Stirling et al., 2005) and reduces severity of root disease incidence (Stirling et al., 2008). Siddique et al. (2007) extensively reviewed that the use of different organic amendments such as compost, plant residue, peat and waste are useful in reducing the pathogen population and disease incidence in cereals and vegetable crops. Although, organic material improves soil organic carbon and soil biology, understanding the effect of incorporating organic materials on soil biology as well as its influence on plant root development is important for producing healthy, less damaged root systems.

This chapter provides about a context of research, literature review on soil health and soil health management approaches, effect of organic materials addition on soil physical, chemical and biological properties, and their impact in crop growth. This chapter also reviews literature on the benefits of using microbial inoculants and their effects on crop growth. At the end of this chapter, a summary with research aims and hypothesis are provided.

1.2 Soil health

A healthy soil is defined as one that can support and sustain plant and animal production, promote plant health, and support water and nutrient cycling (Doran & Zeiss, 2000). Therefore, agriculture practices should focus on crop production practices that maintain soil health with little or no damage to plant health and the surrounding environment. The current vegetable production practices in Australia, with intensive and frequent tillage, no residue retention, and high chemical fertilizer and routine pesticide application, have created several soil constraints to crop productivity.

Commonly observed soil constraints in current vegetable production systems in intensive cropping systems in Australia and elsewhere are soil compaction, poor aggregation and soil crusting, high soil borne pathogen and root disease, and low water and nutrient retention (Gugino et al., 2009). For example, Nawaz et al. (2013) presented that 4 million ha of soil is degraded with soil compaction problem in

Australia. Similarly, the intensive crop production in Australia has suffered from several soil borne diseases caused by *Sclerotinia* spp., *Fusarium* Spp., *Rhizoctonia* spp and root-knot nematodes (McMichael, 2012). So et al. (2009) show lower soil porosity, hydraulic conductivity and reduced soybean yield in conventional tillage than in the no-till farm in 14 years long experiment in Australia. The reduced crop yield in conventionally managed farm is associated with the reduced soil organic carbon (So et al., 2009). These soil constraints are reflected by declining soil health and subsequent affecting on crop growth and productivity. For instance, in Tasmania, Australia, 83% of the vegetables growing paddocks are severly affected by *Rhizoctonia solani* severly affecting seedling establishment when tested with bean (Pung et al., 2007).

Soil health has been assessed by soil physical, chemical and biological indicators. These indicators are selected based on their capacity to change the soil function when soil management practices are changed (Andrews et al., 2004). Commonly used soil health indicators include soil bulk density, porosity, soil aggregate stability, field infiltration, root health, active carbon, soil organic carbon content, microbial respiration, phosphorus, nitrate nitrogen, pH and potassium (Gugino et al., 2009). Andrews et al. (2004) suggest microbial biomass carbon, potential mineralizable nitrogen, available water capacity, macroaggregate stability and electrical conductivity as potential soil indicators that affect soil function with the change in management practices. Pankhurst et al. (1995) present that not all soil health indicators are universally applied showing that carbon mineralization and microbial biomass is affected by the management practices (tillage, stubble management, crop rotation and nitrogen fertilisation) in Australia. They also present that total bacteria, fungi, and actinomycetes, cellulose decomposing bacteria and fungi, soil phosphatase activity, and N mineralisation are less affected by the management practices thus has limited potential as bioindicators. The changes in the levels of those soil health indicators and subsequent reduction in plant growth and crop productivity are often associated with the decline in soil health To improve the level of those soil health indicators integrated approaches of improving physical, chemical and biological properties of soil are being suggested.

1.3 Soil health management approaches

The term ‘soil health’ encompasses the well-studied impacts of soil physical and chemical properties on plant growth as well as the less understood effects of soil biology on disease incidence and plant vigour. Attention to ‘soil health’ has been focused towards the improvement of soil physio-chemical attributes as well as biological components through the adoption of alternate soil management approaches and minimizing tillage and chemical inputs. . Hence, a wide range of soil health management approaches include increasing soilorganic matter by adding organic materials, using green manure, cover crops, no tillage, crop rotation (Pattison et al., 2010), and microbial inoculants, are encouraged with the aim of improving soil physical, chemical and biological properties in Australia.

Soil health management approaches directly or indirectly affect soil physical, chemical and biological properties through the addition of nutrients or improving soil microbial activity (Vinhal-Freitas et al., 2010; Zai et al., 2008). Soil organic matter is derived from root exudates, decayed roots, plant and animal material that is transformed by the microbial population and is often protected within aggregate structures. Organic maerial is the source of soil organic carbon (SOC), which is presented as percentage of carbon present in the soil including the microbes. SOC represents 58% of soil organic matter, and SOC is positively correlated with the soil organic matter (Bianchi et al., 2008). SOC is sensitive to land use type and management activities, therefore has been used as an indicator of soil health (Liu et al., 2014). Green manures, cover crops cultivation, addition of organic materials to soil either as compost, fresh crop residue or crop stubble are the accepted strategies to increase the SOC.

1.3.1 Green manuring and cover crops

Green manure crops are grown in the field and are either winter killed or chemically killed to increase the soil organic matter and nutrients. Organic matter and nutrient supply from the green manure crops depends on the crop type and the growth stage when incorporated into the soil (Hirpa et al., 2009). Use of green manuring crops increase the SOC (Veenstra et al., 2006). Green manure crops incorporated and transformed in to soil provide a larger proportion of soil pore space and soil aeration compared to the unamended soil (Sultani et al., 2007). Green manure play a positive functional role in nutrient cycling and disease suppression. Soil microbes use the incorporated plant material as an energy source and nutrients becomes available to the plants through mineralization.

Growing green manure crops of pathogens non-host species may decrease the pest and pathogen carrying over to the following crops. Some green manuring crops such as *Brassica* spp. are also utilised as natural fumigators, reducing disease and pest population and damage to the crops. For instance, growing Indian mustard as a green manure crop is effective in reducing powdery scab disease. Rapeseed and canola as green manure crop, are effective in reducing incidence and severity of *Rhizoctonia* diseases in potato (Larkin & Griffin, 2007). *Brassica* spp., as green manure, produces glucosinolates that break down to isothiocyanates, which are toxic to soil borne pathogens (Simolinska et al., 2003).

Similarly, cover crops, which can be same as green manuring crops, improve soil nutrients retention and protect the fertile topsoil against erosion (Hoorman, 2009). Cover crops help in conserving soil moisture in summer and protect soil from crusting and compaction by rain drops in the rainy season (Williams & Weil, 2004). Growing cover crops with deeper root systems are considered beneficial in reducing soil compaction (Williams & Weil, 2004).

1.3.2 No-tillage or minimum tillage

Tillage provides the disturbance and redistributes the organic material in the top 15-20 cm soil layer. The distribution of organic materials affects aeration and nutrient cycling. Tillage temporarily increases the soil pore space and increases the availability of oxygen and water movement, but, in the long term, repeated tillage reduces soil pore space, increases soil compaction and reduces air and water movements, and reduces soil aggregate size (Boogar et al., 2014; Mathew et al., 2012; So et al., 2009). To reduce the problems associated with the repeated tillage, no-till or minimum tillage practices are growing. In no-till farming, crop residue is left on the soil surface and soil is not disturbed at the time of planting. Crop residue left in the soil surface after the crop harvest increases the SOC in no-tillge or minimum tillage practice compared to conventional tillage (Mathew et al., 2012; Spiegel et al., 2007). As crop residue is left on soil surface, SOC, nitrogen mineralization, soluble phosphorus and potassium were increased in the top 0-10 cm both in minimum and reduced tilled treatments compared to conventional-tilled plots (Spiegel et al., 2007). The increased soil nutrients on top soil layer in no-till and reduced-till is because organic materials are either left on surface or mixed on the topsoil layer, while in conventional organic materials are incorporated in deeper soil depth thus lesser nutrients are available on topsoil layer (Spiegel et al., 2007).

Tillage affects soil aeration, temperature and moisture content in soil, which in general positively affects soil microbial activity, for example, Linn & Doran (1984) reported that the higher soil moisture on top-soil (0-7.5 cm) in no-till is responsible for the higher soil respiration compared to the conventional tillage. The higher microbial activity with favourable soil moisture and temperature in topsoil increases microbial biomass in no-till or minimum tillage compared with conventional tillage (Mathew et al., 2012; Roldán et al., 2005). The reduced soil biological activities in conventional tillage is due to low organic matter and poor soil structure as a result of repeated soil cultivation (Mohammadi et al., 2012). Thus, to increase SOC in the intensive tillage practices, more organic materials are advised to use. But, continuous organic material addition does not always improve the SOC level, as was demonstrated in long-term trials in tropical areas (Bajgai et al., 2015; Reicosky et al., 2002). Soil cultivation breaks down the soil aggregates, exposing the soil organic carbon to microbes (Król et al., 2013), stimulating microbial growth and respiration and supporting increased turnover of organic substances in tilled soil, thus not increasing the SOC (Goebel et al., 2005; Reicosky et al., 2002).

The residue left on the soil surface after harvest provides substrate to the microbes and increases soil microbial activity, the increase in microbial activity is one of the indicators of healthy soil. Lesser disease incidence in the no-till farming compared to the intensive or conventional tillage is associated with increased microbial activity due to high organic carbon availability (Perez-Brandánet al., 2012).

1.3.3 Crop residue

Crop residue management in the field depends on the farming practices. Crop residues are either surface-managed or incorporated into the soil depending on the tillage practices. In conservation farming organic residues are left on soil surface, while in the industrial and intensive farming practices the crop residues are often removed from the field. In vegetable farming, removing the crop residues or the stubble after the previous crop harvest is the most common practice and can lead to reduce SOC levels. Hence, to improve the SOC in intensive vegetable farming, the use of compost or other organic materials incorporated in the soil at the time of soil cultivation can increase SOC levels. Stubble retention is most commonly practiced in broadacre cropping systems in the Australian grain growing regions (Llewellyn & D'emden, 2009). Adoption of stubble retention increased the SOC compared to the stubble removal in no-till farming (Chowdhury et al., 2013). In no-till farming with the crop residue left on the soil surface, SOC content shows stratification, showing higher at the topsoil and reducing with the soil depth (Mathew et al., 2012; Moussadek et al., 2014). Stubble retention also reduced the bulk density and increased the available water holding capacity of soil when compared with the stubble burning (Valzano et al., 2001) or stubble removal (Donk et al., 2012). Stubble retention in the field improves the soil health such as increasing soil nitrogen, phosphorus and potassium content in soil, and also increases the crop yield when compared with the stubble removed in rain-fed areas (Huang et al., 2012).

Use of crop residue in field such as in no-till or in minimum tillage reduces the disease severity in the crops compared to the no residue left field, for instance the fusarium leaf blight diseae severity in soybean (*Glycine max*) is significantly lower in no-till plots with crop residue left on soil surface compared to the adopting conventional tillage such as ploughing and/or harrowing (Joseph et al., 2016). Joseph et al. (2016) concluded that the increase in microbial activity, diversity and increased nutrient supply in no-till plots suppresses disease development. They further supported that the cooler temperature in no-till with the crop residue on soil surface compared to the other tillage favours reduced disease development than at higher soil temperature, where high soil temperature favours the disease severity.

The adoption of residue retention improves the soil organic matter, but the soil cultivation practice (e.g. type of tillage practice) determines the rate of soil organic material loss. For example, no tillage with residue significantly increased the total soil organic carbon in 0-5 and 5-10 cm soil depth compared to the chisel plow tillage for three years in Iowa, USA (Al-Kaisi & Yin, 2005). Hence, the organic material management practice should be linked with the tillage practice.

1.3.4 Crop rotation

Crop rotation utilises different crops one after another. Crop rotation reduces insect pests and diseases by removing the preferred host from the field, for example rotating cereal crops after legumes decreased damage and severity of nematode and fungus root rot pathogens compared to the cereal after cereal (Alvey et al., 2001; Bagayoko et al., 2000; Smiely et al., 1996). Similarly, growing snap bean after corn reduces the root rot severity and increases the crop yield of snap bean compared to bean after bean (Abawi & Widmer, 2000).

Crop rotation reduces the pathogen population by altering the soil environment by adding organic carbon into soil through the increase in cropping frequency or inclusion of legume in the rotation thereby increases the soil microbial biomass and respiration (Campbell et al., 1991), which is beneficial in reducing the disease severity. Increase in crop rotation causes shift in the soil environment such as improves soil pH, stabilize soil temperature and improves soil structure compared to growing same crop in rotation (Venter et al., 2015). The improvements in soil characteristics are related with the continuous covering of soil with crops and increase in the soil organic matter (Kennedy, 1999). For instance, Gregorich et al. (2001) show that maize monoculture contributed less soil organic carbon compared to the maize, oat and alfalfa in rotation, which is due to more living roots present in the soil. Robertson et al. (2015) presented that pea, fallow and wheat rotation in semi-arid climate of Victoria, Australia supplied significantly higher soil carbon in zero tillage compared to the traditional tillage, but for the same tillage practice pea and wheat in rotation did not have difference in soil organic carbon.

Growing cereal crops in rotation with legumes also increased the soil fertility, for instance millet with cowpea or sorghum with groundnut produced higher mineral nitrogen compared to the continuous cereals in rotations, which was attributed with the nitrogen fixation by the legume crops (Bagayoko et al., 2000). Also, the increased microbial community in legume rotated with cereal crop increased the phosphorus availability and uptake by the cereal crops (Alvey et al., 2001).

1.3.5 Use of microbial products

Soil microbes are considered critical in maintaining soil health and a functional soil ecosystem. Considering the importance of microbes, efforts are being made to enhance soil microbial activity and diversity through the soil management options. Several microbial products are commercially available in the market, either as liquid or solid formulations with different carriers. Microbial products being used in agriculture farms range from compost tea to single or a mixture of pure cultures of different microbial strains. They have been used in wider context from increase nutrient turn over e.g. nitrogen mineralization and phosphorus solubilisation and suppress disease (Nisha et al., 2007; Wu et al., 2005). Many of these soil additives and microbes are effectively being used in soil borne disease management. The mechanism how these microbes decrease pathogen density and disease severity are discussed later in this chapter.

1.4 Factors affecting SOC content

Environmental factors that affect the organic material decomposition mainly include rainfall and temperature. Those environmental factors affect on soil water content and soil temperature. Organic material decomposition increases with the increase in soil temperature (Stott et al., 1986). The lower microbial activity at lower soil temperatue is attributed with the reduced soil microbial activity, thus in temperate regions the lower soil microbial activity slows down the mineralization of SOC, thus total SOC content is larger in temperate climate than in tropical climate (Six et al., 2002). Further, the organic residue decomposition also increases with the soil moisture availability i.e. change in soil water potential, for example, wheat residue incorporated in soil at $20^{o}C$ at -33 kPa water potential is higher in decompostion than

-150 kPa or -5 kPa soil water potential (Stott et al., 1986). The increase in organic material decomposition at higher temperature and soil water potential is related with the higher microbial activity, presented as increase in soil respiration (Stott et al., 1986). The soil microbial activity depends on the soil water content, for example, soil with 62% soil water content produce 3.4 times higher CO_2 production compared to the 44% soil water content (Linn & Doran, 1984).

Another factor that affects the SOC is the soil type or the soil texture. Soil with a higher proportion of clay particles can hold more organic carbon compared to the other form of mineral soils such as sandy or loamy soil (Burke et al., 1988). The increased soil organic carbon in clay soil is associated with the exchangeable cations (e.g Ca, Fe, Al) that form a cation-organic linkage and thus tightly bound the organic carbon (Krull et al., 2001). The higher organic carbon content in clay soil is also related with the larger specific surface area of the clay particles, which possess larger surface area compared to sandy or loamy soil, onto which organic carbon can be adsorbed (Krull et al., 2001). The adsorption capacity of different soil type differ the organic material decomposition. Studies show that soil organic matter in the sand-size fraction is more susceptible to decomposition, and has higher turnover than in the silt- or clay-size fractions (Angers & Maheus, 1990; Dalal & Mayer 1988). Or, in other words, the higher adsorption capacity of clay particles makes soil organic matter less available for the microbial breakdown.

The management factors such as types and intensity of tillage and crop residue application affect soil organic carbon content, which was discussed in the previous sections. Beside those, land-use types also affect the SOC content, for instance, when a wheat–maize system was converted to low biomass producing land use such as horticultural crops, the SOC was significantly reduced in sandy, sandy loam and loamy soils (Kong et al., 2009).

The benefits of organic material additions to soil for increasing porosity, aeration and reducing the soil compaction, and supplying nutrients to the plants has been widely discussed (Bullock et al., 2004; Lehman et al., 2015; Marinara et al., 2006), but the effect of organic materials on soil biology and response to plant root development is less understood. However before moving to address the question, a short review on the effect of organic matter on soil physical, chemical and biological properties and their responses to the plant growth are presented.

1.5 Organic matter addition and soil physical properties

Organic material addition in soil increases the soil organic carbon content, the cation exchange capacity and reduces the bulk density compared to the synthetic fertilizer application (Bulluck et al., 2002). Applying organic materials to soil also increases soil porosity, infiltration rate and soil aggregate size, but the relative impact of different organic materials to soil physical properties depends on the type and rate of application (Barzegar et al., 2002). The fungal hyphae and the fine roots present in soil also constituents the part of organic matter. Fungal hyphae and roots function binding agent that supports aggregation of soil particles (Borie et al., 2008; Wu et al., 2014). For example, mycorrhiza fungi produce a glycoprotein, glomalin, which function as cementing the soil particles and increases the soil aggregate size (Wright & Upadhyaya, 1998). Similarly, soil with higher root length density is having larger and stable soil aggregates than without roots, which is due to the mechanical armouring (Graph & Frie, 2013). These fungal hyphae and plant root itself supply organic carbon. Increased carbon content with the organic material applications in soil functions, as a bonding agent between soil particles and hence increases soil particles size (Król et al., 2013). Organic applications also reduces soil compaction thereby influences the air, water and heat dynamics within and between the soil strata (Leipic & Hatano, 2003) that impact the crop production.

1.6 Soil physical properties and plant growth

Plant roots are the first site of interaction between the soil system and the plant. Only a well-developed root system can supply the water and nutrients to support strong plant growth. However, several soil factors that are influenced by soil management activities affect plant root development. One of the soil physical factors in industrial agriculture is soil compaction that increases mechanical resistance to root penetration, which reduces plant growth, root length and root dry weight (Merotto & Mundstock, 1999; Silva et al., 2004). Soil compaction also changes the root anatomy, for example, soil compaction reduces the cortex thickness and area of the vascular cylinder, and therefore negatively affects the plant physiological processes such as transpiration (Lipiec et al., 2012). Besides increasing mechanical resistant, soil compaction increases the soil bulk density, reduces soil porosity and capillary water supply (Kuht & Reintam, 2004). Lower oxygen availability in a compacted soil profile reduce leaf growth, transpiration and photosynthesis (Meyer et al., 1987). Increased soil compaction reduces the seedling emergence, plant height, grain yield, which was attributed to the increased disease incidence in the compacted soil compared to the un-compacted soil (Moots et al., 1988).

Although root morphological parameters are largely affected by soil physical conditions, little attention has been given linking how root morphological parameters are affected with the soil management practices (e.g. addition of different types and rate of organic material) that are applied to improve the soil health.

1.7 Organic matter addition and soil chemical properties

Stubble or residue incorporation or compost addition is promoted to improve soil chemistry and the nutrient supplying capacity of soil. Organic amendments improved the soil pH, cation exchange capacity and total soil carbon compared to the synthetic fertilizer applied soil (Bulluck et al., 2002). Soil amended with organic materials had a 2-3 fold higher concentration of calcium, magnesium and potassium than soil without organic amendments (Bulluck et al., 2002). Organically managed fields applied with poultry compost manure as a fertilizer for three years maintained higher total organic carbon, available phosphorus and nitrogen compared to the field without poultry compost (Marinara et al., 2006).

Nitrogen, phosphorus and sulphur are major nutrient elements available to the plants after organic material additions, and they are required for plant growth. Organic materials such as compost applications increased the nitrogen availability in soil through mineralization (Chaoui et al., 2003). Although organic material applications increase plant available nitrogen, rate of nitrogen mineralization from the amended organic material depends on the types of organic material amended, soil microbial population and the soil condition (Anggria et al., 2012). Organic materials with high lignin content were degraded slower than those with lesser lignin content (Khalil et al., 2005), therefore the slow decomposition provides late or slower releases of nitrogen than early decomposing organic materials (Carvalho et al., 2013). In addition, the net nitrogen mineralization rate and nitrogen availability to the plants from the organic matter applications depends on the carbon to nitrogen ratio (C:N) of the organic matter (Hodge et al., 2000). Abbasi et al. (2015) provided experimental evidence that nitrogen mineralization from different crop residues are determined by the initial nitrogen concentration, which is positively correlated, and net nitrogen mineralization in soil incorporated with the crop residue was negatively correlated with the lignin content.

Organic material with C:N ratio of 30:1 is considered a balanced diet for microbes while above that C:N ratio microbes utilize extra nitrogen for each carbon molecule to acquire the energy source from the substrate. In this condition, the microbes compete for nitrogen source with the plant. Microbes are stronger competitor than plants, hence; limited nitrogen is available to the plant for growth (Hodge et al., 2000). In the short term, the net mineralisable nitrogen was arrested and nitrogen become unavailable for plant growth following use of organic materials having a C:N ratio greater than 30 (Hodge et al., 2000). For instance, total mineral nitrogen in soil incubated with *Zea mays* roots or shoots were significantly lower than in soil incubated with *Glycine max* shoot or *Trifolium repens* shoot, at different times after the crop residues were added (Abbasi et al., 2015). Hence the quality of organic material becomes important for the plant growth. But, the response of crop residue with high C:N depends on the initial nitrogen content in soil or the external nitrogen application, for example if excess nitrogen is available in soil applied with high C:N ratio organic materials, less nitrogen is immobilized in soil and nitrogen become available for plant growth (Mary et al., 1996).

1.8 Soil chemical properties and plant growth

As the plant is dependent on the soil system for its growth, it is affected by all types of soil processes including chemical changes. Changes in soil pH, and nutrient condition affect the crop growth and yield. For instance, soybean grain yield at pH lower than 6 was significantly lower than at above 6.5 pH (Peters et al., 2005). Similarly, a percentage reduction in number of plants from emergence to harvest and sorghum grain yield was significantly lower at acidic soil pH (< 5.42) compared to neutral pH (Butchee et al., 2012). Acidic soil pH also negatively affected the plant growth by decreasing the phosphorus, calcium and molybdenum and increasing the toxic level of elemental aluminium and manganese (Hart et al., 2013).

Plant growth is affected by soil available nutrients such as nitrogen, phosphorus, potassium and sulphur. A high nitrogen supply increased the crop shoot weight, root weight compared to the lower nitrogen apply (Cechin et al., 2004; Gharakand et al., 2012). For instance, the final harvest dry matter in stem, leaves and head were 21, 14 and 25% higher, respectively, in sunflower plants watered with 228 ppm nitrogen supply than in 28.2 ppm nitrogen supply (Cechin et al., 2004). Lower nitrogen application rates also significantly reduce the leaf area, chlorophyll content and photosynthesis, therefore resulting in less dry matter production (Zhao et al., 2005). Zhao et al. (2005) showed that applying 20% and 0% nitrogen application in sorghum (*Sorghum bicolor* L) produced 16-35% and 31-34% less photosynthetic rate, respectively, compared to the 100% nitrogen applied treatment. They presented that the total dry matter in 20% and 0% nitrogen reduced by 15% and 41%, respectively, compared to the 100% nitrogel application. Nitrogen application also increases the root length, density and root surface area per plant compared to the no nitrogen application (Xue et al., 2014). Research shows increased root proliferation with increased nitrogen application (Asghar & Kanehiro, 1977). Plants use the proliferated root to capture nutrients from soil, and therefore maximise the nitrogen uptake (Robinson et al., 1999). In contrast, Bonifas & Lindquest (2009) presents that the root length density did not vary among without nitrogen and increased nitrogen application rates both in corn and velvetleaf.

Effects of nitrogen on Arabidopsis root development are categorised by localized proliferation of lateral roots when nitrogen availability is patchy, and a systemic inhibitory effect on lateral root initiation when high nitrogen concentration is homogenously available (Zhang et al., 2007). Zhang & Forde (2000) showed a localized stimulatory effect on *Arabidopsis* lateral root due to higher potassium nitrate concentration without affecting the primary root length, compared to a lower nitrate concentration. A laboratory experiment with sucrose and nitrate solution with

Arabidopsis showed high C:N ratio negatively affects the number of lateral roots and lateral root growth in *Arabidopsis* (Malamy & Ryan, 2001). The response of increased lateral root number could be due to the expression of stimulatory effect to certain genes in the patches of high nitrogen availability while at a uniformly high nitrate concentration the response was down-regulated (López-Bucio et al., 2003). Similarly, at high C:N or low C:N ratio, the expression of certain genes that regulate the lateral root initiation were also affected (Gao et al., 2008).

Similar to nitrogen, the role of phosphorous in root development was demonstrated by Mollier & Perellin (1999), who showed that phosphorus starvation affects the root and shoot growth in maize compared to the control without phosphorous starvation. Phosphorus starvation reduced the number of first order laterals per cm of the axile root significantly when compared with the control (Mollier & Perellin, 1999). Although, phosphate availability increases the total root system length, including the length of the primary root axis, the increased phosphate concentration in growing media reduced the mean lateral root length (Williamson et al., 2001).

All 17 essential are important for plant growth. The role of C:N ratio in nitrogen availability is well established (Abbassi et al., 2015; Hodge et al., 2000). Thus the effect of different C:N ratios on root system development in vegetable crops and its response to the soil biology become important from a crop production point-of-view. Therefore, this study will focus on how different C:N ratios impact the capsicum root development and soil microbial activity.

1.9 Organic matter addition and soil biology

Soil organic matter provides the energy source to the soil microbiota and soil microbes function as a transient nutrient sink and release nutrients from organic matter. Microbes utilize the organic materials added in the soil or the organic carbon shovel out of the plant root system in the form of exudates. It is estimated that about 20% of carbon fixed by plants are removed to the soil environment by the plant root systems (Gregory & Atwell 1991). These organic matters provide food and shelter for a diversity of microbes directly through energy supplying or indirectly by modifying microhabitats. Organic matter addition increases the soil organic carbon supply, and increased carbon is related with the increase in microbial activity and biomass (Alvarez et al., 1995a). Generally soil microbial activity, biomass and diversity are a function of the amount of soil organic matter content; therefore, increased microbial activity or diversity is considered as an indicator of healthy soil.

To improve soil biology use of compost in crop production is gaining interest because compost can be locally prepared using the farm waste such as crop residue, animal manure, household organic waste, and they are as effective as inorganic fertilizers in increasing crop yield (Basso & Ritchie, 2005). Compost is decomposed, stable organic material produced by the process of biological oxidative transformation (Bertoldi et al., 1983). Composting involves four different phases; mesophilic, thermophilic, cooling and curing phase. At the thermophilic phases the temperature remains very high (> 45^{o}C), decomposes organic materials by the thermophilic bacteria and kills disease-causing plant pathogens as well (Noble & Roberts, 2004). In the cooling phase, many beneficial microbes starts colonizing and digesting resistant organic materials then, harmful pathogens are spontaneously killed or removed by the beneficial microbes at the curing phase due to competition for nutrients (Ryckeboer, 2003). The beneficial microbes in compost include different species of fungi and bacteria such as *Trichoderma* sp. *Penccillium* sp., *Bacillus* sp., *Pseudomonas* sp. and many others depending on the substrate used in the composting (Bertoldi et al., 1983; Boulter et al., 2002; Ryckeboer et al., 2003; Yamamoto et al., 2009).

The benefits of compost use in cropping soil is described as; increase SOC and supply nitrogen, increase in soil aggregate stability, increase in biological activity as indicated by the increase in soil respiration and increase in the rate of mineralization (Bouajila & Sanaa, 2011). Compost application also increases the biological community by increasing mycorrhizal spore number, mycorrhizal root colonization percentage in crops and increase in the glomalin content in soil (Valarini et al., 2009). The increased glumalin content indicates the increase in the mycorhiza activity, in which glumalin function as the cementing the soil particles and increase in the water stable soil aggregates (Valarini et al., 2009). The beneficial effect of compost application increases with the increase in rate of application, but the benefits from the compost depends on the quality of the compost. For example, the well decomposed or cured compost produce significantly less amount of ammonia and volatile organic acids compared to the semi-cured and uncured compost, which in larger amount are toxic to the crop and soil microbes (Brinton, 2003). Compost quality, which is measured in term of maturity and stability, is determined by the factors like aeration rate and ratio of carbon to nitrogen (Bertoldi et al., 1983; Nada, 2015).

It has been shown that the application of crop residue, manures or compost increases the soil microbial activity and microbial biomass (Hui et al., 2004; Zhen et al., 2014). For example, microbial biomass carbon and microbial respiration was significantly higher in urban waste compost applied soil compared to the control without urban waste (Ros et al., 2003). However, microbial growth and its activity depend on the types or quality of organic matter substrate. For example, microbial biomass carbon is higher in manure compost than in sawdust or rise husk (Chowdhury et al., 2000), which was the result of the easily decomposable nature of manure compost compared to other amendments. Likewise, when cotton gin residue (CGR), animal manure, rye vetch green manure and control with fertilizer application were used to understand microbial activity and nutrient availability, the microbial biomass carbon and soil microbial respiration was significantly higher in the CGR followed by animal manure and rye vetch (Tu et al., 2006). Among different soil amendments, the CGR showed higher microbial respiration and next was for animal manure and least in rye vetch manure or the control treatment (Tu et al., 2006). High microbial biomass and activity was related to increased biomass turnover and degradation of the non-microbial organic materials, thereby increasing nutrient mineralization (Tu et al., 2006).

The variation in soil microbial activity and biomass was also found with several soil factors such as soil pH, soil moisture, soil temperature, and presence of heavy metals. For example, at low or acidic soil pH (< 4.5) microbial activity decreased and increased towards the neutral pH, which is associated with the increased available soil organic carbon with the increase in soil pH (Rousk et al., 2009). Both soil moisture and temperature are critical for increasing soil microbial activity, for example, Lopes et al. (2005) shows that the CO_2 evolution in cultivated clay, phaeozem in Russia was positively correlated with the soil moisture at summer period, but in winter and spring the CO_2 evolution was insignificant with soil moisture content. Also, effect of heavy metals such as cadmium, chromium and lead although decreased with the organic matter decomposition time but reduced the soil respiration significantly with the increase in the concentration of chemicals (Verma et al., 2009).

1.10 Soil organic matter and plant growth

Better plant growth and crop yield are reported in treatments with organic matter addition (Ei-Mougy et al., 2013; Nawar, 2008). Organic matter, such as compost or their extracts have enhanced crop growth through direct addition of nutrients (Moyin-Jesu, 2007). Microorganisms present in compost are also known to support plant growth through the enhanced nutrient availability and nutrient uptake by the plants (Chen et al., 2001; Hameeda et al., 2008; Hayat et al., 2013) or through the enhanced defence mechanisms (Adam et al., 2014).

Adding compost to soil supports healthy crop growth through disease suppression in the soil system and reducing the plant root disease incidence or pathogen density. However, the composition of microbes present in compost is determined by the source of composting materials (Castaño et al., 2011). Compost contains several beneficial microbes including different species of *Bacillus* and *Pseudomonas* spp. (Boulter et al., 2002; Hayat et al., 2013; Sabet et al., 2013). The compost application is beneficial in reducing the crop root rot disease incidence and soil-borne pathogen population (Sabet et al., 2013). For example, compost prepared from mixture of aromatic plants reduced the cucumber root rot diseases 54.8% for *Fusarium solani*, 71.6% for *Rhizoctonia solani,* 68.3% for *Sclerotium rolfsii* and 62.5% for *Pythium ultimum* (Sabet et al., 2013). Similarly, use of compost prepared from mixture of rice straw and animal manure reduce the root rot disease 74.3% for *F. solani*, 78.5% for *R. solani*, 76.7% for *S. rolfsii* and 72.6% for *P. ultimum* (Sabet et al., 2013). Nawar (2008) shows the use of rice straw compost in pathogen infected green been growing soil reduce the root rot disease incidence by 63 and 57% at pre-emergence and post-emergence seedling, respectively, compared to the without compost applied soil. Sabet et al. (2013) also show that the different types of plant and animal-based composts significantly increased the root length, shoot length and their dry weight in cucumber seedlings in pathogen infested soil compared to the pathogen only applied soil. Bareja et al. (2010) showed no difference in the root and shoot characteristics but significantly lowered root rot severity following the use of different plant-based compost amendements in soil compared to the compost unamended soil.

Not only the compost use, crop residue such as cane residue incorporated soil produced significantly higher sugarcane biomass per plot compared to the unamended soil, which was linked with the reduced population of lesion nematode (*Pratylenchus zeae*) in sugarcane residue incorporated soil (Stirling et al., 2005). Stirling and Eden (2008) showed increased capsicum fruit yield per plant in commercial compost or cane residue applied treatment compared to the control without compost or cane residue, in which compost or cane residue applied treatment had lesser root gall severity caused by root-knot nematode pathogen *Meloidogyne incognita*.

The above-presented evidences show that the organic amendments applied treatments increased the soil microbial activity with less disease incidence and severity to the plant root. As the research is concentrated on the root system development in capsicum and chilli crops, a short review on how beneficial microbes support in reducing the root disease incidence and improve crop growth are presented here.

1.11 Mechanisms of microbial crop protection

Beneficial microbes added to soil either through the addition of organic amendments or through the application of commercial microbial product can reduce plant disease infestation and improve crop growth. The mechanism that beneficial microbes protect the crop from the plant diseases are broadly categorised into direct and indirect antagonism; in which direct antagonism include direct physical contact or selectivity with the pathogen (Junaid et al., 2013). This includes parasitism and competition between the bio-control agent and the pathogen.

Plant roots excrete exudates and soil-inhabiting microbes use them as food and energy, creating a symbiotic relation between beneficial microbes and the plant root system. Nutrient limited pathogens also compete with the saprophytic microbes for the same nutrients. Beneficial microbes are able to colonise the root surface more rapidly than pathogens (Elad & Chet 1987). Widespread coverage of the root surface by beneficial microbes reduces access to the root by disease causing pathogens and causes nutrient starvation for those pathogens by restricting the sites for infection (Elad & Chet 1987).

Predation is another mechanism where beneficial microbes kill the pathogenic microbes, therefore reducing its population and degree of pathogenicity. Certain microbes attack the pathogen at different stages of their life cycle through parasitism. Parasitism is commonly found among fungi and nematode pathogens. For instance, *Trichoderma harzianum*was is effective in reducing the number of galls per plant and number of egg masses per plant by at least six-fold and four-fold, respectively, in tomato seedlings when compared with the *Meloidogyne Javonica* applied treatment (Naserinasab et al., 2011). *T. harzianum* was also effective in reducing the diseased tomato seedling by more than 70% when *T. harzianum* was included in the seedling growing media (Sivan et al., 1984). It was presented that *T. harzianum* showed antagonism through parasitism on the fungal mycelium and also to the nematode eggs (Naserinasab et al., 2011; Siameto et al., 2010).

Some microbes produce chemical compounds or antibiotics that affect the pathogen growth, a process referred to as antibiosis. For instance, *Bacillus subtilis* QST713 produced iturin that affected the population and growth of *Rhizoctonia solani* a casual organism for damping off (Paulitz & Blanger, 2001). Similarly, the genetically modified *Pseudomonas putida* WCS358r produced phenazine, an antifungal compound, and were found to supress the pathogenic fungal population in the rhizosphere of wheat (Glandorf et al., 2001).

Beside antibiotics, microbes also produce iron-binding compounds called siderophores. Siderophores with their high affinity to the available iron in the rhizosphere, making iron unavailable for the growth of plant pathogens in the root zone. For instance, rhizobacteria such as *Pseudomonas fluorescens* (MPF47) and *P. aeruginosa* (MPM1) produced siderophores of hydroxamate type that reduced the mycelium growth of *Rhizoctonia solani* by 71 and 51% compared to the control without *Rhizobacteria* (Solanki et al., 2014). The siderophore production was increased in treatments with iron deficient conditions compared to the iron supplemented treatment. Those bacteria reduces the root disease index by 73 and 60% compared to the *R. solani* infected control, along with promoting significantly increased root, shoot length and tomato fruit yield in the glasshouse experiment (Solanki et al., 2014).

Some microbes also produce compounds or enzymes that directly lyse the pathogen. *Lysobacter* sp. strain SB-K88 derived xanthobaccin A inhibits the mycelial growth and induces lysis of the zoospore of *Aphanomycescochlioides*, a fungal pathogen causing damping-off disease in sugar beet (Islam et al., 2005). Microbe releases hydrolytic enzymes such as chitinase, glucanase and protease support in controlling the fungal pathogens, for example, several strain of *Pseudomonas* species produces the hydrolytic enzymes chitinase and glucanase that are responsible for the degradation of the fungal hyphal wall, thus reducing the root rot disease incidence caused by *R. solani* in tomato in glasshouse experiment (Solanki et al., 2014). Similarly, *Lysobacter enzymogenes* strain C3 produces an enzyme glucanase that degrades the cell wall of fungi and oomycetes (Palumbo et al., 2005).

The microbes associating with the plant root system may induce an enhanced defensive capacity within the plant, with these defence responses divided into acquired systemic resistance (ASR) and induced systemic resistance (ISR). The resistance developed by the plants against the pathogenic bacteria with the use of beneficial microbes are usually associated with induced systemic resistance (ISR) (Choudhary et al., 2007). In ISR, the microbe induces resistance to the plant system after inoculation. The reduction in number of galls or the egg masses of *Meloidogyne incognita* in the tomato plant root system induced by inoculating *Bacillus subtilis* in a split root system compared to the uninoculated control provides evidence that the bacteria improved plant growth by indirectly reducing the disease severity (Adam et al., 2014).

Microbes used as biocontrol agents may either be present as free-living around the root system or symbiotically attached to the plant root system. For example, many of the *Pseudomonas* and *Bacillus* species bacteria are reported forming a multiple layer of the bacterial cells around the plant root and are encased into a polymeric layer, which is collectively called a biofilm (Bogino et al., 2013). This biofilm physically protects the plant root against fungal and bacterial pathogens (Bais et al., 2004; O' Toole & Kolter, 1998). The mechanisms that the biofilm protect the plant root against the soil borne pathogens include quorum-sensing and antibiotic production (Wood & Pierson, 1996). Quorum sensing is the specific cell-to-cell communication in bacteria, which controls the expression of genes required for the specific pathogenicity. For example, the *P. aureofaciens* 30-84, which is responsible for the control of take-all disease in wheat with the production of phenazine antibiotic controlled by the diffusible signal regulated by PhzR and Phz1 (Wood & Pierson, 1996). Bais et al. (2004) presented that biofilm producing bacterial *B. Subtilise* strain 6051 around the *Arabidopsis* root surface produced surfectin, which is responsible in protecting the *Arabidopsis* root from pathogen *P.syringae* pv tomato DC3000 by the antibacterial property of this chemical.

1.12 Plant root system study

During early plant embryonic development, the embryonic root apical meristem constitutes the stem cells that eventually give rise to the root system (Sabatini et al., 2003). The meristemetic cells integrate the signals from various phytohormones and environments that regulate specific root development. The interaction between the developmental processes and environment brings variation in the orientation of the root and its morphological features (Jovanovic et al., 2008). The parameter that determines the root morphological characteristics determines the exploration of the soil volume by the plant root system. Plant root characteristics and growth rate are affected by the soil characteristics, which is largely affected by the management options in the crop field condition.

Many root studies have been conducted in the laboratory, dissecting the molecular and hormonal basis of main or lateral root growth (Chang et al., 2013; Moriwaki et al., 2011). Others have focused on the environmental regulation of the root development, for example on the nitrogen dependent nature of root development patterns (Araya et al., 2014a, 2014b; Malamy & Ryan, 2001). Lateral root development patterns have also been shown in media based studies using the model plant *Arabidopsis* to be influenced by auxin-dependent organ development processes (Moriwaki et al., 2011). Detailed understanding of root development processes has been gained from many studies using *Arabidopsis* grown in artificial growth media, but far fewer have been undertaken under the much more variable field conditions to which crops are exposed, so the degree to which the various regulatory systems are influenced by the conditions existing in the field are still to be determined.

The root growth of crop plants is mostly expressed in terms of root length, for example relating to effects of soil physical characteristics such as reduced root length occurring with increasing soil compaction levels (Ramazan et al., 2012). The changes in root diameter and cross section area of cortical cells have also been assessed the compacted soil (Atwell, 1990). Differences in root length and number of lateral roots at different soil depth have been used in phenotyping different varieties of crop for agronomic use (Nagel et al., 2015). Similarly, study of variation in root length is used to identify the crop varieties that are efficient in nutrient acquisition from different soil depth (Liao et al., 2001).

Root growth study parameters include root length, root volume and root dry weight, and they are assessed in relation to availability of soil nutrition such as nitrogen application rate (Gharakand et al., 2012), or the effect of availability and deficiency of nutrients on root growth such as primary root length, lateral root length, total root length, and number and length of root hairs (Sarker & Karmoker, 2009). Crop root traits have also been studied to identify suitable crops for adoption in arid and semi-arid environments. Among crop varieties, in the water stress condition the total root length increased in the drought tolerant varieties and also the dry-matter partitioning increased towards the root (Rauf & Sadaqat, 2007). The root growth study in term of root length per viewing area and soil depth in maize and sunflower were decreased in full-irrigated conditions compared to water-deficient conditions (Comas et al., 2013).

Crop root system architecture, which defines the orientation of the different types of root (Lynch, 1995), is another root development parameter used in root system research. It deals with the distribution of the root system in the soil space (Hodge et al., 2009). The difference in plant root system distribution in soil has been utilized in studies of resource capture (moisture and nutrients) from different soil strata among different crop species (e.g. between the dicot and monocot species) using mixed or intercropping techniques in the tropics and sub-tropics.

1.13 Root system assessment

Roots are the underground so it is hard to understand the developmental patterns that determine root growth rate and architecture. Since, plant roots are not directly accessible, researchers use both destructive and non-destructive methods for it's study..

The most common method of root system study involves excavation or uprooting of total root system. This method is appropriate for shallow rooted small plants, but is tedious and time consuming for replicated sampling or field study of deep-rooted plants (Bohm, 1979). Excavation of undisturbed cores with or without pinboard matrices is another practice of getting plant root from the soil, but taking whole soil blocks for soil removal may introduce errors (Bohm, 1979).

Use of 'in-growth' cores is another method of studying root development in the field. This method uses root free soil from the same field or a similar soil, with a mesh bag filled with the soil and inserted into the plant root zone for subsequent extraction and analysis of roots rowing into the bag (Steingrobe et al., 2000). This method may alter the physical and chemical properties of soil inserted into the mesh bag, which may enhance or reduced the root development in the mesh bag compared to the field state root development (Steingrobe et al., 2000). Core extraction is another method, in which soil cores are removed with the use of a soil auger or semi-circular cutting blade (Metcalfe et al., 2007). Soil core method does not represent the whole plant root system and also may cause variation in soil volume, but this method is still convenient and relatively quick to perform (Metcalfe et al., 2007).

Trench wall/root window techniques provide an opportunity to directly monitor root growth and short-term root growth dynamics. This method does not provide information on root elongation rate and total root extension (Bohm, 1979). Rhizotrons are similar to trench wall/root window but differ by providing a setting that allows periodic root monitoring throughout the crop season. A transparent plastic sheet or glass set on one side of the rhizotron in which root development is traced at time intervals allows assessment of the rate of root development total root development and root turnover (Metcalfe et al., 2007).

In the field condition, use of transparent mini-rhizotron tubes with video imaging facilities is becoming popular, as it is a simple method to generate 2D information on root morphology, root growth and turnover. In mini-rhizotron method, a soil core is removed just below or on the side of plant and a tube with video imaging facility is inserted (Judd et al., 2015). This technique does not provide the total root development of the plant and also needs repeated measurements (Judd et al., 2015). Sophisticated techniques such as computer-aided X-ray and gamma-ray tomography and Ground Penetrating Radar (GPR) are also in use but they have several limitations to use in the field conditions as they can only applicable for the small root systems (Neumann et al., 2009, Tracy et al., 2012).

1.14 Root morphology measurement

Multiple root system parameters including root length, root diameter, surface area and volume of root, root branching and root viability are important in root system development studies. Usually root length, surface area and root volume are presented to express the root system development considering they are the key indicators for the uptake of nutrients and water from the growing substrate (Himmelbauer et al., 2004). A simple method of measuring root length is using a ruler (Ling et al., 2013), but this method is tedious and biased when working in large-scale samples. The intersection method calculates the root length by counting the number of intersections that roots laid out on lined paper with certain pattern of lines (Leskovar and Cantliffe, 1993; Newman, 1966; Tennant, 1975). Root surface area can be calculated based on the length and diameter of roots but this measurement is tedious and not frequently used in root study. Root volume measurement can be performed using the water-displacement method (Pang et al., 2011). Although displacement methods produce similar root volume when compared with the digital method such as WinRHIZO, other root morphological parameters cannot be measured with this method (Pang et al., 2011).

While many different methods have traditionally been used in root studies, image analysis methods have more recently become the standard approach to measurement of root morphological characters. Several free software programs such as DART and Image J, and commercial software including Delta-T, WinRHIZO, ROOTEDGE, Rootfly, Root Tracker, RootView, and Root Image Analyzer are available for root system study (Bot et al., 2010). These software packages are based on the analysis of the scanned image of the root system. ROOTEDGE and WinRHIZO are the most widely used programs in root studies from field crops to forest species (Fenta et al., 2014; Gao et al., 2015; Kechavarzi et al., 2007; Makita et al., 2012; Ramos et al., 2010). A comparison between different methods of root morphology measurement showed an insignificant difference between manual and image analysis methods (WinRHIZO or ROOTEDGE), with both image methods producing reproducible results for root length, diameter and surface area (Himmelbauer et al., 2004).

1.15 Measurement of soil biology

Soil system is a complex interplay of the microbes and the edaphic factors. Microbes constitute one of the major proportions of the living system in soil, and are responsible for the breakdown of organic matter and nutrients cycling (Aislabie & Deslippe, 2013). Widely used indicators to measure the soil biology consist of measuring the change in the mass, community structure and microbial activity in the soil. They are commonly expressed in term of microbial biomass, microbial diversity and microbial activity.

1.15.1 Microbial biomass

Microbial biomass is the living component of the soil system that includes the bacteria, eukaryotes and archaea. Microbial biomass function as a transient nutrient sink that provides nutrients from the organic matter to the plants. Microbial biomass is usually considered over other biological indicators of soil health because it is too sensitive to any changes in soil properties and agronomic practices (Araújo et al., 2008). Microbial biomass has been measured using fumigation-incubation, fumigation-extraction and substrate induced respiration methods (Barajas-Aceves, 2005; Beck et al., 1997). Fumigation-extraction methods is the most commonly used methods, in which soil samples are fumigated with chloroform and microbial biomass is measured by subtracting the biomass carbon of non-fumigated soil from the fumigated soil (Vance et al., 1987).

1.15.2 Microbial activity

Microbial activity describes the rate of microbiological process in the soil system. Microbial activity provides information on how the microbes are involved in the carbon and nitrogen cycling. Microbial activities are also calculated from the soil respiration such as fluxes of CO_2 trapped by NaOH (Barajas-Aceves, 2005). In-situ soil respiration is measured as efflux of CO_2 using dynamic chamber systems connected to an infrared gas analyser (SRC-1, EGM-3; PP systems, UK) (Vinther et al., 2008). Besides measuring CO_2 fluxes, microbial activity has also been measured by using fluorescein diacetate (FDA) hydrolysis and dehydrogenase activities (Lopes et al., 2010). Although FDA is simple and rapid method of measuring soil microbial activity, it has limitations causing non-biological hydrolysis of FDA at very low or high pH, and showing lower FDA activity in sandy and clayey soil (Schnuerer & Rosswall, 1982). Soil enzyme activity like β-glucosidase activity, which is active in crop residue degradation, is also suggested as one of the important indicator in the change of soil microbial activity (Stott et al., 2010).

1.15.3 Microbial diversity

Microbial diversity measures the relative abundance of microbes. Microbial diversity provides information related to the community composition and dynamics of the microbes (Keshri et al., 2013). In agriculture, measuring microbial diversity studies can be used to understand how the soil microbial status changes with time and space. This information can be correlated with the soil fertility and crop productivity and in general with the overall soil health condition. Microbial diversity is measured using simple microscopic counting to biochemical based and molecular techniques.

1.16 Measuring microbial diversity

Biochemical based methods of microbial diversity includes plate counts, community level physiological profiling (CLPP) and fatty acid methyl ester analysis (FAME) which is also known as phospholipid fatty acid analysis (PLFA), while molecular based methods includes guanine plus cytosine (G+C), nucleic acid reassociation and hybridization, DNA microarrays and DNA hybridization, Denaturing and temperature gradient gel electrophoresis (DGGE and TGGE), single strand confirmation polymorphism (SSCP), amplified ribosomal DNA restriction analysis (ARDRA) or restriction fragment length polymorphism (RFLP) and few others have been reviewed extensively and none of them are free from drawbacks (Kirk et al., 2004). Molecular method also includes the DNA sequencing based community analysis such as pyrosequencing based community analysis (Fakruddin et al., 2012). Although molecular methods can handle large number of samples, are reproducible and can detect structural changes in microbial community, many of them depend on lysing and extraction efficiency, so microbial community may be changed with different sample handling strategies, and also some methods only detect the most abundant species (Kirk et al., 2004).

The plate count method is a widely used biochemical method of enumerating microbial diversity. Although this technique is relatively quick and inexpensive, the method is biased to the microbial population that are culturable and to the rapidly growing species (Kirk et al., 2004). Plate count method can culture less than 1% of the microbes present in the agricultural soil (Torsvik et al., 1990). This method has been widely used to estimate the microbial population in soil samples and also used as a basis for estimating microbial population for other biochemical methods such as CLPP.

Fatty acid methyl ester analysis (FAME) is a non-culture based biochemical method to study microbial diversity. FAME differentiates the microbial community composition based on fatty acids group, considering that certain group of microbe contains specific fatty acid group and the change in the fatty acid profile provides the information about the change in the microbial community structure (Kirk et al., 2004).

CLPP utilizes the functional capabilities of the microbial population based on the utilization of the carbon sources. CLPP uses Biolog plates. The Biolog Ecoplate consists of 96 wells with 31different carbon sources and a control well, replicated three times on each plate. Tetrazolium violet is used as an indicator of the reduction process that occurs in wells where the microbes are able to break down the carbon source. Sole carbon sources present in the Biolog Ecoplates are carbohydrates (7), carboxylic acids (9), amino acids (6), Polymers (4), amines (2) and others (3) (Preston-Mafham et al., 2012). Similarly, FF plates are used to quantify the fungal diversity based on the 95 different sole carbon sources. The 95 different sole carbon sources in the FF plates are divided into the groups of carbohydrates (44), carboxylic acids (17), amino acids (14), polymers (5), amines (5) and others (10) (Preston-Mafham et al., 2012). In FF plates Iodonitrotetrazolium violet is used as the redox dye, an indicator of respiration of sole carbon source that turns a reddish orange colour when microbes utilize the carbon. The colour changes in each well over a certain time period is quantified using a plate reader. The differential rate and intensity of colour produced in the well from the reduction of dye is used as an indicator of utilization pattern of different microbial community for the specific carbon source.

CLPP is an important tool to differentiate microbial community and has been successfully used to assess the microbial diversity of forest soil (Pignataro et al., 2012), grassland (Liu et al., 2008) and in agriculture soil treated with chemical fertilizer and herbicides (Liu et al., 2007). This method is reproducible but only tells the diversity of culturable fraction of microbial community, favours fast growing microbes, and the rate of carbon utilization is density dependent and only represents organisms that are capable of utilizing available carbon sources (Kirk et al., 2004). Nevertheless, CLPP has become a valuable tool studying a functional diversity of microorganism and quantify the microbial diversity including richness and evenness of microbes within the community or between the sites.

1.17 Expression of microbial diversity

Microbial diversity can be expressed in term of inventories of taxonomic groups, phylogenetic trees or by using diversity indices (Trosvik et al., 2000). The diversity indices are commonly used in studying ecological diversity, and they are now adapted to different areas including expressing soil microbial diversity. Commonly used diversity index in ecology are Shannon diversity index, OTU richness estimator, Margalef index, Q statistic for the species richness, and Shannon evenness index, and Simpson's index to measure the evenness and dominance (Hill et al., 2003). Shannon diversity index and evenness are the most commonly used indexes. Shannon diversity index provides the species richness e.g. the number of microbial species relative to the total number of microbes in a community (Hill et al., 2003). This does not provide information about the individual species dominance. Evenness describes the species dominance, which provides information on a species relative to the other species present in a community.

1.18 Summary and research aims

The intensive cropping systems based on conventional tillage practices and, reduced soil organic material has created several soil health related problems in Australian cropping soil. One of these is reduced soil microbial activity and microbial community diversity. To improve this condition of the soil in conventional farming systems, use of different organic amendments are encouraged. Organic amendments including crop residue and compost added in soil are beneficial to improve the soil structure, soil aggregate stability, reduce soil compaction and increase biological activity. The increased soil biological activity is tending to reduce disease severity and soil borne pathogen populations that causes root rot diseases in vegetable crops. However, management of organic amendments in cropping systems does not offer the precise control over the soil system that other intensive management practices have delivered. In order to generate more repeatable results from the use of soil organic amendments, it is important to understand the effect of organic material application on soil biology. Therefore this study will investigate the effect of organic amendments, which is considered beneficial to improve the soil health, on soil biology (soil respiration) and its influence on the root system development in vegetable crops through a series of field, pot and laboratory research. To address this broader objective the research is divided in to eight chapters with specific aims and hypothesis.

Chapter 1. Introduction and literature review. This chapter provides a brief rationale of the research in the field of soil health and root development and general review of the literatures on soil health, soil health management approaches, organic material addition and their effect on soil physical, chemical and biological properties, and their impact in crop growth.

Chapter 2. Materials and Methods. This chapter provides general overview of the materials and methods used in different research chapters including soil characteristics, organic materials used, field preparation, crop management, soil nutrient analysis, root sampling and root morphological measurement. The specific materials and methods used in different research chapters are explained in detail in the respective chapters.

Chapter 3. Effect of organic material application on capsicum and chilli root growth. This research aimed to establish the effect of different types and rate of organic amendments on capsicum and chilli root system development. This research tested following hypothesis i) organic material addition increases soil microbial activity (soil respiration) ii) organic material applied treatment produce longer root system iii) increasing the application rate of compost produce longer and healthier root system.

Chapter 4. Effect of varying carbon to nitrogen ratio (C:N ratio) on root growth and soil microbial activity. As chapter 3 showed that soil incorporation of uncomposted cane residue to improve soil organic carbon significantly reduce the root growth, one of the mechanism could be very high C:N ratio. Thus, this research, aimed to investigate how the varying C:N ratio in soil impact on the root growth and soil microbial community. This research tested the following research hypothesis; i) very high and low C:N ratio negatively affect the crop root length and ii) the root development at very high and low C:N ratio in field crops is microbially mediated.

Chapter 5. Effects of different types of compost on chilli seedlings root growth and their influence in the field. Chapter 3 field research shows different rate of compost application in field affect root growth in chilli crop. Thus, this research chapter aimed to assess performance of vegetable seedlings of same age, grown in different media, in open field conditions. The hypotheses tested were; i) chilli seedlings grown with compost media produce longer root length compared to seedlings grown in conventional peat-based seedling growing media, and ii) Compost based transplants produces a larger root system and higher crop yield when transplanted into open field conditions compared to peat-based transplants under similar field management practices.

Chapter 6. Effect of microbe inoculation on capsicum seed and seedlings on reducing the allelopathic effect of crop residue and their leachate on crop seed germination and root growth. One of the experiment in chapter 4 suggested that leachate from the cane residue could have negatively impacted root growth. Thus, this research chapter aimed to examine the allelopathic effect of different crop residue leachate on seedling emergence, root development, crop growth and yield, and whether microbes can be utilized to minimize the negative effect of organic matter biologically. Therefore, this research tested the following research hypotheses; i) increased concentration of crop residue leachate reduces crop seed germination and root development ii) application of microbes reduces the allelopathic effect of cane residue leachate on seed emergence iii) field application of microbes reduces the allelopathic effect of organic matter thereby enhance plant growth and root development.

Chapter 7. Effect of microbial inoculation on reducing root damage caused by crop residue leachate. Chapter 6 showed that cane residue leachate negatively affects crop seed germination and root growth, and that negative effect can be reduced by microbial inoculation on seeds or seedlings at the time of seedling sowing or transplanting. This research chapter aimed to investigate how does the microbes reduce the root damage caused by the cane residue. Therefore, this research tested the following hypothesis; i) inoculating casicum seedling with beneficial microbes reduces the root damage caused by the allelopathic effect of cane leachate, ii) increasing capsicum seedling inoculation time with beneficial microbes reduces root damage by allelopathic effect of cane leachate and iii) microbes are more effective at higher temperature to reduce the root damage caused by allelopathic effect of cane leachate.

Chapter 8. General discussion. This chapter sum-up all the research and their outcomes in relation to the field application along with final conclusions.

2 General Materials and Methods

This chapter describes methods common to the series of experiments carried out in this research. Descriptions of overview of experiment, overall climate of the Bundaberg region, soil type, pest control, organic amendments, planting materials, types of experiments, cultural practices, statistical procedures and others are included. Details of specific methods used are presented in the materials and methods section in each experimental chapter in the thesis. A summary of the experiments completed in the research is presented in Table 2.1.

Table 2.1 Overview of the experiments.

Chapter no	Environment (soil type)	Location, Coordinates	Start-end Date	Brief description
Chapter 3	Field (Red Ferrosol)	Dept. Agriculture and Fisheries, Bundaberg 24°51’02.04” S, 152°24’10.16”E	May-Nov 2012	Measured the root morphological parameters of capsicum and chilli grown with different types and rate of organic matter application.
	Field (Hydrosol)	AustChilli, Bundaberg 24°56’10.01”S, 152°24’10.93”E	Jul-Nov 2013	Measured effects of compost amendment on chilli root morphology and crop yield.
	Field (Hydrosol)	AustChilli, Bundaberg 24°56’10.01”S, 152°24’10.93”E	Jan-June 2014	Measured effects of compost amendment on chilli root morphology and crop yield.
	Pot (Hydrosol)	CQUniversity, Bundaberg 24°54’03.93”S, 152°18’44.45”E	Jul-Sep 2013	Measured root morphological parameters and root health of capsicum seedling grown in different rate of compost applied to field soil.
Chapter 4	Pot (Hydrosol)	Dept Agriculture and Fisheries, Bundaberg 24°51’00.69” S, 152°24’03.93”E	Jan-Feb 2014	Measured the root development of capsicum seedling grown at different C:N ratio adjusted with varying nitrogen.
	Pot (Red Ferrosol)	Dept. Agriculture and Fisheries, Bundaberg 24°51’00.69” S, 152°24’03.93”E	June-Oct 2014	Measured the capsicum root development and crop growth with different C:N ratio adjusted at varying carbon and nitrogen application.
	Laboratory	CQUniversity, Bundaberg 24°54’03.93”S, 152°18’44.45”E	Nov 2014	Measured average well colour development, shannon diversity index and evenness for the soil microbes from different C:N ratio

Chapter no	Environment (soil type)	Location, Coordinates	Start-end Date	Brief description
Chapter 5	Nursery	Wide Bay Seedling, Bundaberg 25°39'51.99"S, 152°34'24.35"E	June-July 2014	Measured the root morphological parameters of chilli seedling grown in conventional and compost based substrate.
	Field (Hydrosol)	AustChilli, Bundaberg 24°56'10.01"S, 152°24'10.93"E	July-Nov 2014	Measured the root morphological parameters and yield of chilli in the field from seedling grown in conventional and compost based substrate.
Chapter 6	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Dec 2013	Measured germination percentage and seedling root length of different crops at varying concentration of crop residue leachate.
	Pot (Hydrosol)	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Mar 2014	Identified the effective concentration of cane residue leachate for allelopathic effect on capsicum seedling emergence.
	Pot (Hydrosol)	Dept. Agriculture and Fisheries, Bundaberg 24°51'00.69" S, 152°24'03.93"E	July-Aug 2014	Identified the effect of different commercial microbes in inhibiting the allelopathic effect of cane residue leachates on capsicum seedling emergence.
	Field (Red Ferrosol)	Dept. Agriculture and Fisheries, Bundaberg 24°51'00.61"S, 152°24'03.89"E	Aug 2014-Feb 2015	Measured the crop growth and root morphological parameters in transplanted capsicum seedlings treated with different microbial solution in cane residue applied soil.
Chapter 7	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Jan-Mar 2015	Measured the damage on capsicum seedling with different exposure time on cane residue leachate.
	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Mar-May 2015	Measured the root damage on capsicum seedling exposed to different concentration cane residue leachate.
	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Mar-May 2015	Measured the root damage on microbial inoculated capsicum seedling exposed to the cane residue leachate.
	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	Mar-May 2015	Measured the proportion of healthier and damaged capsicum seedling root after varying time of exposure to microbial solution and cane

Chapter no	Environment (soil type)	Location, Coordinates	Start-end Date	Brief description
				residue leachate.
	Laboratory	CQUniversity, Bundaberg 24°54'03.93"S, 152°18'44.45"E	April-May 2015	Measured the effect of temperature on capsicum seedling root damage after exposing seedling to microbial solution and cane residue leachate.

2.1 Climate

Bundaberg is located within the Wide Bay Burnett region in Queensland, Australia. Bundaberg experiences a subtropical climate with a hot summer and mild winter. This climate allows field production of several vegetable crops all year round. The mean maximum and minimum temperature and average rainfall during the experiment period, 2012 - 2014, in the Bundaberg are presented in Fig. 2.1, 2.2 and 2.3. The climate data were recorded by the Australian Government, Bureau of Meteorology at the Bundaberg aero site, located near to the experiment sites. The mean daily temperature in the experiment area remains higher from October to March, and lower from April to September with occasional record of high temperature in the summer season. The monthly average precipitation varies with the year but in general the maximum precipitation is concentrated early in the year, which coincides with the high temperature summer season.

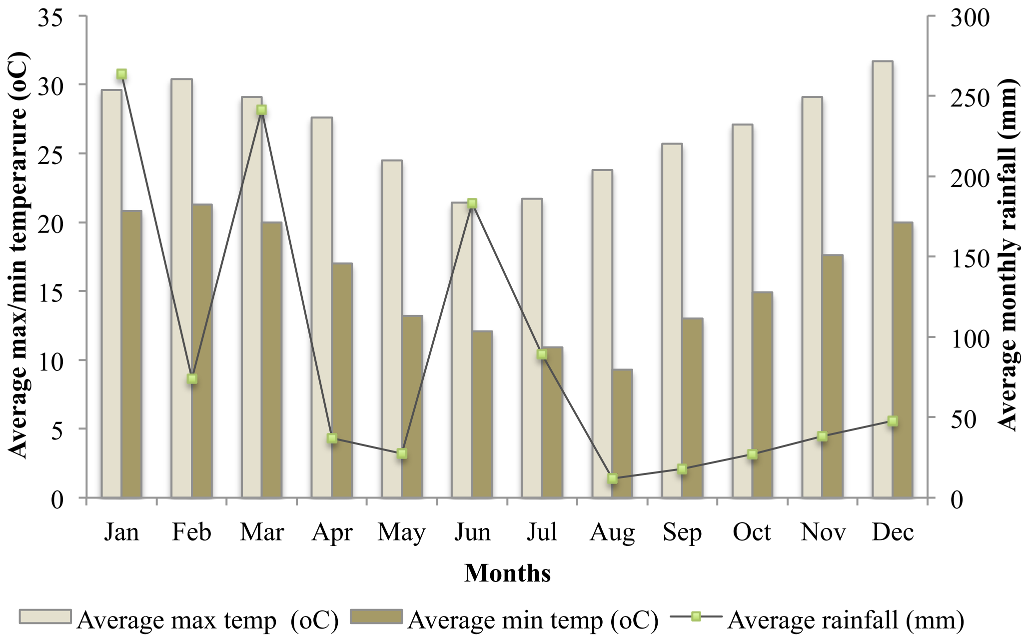

Figure 2.1 Average monthly maximum and minimum temperature and rainfall for the year 2012 in Bundaberg, Queensland, Australia (BoM, 2016).

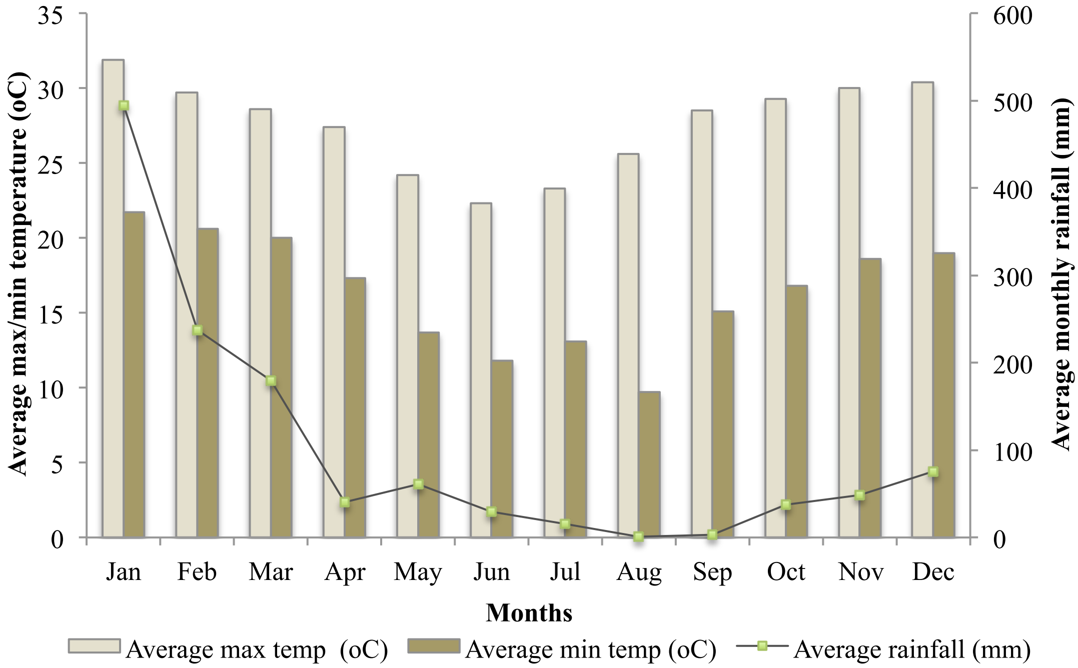

Figure 2.2 Average monthly maximum and minimum temperature and rainfall for the year 2013 in Bundaberg, Queensland, Australia (BoM, 2016).

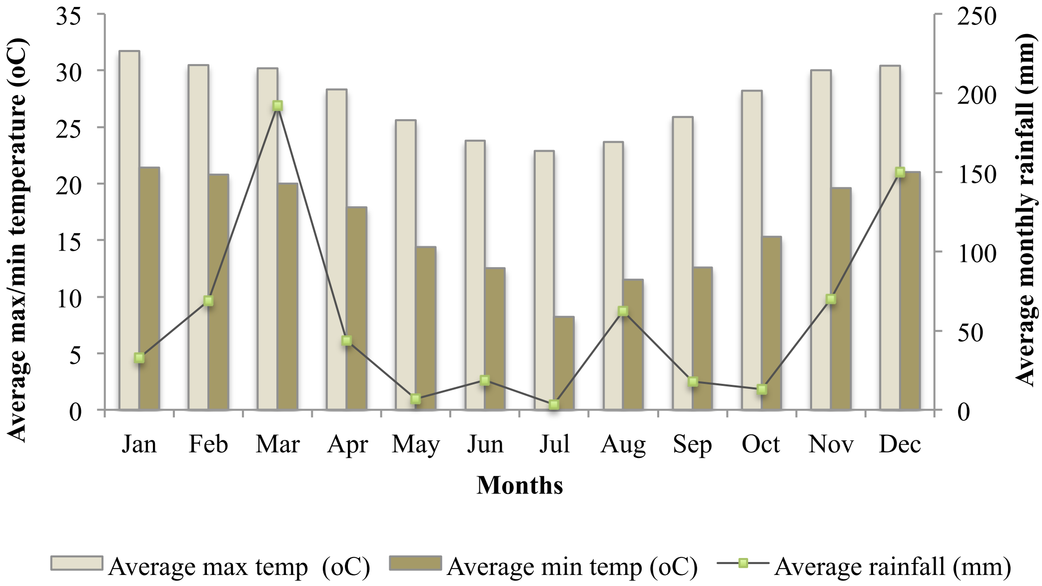

Figure 2.3 Average monthly maximum and minimum temperature and rainfall for the year 2014 in Bundaberg, Queensland, Australia (BoM, 2016).

Long-term average monthly temperature data (1959-2015) shows similar trend in average monthly temperature as individual experimental years, showing decrease in both average maximum and minimum temperature from January to July and again increase from July to December (Fig 2.4). The experimental year 2012 experiences warmer in February, November and December, while the months January, May, June, August and October were colder than the long-term average. In 2013, it was warmer in January and from July to December, but colder in May compared to the long-term average. In 2014, January, March to June, and August, November and December was warmer months compared to long-term average monthly temperature.

The long-term monthly average rainfall (1942-2015) pattern was not similar to average monthly rainfall pattern in the individual experimental year (Fig 2.4). In 2012, January, March, June and July were wetter and other months were drier than the average long-term rainfall data. Similarly, in 2013, from January to March were wetter, and April to December experiences drier season compared to the average long-term rainfall. In 2014, March, August and December received more rainfall and the remaining month got lesser rainfall compared to the long-term data.

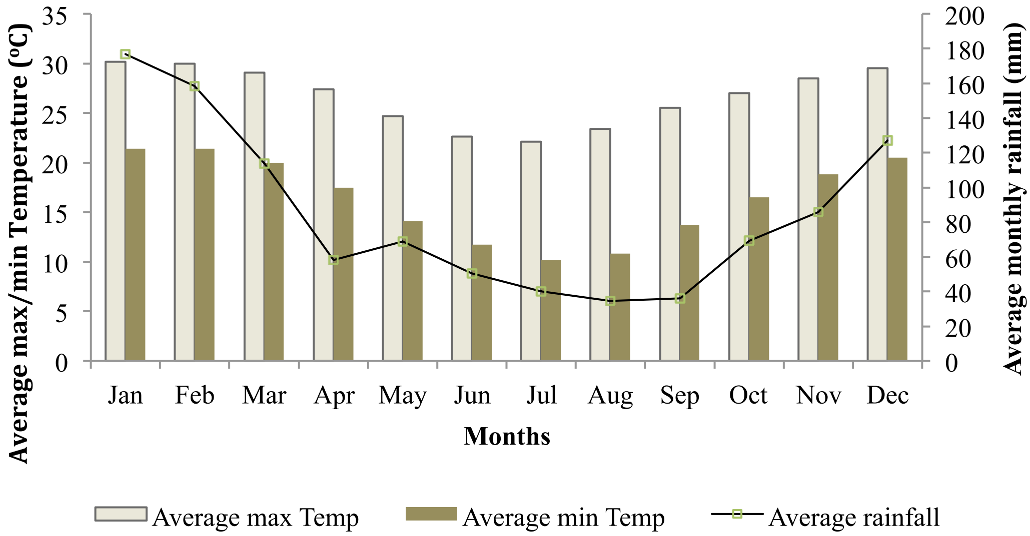

Figure 2.4 Long-term average monthly maximum and minimum temperature (1959-2015), and rainfall (1942-2015) in Bundaberg, Queensland, Australia (BoM, 2016).

2.2 Soil type

The field experiments were conducted in Bundaberg, Queensland, in two different sites with contrasting soil types. The field experiments were conducted either in a Department of Agriculture and Fisheries (DAF), Bundaberg, Queensland, or in commercial chilli field at AustChilli Bundaberg, Queensland at different times of the year. Soil for the pot experiment conducted either in the polyhouse at DAF or shade at CQUniversity was also taken either from DAF or AustChilli sites. The soil at DAF has dark reddish colour, clay texture and firm consistency when dry, classified as Red Ferrosol in the Australian soil classification system (Isbell, 2002). Soil in the AustChilli field was with grey colour, sandy loam topsoil, and grey to yellow colour sandy clay loam subsoil classified as Hydrosols (Isbell, 2002). The soil used in different field and pot experiments were either from DAF or AustChilli site, and their selected properties are presented in Table 2.2.

Table 2.2 Selected soil properties at DAF and AustChilli field sites at 0-10 cm soil depth.

Soil properties	Units	Red Ferrosol (DAF)	Hydrosols (AustChilli)
Colour		Red brown	Grey
Sand	%	12$^{\$}$	73
Silt	%	17$^{\$}$	14
Clay	%	73$^{\$}$	13
pH (1:5 water)		6.10	6.1
EC (1:5 water)	dS m^{-1}	0.22	0.03
Bulk density	g cm^{-3}	0.86	1.06
Organic carbon	g kg^{-1}	14.2	4.40
NO_3-N	mg kg^{-1}	22.7	3
NH_4-N	mg kg^{-1}	4.6	0.4
K (Am. Acet.)	mg kg^{-1}	226.0	86.0
Ca (Am. Acet.)	mg kg^{-1}	1044.0	443.0
Mg (Am. Acet.)	mg kg^{-1}	247.0	50.0
Na (Am. Acet.)	mg kg^{-1}	97.0	7.0
Zn (DTPA)	mg kg^{-1}	4.0	1.38
Cu (DTPA)	mg kg^{-1}	4.7	7.20
Fe (DTPA)	mg kg^{-1}	21.0	67.2

$^{\$}$ Christianos et al. (1990).

2.3 Pest control

Capsicum and chilli crops in the field experiments were controlled and protected from different insect pests and diseases by spraying insecticide and fungicide based on regular crop scouting and past pest and disease incidence history. Several insect pest and diseases were common both in capsicum and chilli plant.

In capsicum and chilli field bacterial leaf spot (*Xhantomonas campestris* pv.*vesicatoria*) and powdery mildew (*Leveillula taurica*) were common diseases in the above ground plant parts. Powdery mildew was more frequent in the cold weather (May to September) in the field. Preventive control measures were commonly used in the field based on the previous year disease infestation. The bacterial leaf spot was controlled by using copper hydroxide (Blueshield, 500 g kg^{-1} a.i., Bayer) or cuprous oxide (Nordox, 750 g kg^{-1} a.i., Nordox) applied as 1 and 2 kg ha^{-1} respectively. Both *Bacillus subtilis* (Rhizomax, Biofilm) and *Bacillus amyloquifacens* (Lolipepta, Biofilm) were also used at 15 L ha^{-1}: as trickle irrigation to control leaf spot alternating with the copper applications. Powdery mildew was controlled by spraying sulphur (Microthiol, 800 g kg^{-1} a.i, Nufarm) or penthiopyrad (Fontelis, 200 g L^{-1}a i., DuPont) with the application rate of 2 kg ha^{-1} and 1.75 L ha^{-1} respectively. Anthracnose disease caused by *Colletotrichum acutatum* was controlled by using chlorothalonil (Cavalry Weatherguard, 750 g L^{-1} a.i., Adama) with the application rate of 2 L ha^{-1}. The more common stem rot disease near the ground surface caused by *Sclerotium rolfsii*, which was controlled by spraying procymidone (Sumisclex, 500 g L^{-1} a.i., Sumitomo) with the application rate of 1:1000 L of spraying water. Biological prevention of this disease was carried out with *Bacillus subtillis* (Rhizomax, Biofilm) applied at 15 L ha^{-1} application rate as after planting prevention.

Heliothis (*Helocoverpa armigera*), mites (*Tetranychus urticae*) and aphids (*Myzus Persicae*) were more common insect pests in the capsicum and chilli field. Heliothis was controlled by using methomyl (Lannate, 225 g L^{-1} a.i., Crop Care) spraying at 2 L ha^{-1}. Mites were controlled by using abamectin (ABA 18, 18 g L^{-1} a.i., Genfarm) and aphids were controlled by using primicarb (Aphidex, 500 g kg^{-1} a.i., Adama) at the application rate of 300 mL ha^{-1} and 750 g ha^{-1} respectively. Fruit fly (*Bactrocera tryoni*) in chilli plants was controlled by spraying trap; maldison (Hy-Mal, 1150 g L^{-1} a.i., Crop Care), and in case of large population dimethoate (Rover, 400 g L^{-1} a.i., SIPCAM) was sprayed at 750 mL ha^{-1}.

Weeds in the plant bed were controlled using plastic mulch. Some weeds emerged from the seedling plant hole in the plastic mulch, were hand pulled. Inter-row space weeds were removed manually using a chip hoe. Weeds around the field boarder were controlled using herbicide at the time of minimum airflow to minimize the herbicide-drifting problem to the crop plants.

Both hydraulic and air-curtain boom sprayers were used for insecticide and fungicide application. The sprayers were installed on the back side of the manually operated tractor and sprayed in the field.

2.4 Organic amendments

The organic materials used in the experiment were either bought from local agricultural suppliers or collected from the field. The majority of experiments utilised cane residues, also called cane trash (*Saccharum officinarum* var. Q240), the leaves of the plants left in the field after the sugarcane crop harvest. As vegetable crops in the Bundaberg region are commonly grown in rotation with sugarcane crops, the incorporation of cane residue into soils to retain or improve soil organic matter levels is a common practice.

Dry, baled cane residue and lucerne (*Medicago sativa* var. T15651) were bought from the commercial mulch supplier (Bunnings Pty Ltd, Bundaberg). Sorghum (*Sorghum halepense* var. Cowpow) leaf and stem material was collected from the AustChilli field where it was grown as a cover crop. The harvested sorghum material was chopped into small pieces and air-dried. Cane residue, lucerne and sorghum were powdered using a grinding machine with 2 mm mesh size (Christy, England) at the Department of Agriculture and Fisheries (DAF) at Bundaberg, Queensland.

Two different types of compost were used in the research. Sawdust and cane residue based compost used in the experiments was bought from Australian Prime Fibre, Childer, Queensland, Australia, and leaf litter based compost used in the experiments was obtained from Wide Bay compost, Queensland, Australia. A commercial formulated compost was used while raising chilli seedling in nursery.

2.5 Planting materials

The research used capsicum (*Capsicum annuum* L. var. Warlock and California Wonder) and chilli (*Capsicum annuum* L. var. Caysan) as model crop plants to examine interactions between soil parameters, plant root development and crop performance. These crops were chosen as they have relatively weak root systems compared to other vegetable crops and may benefit from soil amendments that promote stronger root system development. The experiments in the research used either direct seeded or transplanted nursery-raised seedlings. All seeds used in the research were purchased from commercial seed supply companies and were tested to ensure high germination percentage and seedling vigour.

Seeds were used for the assessment of the effect of organic matter leachate on germination and seedling root morphological parameters in the laboratory, and to assess the effect of cane trash leachate on seedling emergence. Capsicum and chilli seedlings were used to assess root system development and crop performance in the field with the application of different organic matter treatments. Seedlings were also used in the pot experiments in the polyhouse to investigate the root development with different C:N ratio. The experiments in the laboratory were generally followed by the experiments in field conditions to validate laboratory experiment by the field research so that treatment effects are more predictable and practical.

The capsicum (var. Warlock) and chilli (var. Caysan) seedlings used in the field experiments in chapter 1, 5 and 6 were raised in a commercial seedling nursery (Wide Bay Seedling, Queensland) in plastic plug trays of 18 mm^3cell volume. A mixture of peat and vermiculite was used as the seed germination media. In the nursery, seedlings were germinated at 28^{o}C and transferred to greenhouse conditions with controlled temperature and relative humidity. Before supplying the seedling for transplanting, they were hardened by exposing to sunlight for a week. Six weeks old seedlings of height 0.13 to 0.15 m, supplied by the nursery, were used in the field experiments.

Seedlings for the pot trials in chapter 4 were prepared at the Central Queensland University (CQUniversity), Bundaberg campus shade house. Capsicum (var. California Wonder) seeds were sown in plastic plug trays, as described above, filled with seedling raising mixture Osmocote (Scotts, Australia) that contained organic materials including composted pinebark, living microorganisms plus mineral and fertilizer additives. Seedlings were raised for 40 days, and hardened for a week before transplanted in the pots.

Capsicum seeds (*Capsicum annuum* var. California Wonder) used for the pot experiments in chapter 6 and 7, and feed stock barley (*Hordeum vulgare*) and cabbage (*Brassica oleracia* var. Greyhound) seeds used in the chapter 6 were bought from the commercial supplier. Locally available seeds were tested for germination in the laboratory at CQUniversity Bundaberg campus. For the subsequent germination test in the laboratory in the petri dishes, capsicum seeds from the supplier were treated (when required) with 1% commercially available sodium hypochlorite solution for five minutes and then washed for 8-10 times.

2.6 Microbial products

Freeze-dried microbial product for different microbes either single strain or mixture of different strain or species were bought from commercial microbes supplier (New Edge Microbials Pty Ltd). Microbes were used in the pot and field experiment in chapter 6 to improve the capsicum seedling emergence and root growth, and in the laboratory in chapter 7 to minimize the root damaging effect of cane residue leachate. A range of commercial product/additives used as treatments were *Bacillus* sp. both in freeze dried and liquid formulations, *Pseudomonas* sp., *Glomus* sp., *Azotobacter* sp. and compost leachate and other with mixture of different microbes. The mixed treatments include *Pseudomonas* spp. + *Streptomyces* spp., *Pseudomonas* spp. + *Bacillus* spp. + others, *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. + others. The mixed microbial products will be presented with the name of the main microbes present. Microbial solutions were prepared by mixing one vial of freeze-dried microbes (20 g vial, 5 x 10^{11} CFU bacteria) in 2 L deionised water for the freeze-dried product, and for the powdered and liquid formulations the equivalent amount were added to 2 L deionised water. Microbial solution were prepared by mixing each vial of freeze dried microbial product in 2 L deionised water for the use in chapter 6 pot trial and chapter 7 laboratory experiments, while for inoculating transplant each vial was mixed in 10 L water.

2.7 Laboratory experiments

Laboratory experiments were carried in the CQUniversity, Bundaberg campus laboratory. Crop residues water leachates were used to assess the root development in different crops, most of the time cane trash leachate was used. Cane trash leachate solution was prepared from un-composted, dried, powdered cane trash soaked in deionised water overnight with occasional stirring. Leachates were used to assess its effect on crop seedling root development and seedling emergence. Capsicum seedling root damage was also measured at different concentration cane trash leachate with different exposure time to microbial solution. The individual treatment was arranged on filter paper in petri dish. Petri dishes were arranged in replicated trial. Moisture in the petri dish was maintained applying deionised water. Crop seed germination was measured by counting number of germinated seed. Seeds were considered germinated when radicle appeared out of the seed surface. Root morphological parameters were measured by scanning roots in scanner (Epson Perfection V700, Digital ICE Tech.) and then by using WinRHIZO Pro 2012a software (Regents Instr. Inc., Canada). Root damage by cane trash leachate and effect of microbes was measured after scanning root by using colour-scanning method.

2.8 Pot trials

Pot trials were conducted either in a glasshouse at the CQUniversity, Bundaberg campus or inside a polyhouse at the Queensland DAF research station, Kalkie, Bundaberg. In both cases, pots were kept on a metal-wired table.

The effect of uncomposted cane residue leachate prepared with cold-water extraction on capsicum seedling emergence was conducted at CQUniversity, Bundaberg Campus and DAF, Kalkie, Bundaberg. Capsicum seeds (var. California Wonder) were sown in sandy-loam soil collected from AustChilli field, in styrofoam cups (70 mm top and 50 mm bottom diameter with 80 mm height) and supplied different concentration organic-cane residue leachate. Commercial microbial solutions were applied in the cane trash leachate applied soil to examine whether microbes can be used to inhibit the toxic effect of cane residue leachate on capsicum seedling emergence. The successful seedling emergence was recorded as appearance of two cotyledon leaves on the soil surface.

For the seedling transplanting pot experiment, capsicum seedlings prepared in a multi-plug seedling tray using seedling-raising mixture were transplanted in polythene plastic pot (140 mm top and 110 mm bottom diameter with 145 mm height) filled with red Red Ferrosol soil mixed with dried, un-composted cane trash powder and nitrogen fertilizer to adjust the carbon and nitrogen ratio.

In all pot experiments, moisture was maintained at field capacity. Field capacity was measured in a CQUniversity laboratory by adding water on soil in cup or pot to the sufficient amount that it starts flowing from the bottom of the cup or pot, and keeping them for 24 hours so that all freely available water drains out. Field capacity was measured as the difference in weight of soil after draining out all freely available water and dry weight of the soil. Water in pot experiments was added when water level lowers from the field capacity, which was measured in every two days using weighing balance. Weeds emerged in the pot during the experiment time were hand pulled.

2.9 Field trials - crop management practices

Field sites were prepared with several passes of primary tillage with a chisel plough, a secondary tillage with rotary hoe and a final pass with a bed former. The seedling transplanting beds of 0.15 m height and 0.75 m width were prepared using the bed former. After bed formation, organic matter and/or compost were applied and mixed into the soil. A drip tape of internal diameter 16 mm with emitters in 0.3 m spacing (Rivulis, Australia) for irrigation was laid on one side of the planting row centre line in single row planting methods, and on the bed centre line in double row planting (in chilli field). Drip lines were laid approximately at 0.05 m depth in a furrow made at the time of bed formation. The rows were covered with polyethylene plastic prior to planting. White plastic was used in summer so as not to increase the bed temperature while black plastic was used during winter in order to increase the bed temperature. After plastics were laid down, the field was left for approximately 20 days before transplanting seedlings.

Capsicum and chilli plants were transplanted using a tractor driven waterwheel type transplanter. The rotating wheels dug holes in equal distance on the side of the drip tape. Water was added to the holes through a water pipe attached to the waterwheel, and transplants were then placed by hand into the holes with soil pushed in to ensure soil to root system contact. In single row transplanting, spacing between the plants was maintained at 0.25 m and between rows was 1.50 m, while in double row transplanting, plant-to-plant spacing was 0.15 m and between rows within a bed was 0.35 m.

Irrigation in the field experiment was managed using the drip tape. Irrigation rate and time was managed through monitoring soil water levels at DAF site, while at the AustChilli site irrigation was applied based on a commercially developed application schedule. No evidence of water stressed was noted in any of the field trials. Fertilizers were applied based on soil testing. Basal fertilizer was applied at the time of field preparation while fertigation was during crop development. So soils were not nutrient limited at the time of seeding or transplanting, but the nutrient analysis provided information whether the treatment affected on soil nutrients or not.

2.10 Crop, plant and soil assessments

Fruit harvest both in capsicum and chili was carried by hand. Since both crops are indeterminate in flowering habit, rotational harvesting was carried to assess the total fruit yield. Above ground dry weight of plant was measured by destructive sampling method. Individual plant was excavated from the field or from the pot and separated the above ground biomass from root and fruit when required. The number of plants sampled in each experiment will be explained in detail in individual chapter.

Measurements of soil respiration in the field were taken by using closed respiration chamber using EGM3 (PP Systems, UK) at different time of the crop growth stages. Soil respiration was measured in the noon after irrigating the field, and the measurements were taken in between the two plants in a row. Mulch plastic was removed to the size of soil respiration chamber diameter and then the chamber was inserted to the collar depth when the instrument was ready for the measurement.

Soil physical properties (except Red Ferrosol soil texture) presented in Table 2.2 such as color, texture and bulk density were measured at the Central Queensland University, Bundaberg campus laboratory. Soil chemical properties were measured by sending the soil samples to the accredited soil testing laboratories in Queensland, Australia. In the field, soil samples were collected from 0-10 cm soil depth before seedling transplanting and also at the crop harvest stage. In the pot experiment, soil samples were collected from the 0-5 cm rhizosphere area. Several soil samples were collected from each treatment from the field and from the pot experiments, and a composite samples were prepared by thoroughly mixing the samples by hand. A sub-sample for each treatment was prepared from the composite sample, and sent a single sample from each treatment to the laboratory through express postal services. Soil color was identified using Munsell soil color chart. Soil texture was measured by dispersing soil in water with non-foaming agent, and using a textural triangle as described by Whiting et al. (2015). Soil bulk density was measured by using core ring method as described by Hao et al. (2007), which is calculated as ratio of oven-dried mass of soil to the bulk volume of that soil. Soil chemical properties were analyzed by using the methodologies recommended in Rayment and Lyons (2011), in which pH and electrical conductivity (EC) was measured by 1:5 soil/water suspension, phosphorus content was determined by Olsen method, K, Ca, Mg and Na was measured by ammonium acetate extraction, Cu, Zn, Mn and Fe was measured by using diethylenetriamine penta acetic acid (DTPA), B by using $CaCl_2$ extracting solution, S by calcium phosphate extraction, nitrate nitrogen (NO_3-N) was measured by using water, ammonium nitrogen (NH_4-N) was measured by using steam distillation method and organic carbon content was measured by using loss-on-ignition method.

2.11 Root sampling, cleaning and scanning

Roots were sampled either using whole root system excavation or soil core sampling methods. Whole root system excavation provided total root system of sampled plant while the core sample provided a part of the total root system of a plant. Whole root system excavation was used at the early vegetative stages in 2012 and 2014 capsicum field plots and from the pot trials. When the root system was extensive, especially in the field experiments, whole root excavation was labor and time intensive, therefore; root samples were taken using a soil core sub-sampling method (Bohm, 1979).

Collected roots were washed in the field immediately after root excavation and kept in pre-labeled plastic bags, transported to the CQUniversity laboratory and rewashed in low-pressure tap water. Extra debris remained in the root system were hand removed using tweezers. Cleaned roots were scanned using scanner (Epson Perfection V700, Digital ICE Tech.) either in grey scale or color and root morphological features were measured using WinRHIZO Pro 2012a software (Regents Instr. Inc. Canada).

2.12 Biomass and fruit yield assessment

Above ground biomass (hereafter mentioned as shoot biomass) and root biomass were measured at each time of root sampling for both capsicum and chilli plants, when necessary. Plant and root biomass were taken after drying them to constant weight at 70°C at CQUniversity, Bundaberg campus laboratory. Fresh fruit were harvested from capsicum and chilli plants by using repeated harvest method. Fruit weights were taken using a weighing machine (CPWPlus35, Adam) immediately after the fruit harvest in the field.

2.13 Data analysis

Data were analyzed using Minitab-16 software (Minitab, 2009) and SPSS (IBM SPSS, 2013). Analysis conducted using Generalized Linear Model (GLM) and analysis of variance (ANOVA) to test any treatment difference for the data following normality and with equal variance. Data required for transformation for ANOVA were transformed based on the data skewness. Data sets not appropriate for ANOVA were tested using Kruskal-Wallis one-way non-parametric test. Relationships between variables were tested using Pearson's correlation coefficient (r). Specific data analysis methods used in each experiment is separately explained in the individual research chapter.

3 Influence of organic amendments incorporation on soil biology and root system development in transplanted capsicum and chilli

Abstract

Organic material additions to increase soil organic carbon (SOC) content and encourage increased soil biological activities are increasingly being used in intensive cropping systems to improve soil health and the functioning capacity of the soil. This study examined capsicum (bell pepper), and chilli plant growth and soil biological activity responses to different types and rates of organic amendments to soils to identify relationships between root system development and soil biological activity. Addition of composted and fresh organic materials derived from sugar cane crops increased soil microbial respiration in both field and pot trials. Higher application rates of organic materials resulted in higher soil respiration rates. Significant plant growth responses were observed between different organic amendments, but no correlations between root system development and soil respiration rate were recorded. Fresh cane residue applications produced a significantly shorter root length and smaller surface area, volume in capsicum compared to composted cane residue and an untreated control. Uncomposted cane residue treatment produced 40.6, 45.6 and 53% shorter total root length in vegetative, flowering and fruiting stage respectively, compared to the control without residue application. Compost applications at 22.5 Mg ha^{-1} in chilli reduced total root length, surface area and total root volume compared to a 15 Mg ha^{-1} compost addition. The reduction in total root length was attributed to a lower number of first order lateral roots. While soil respiration rate increased with organic material addition, the soil respiration rate displayed no correlation to root development (root length). The treatments with shorter root length, surface area and root volume in both capsicum and chilli were found to produce smaller aboveground biomass, and significantly lower fruit yield. These results highlight the need for better understanding of shorter-term effects of organic amendments on crop performance to support growers to adopt practices from which they may derive longer-term soil health benefits.

Key words: cane residue, capsicum, compost, soil health, soil respiration, root length.

3.1 Introduction

Increased emphasis is being placed on soil management practices in intensively managed cropping systems to improve the productivity and sustainability of the systems (Abawi & Widmer, 2000; Pankhurst et al., 2003). The increased focus on soil health has emerged in part due to awareness that crop production systems based on high inputs and mechanization can negatively impact on soil health parameters and the environment (Hamza & Anderson, 2005; Steiner et al., 2007; Wood et al., 2006). Declining soil structural and chemical status under high input, mechanized production practices has been widely noted (Bulluck et al., 2002; Marinari et al., 2006) and more recently the detrimental impacts on soil biology have been highlighted (Marinari et al., 2006; Tu et al., 2006).

Management practices to minimize the detrimental impacts on soils of high input systems include the use of cover crops (Williams & Weil, 2004), crop rotations (Aziz et al., 2011), and minimum tillage (Al-Kaisi & Yin, 2005). These practices can improve soil physical and chemical properties along with increasing soil organic carbon levels. It is accepted that in the long term soil carbon level increases with the addition of organic material in the soil (Liu et al., 2013), but the organic carbon stock in cropping soil is affected by the management practices such as crop residue management and tillage (Page et al., 2013). The level of adoption of use of organic material such as crop residue varies between cropping systems such as broadacre cropping and horticulture. In both cereal and vegetable production addition of organic materials has resulted in a slowing of the rate of decline in soil health, but has not reversed the decline (Bajgai et al., 2015). Decreasing soil organic matter levels, measured as soil organic carbon, in intensive cropping systems especially in the tropical areas like in Australia is a commonly used indicator of declining soil health (Bajgai et al., 2014) and the difficulties in increasing soil organic carbon even with the use of practices such as those listed above have been documented (Fronning et al., 2008; Veenstra et al., 2006). That means, the soil organic carbon did not increase in the field even when organic materials were incorporated in soil (Leifield et al., 2009). Although soil organic carbon does not increase with the organic material addition in soil, it may increase the soil characteristics that benefits out of organic materials additions in soil thereby improves soil characters such as aggregate stability.

Addition of organic material to soils, either as amendments or through incorporation of crop residue, is an accepted strategy to improve soil health. An increase in soil organic carbon (SOC) improves many other soil health indicators including decreased soil bulk density and increased soil aggregate stability (Celik et al., 2004; Aggelides & Londra, 2000), increased soil biological activity (Lohila et al., 2003; Ryles & Silver, 2013), increased mineralizable nitrogen (Tu et al., 2006), increased nitrate nitrogen (Ghosh et al., 2008) and reduced pathogen density (Stirling et al., 2005). The soil physical and chemical status benefits have been the main drivers for adoption of the production practices designed to reduce the rate of decline in soil organic matter (Barzegar et al., 2002; Tester, 1990), whereas the combined physical, chemical and biological benefits are listed when organic material additions are being promoted to growers.

The ecology of the soil is very complex, and so it is not surprising that much of the research on the biological benefits of increased SOC levels has utilised specific indicators as a simplified method to assess soil biological status. Soil respiration is one of the most commonly used biological indicators of soil health, and is a measure of soil biological activity. Organic materials additions increase carbon levels in the soil, and soil microbes utilize this carbon as an energy sources for their growth and functioning (Tu et al., 2006). Increased microbial activity and biomass, and the associated increase in combined respiration rate of all soil microbiota, under higher soil organic carbon conditions resulted a positive correlation of soil respiration with soil microbial biomass (Lee & Jose, 2003). As soil respiration rate is relatively simple to measure, it is widely used as an indicator of soil biological activity and as a measure of soil microbial biomass.

Increased soil microbial activity enhances the nitrogen mineralization rate (Zaman et al., 1999), increasing nutrient availability to crop plants, and has also been related to a decrease in root disease severity (Craft & Nelson, 1996). Soils with higher organic carbon content and biological activity therefore have often been concluded to have higher productivity compared to soils with lower microbial activity and soil organic carbon (Bulluck et al., 2002; Ghosh et al., 2012; Kanchikerimatha & Singh, 2001). This conclusion is likely to over-simplify the relationships between soil organic matter, soil biology and crop productivity.

While the mechanisms involved in beneficial interactions between soil microbes and crop growth are not fully understood, the relationship between increased soil microbial activity/biomass and plant performance have been studied in grasslands (Geng et al., 2012; Wang et al., 2007) and field crops (Liang et al., 2014). Wang et al. (2007) documented an increase in root and shoot biomass in grass grown in soils with higher soil respiration rates. Retention of harvest residue from sugarcane crops increases soil respiration rate and millable stalk population in the following sugarcane ratoon crop as well as a higher sugar yield (Kennedy & Arceneaux, 2006). Liang et al. (2014) presented that the cucumber fruit yield was positively correlated with the soil respiration. While these studies do not define a causal mechanism, they do support the widespread belief in the industry that increasing SOC contents increases beneficial biological activity and therefore improves crop performance.

The effect of organic material amendments on crop yield depends on the types and rate of application (Ghorbani et al., 2008; Hou et al., 2012; Kimpinski et al., 2003). Roy et al. (2010) showed that the use of compost and vermicompost from paddy straw resulted in a significantly higher biomass and crop yield for *Zea mays*, *Phaseolus vulgaris* and *Abelmouschus esculentus* compared to mulching with fresh straw. They determined that an increased nitrogen immobilization rates due to uptake by microbes in soils amended with fresh organic materials with a high carbon to nitrogen ratio (C:N ratio) was the cause of the differing crop responses to soil amended with fresh and composted organic materials Conversely, increasing the rate of compost application beyond an optimum level has been shown to reduce crop yield (Lee, 2012). One of the causes of reduced crop yield was determined to be excessive nitrogen supply. Increasing nitrogen supply can increase fungal disease severity (Workneh & Bruggen, 1994), reducing yield. These results highlight the complexity in predicting the effects of various types of organic material and rates on both soil biology and plant growth.

The beneficial effects of increasing soil organic carbon levels are well accepted, but there is sufficient evidence to demonstrate that the response is not uniform. Given the complex nature of soil ecology, and the many potential mechanisms that may be involved in both positive and negative soil microbial interactions with crop plants, alternative research approaches may be useful in developing soil health based crop management strategies. Crop yield depends on sufficient acquisition of water and nutrients through the plant root system at the right time. The root system is the interface between the plant and soil systems, and a healthy soil environment to support optimum root system functionality is required for better plant performance.

Root development in cereals, vegetables and other crops have been extensively studied in relation to soil compaction (Lipiec et al., 2012; Tracy et al., 2012), water availability (Comas et al., 2013), and other soil physical and chemical factors (Gavito et al., 2001; Gil et al., 2000). In contrast to studies of soil physical and chemical factors on root development, studies on soil health and biology have often been focused on pathogen populations, and reducing root disease severity (Abawi & Widmer, 2000; Stirling et al., 2005, 2011; Tilston et al., 2002), or reducing the pathogen population density in soil (Forge & Kempler, 2009; Stirling et al., 2005). The effects of organic amendments have been included in a number of these studies,but attempts have not been made to assess the effects of these amendments on soil microbial activity and root system development simultaneously.

Study of root system development provides an opportunity to understand the complex relationships between soil organic matter content, soil biology and crop performance. Links between organic material addition, soil biological activity (soil respiration), and root development offer a valuable initial step to unserstand the effect of organic material application on soil health and plant root development. Thus, this research aimed to establish the effect of different types and rate of applied organic material on capsicum and chilli root system development. This research tested the following hypothesis; i) organic material addition increases soil microbial activity (soil respiration) ii) organic material applied treatment produce longer root length iii) increasing the application rate of compost produce longer and healthier root system.

3.2 Materials and Methods

3.2.1 Field experiments

The effect of composted and uncomposted organic amendments at varying ratio on capsicum, and chilli root system development were conducted in the 2012, 2013 and 2014 crop production seasons. The trial in 2012 was conducted in Red Ferrosol soil at the Department of Agriculture and Fisheries (DAF), research station at Bundaberg, Queensland (24°51'02.04" S, 152°24'10.16"E). In 2013 and 2014 trials were conducted in Hydrosol on a commercial farm at AustChilli, Bundaberg (24°56'10.01"S, 152°24'10.93"E).

The 2012 field trial was a replicated factorial design incorporating two organic amendment treatments and a control and, two cultivation levels. Organic amendment treatments were uncomposted cane residue (30 Mg ha^{-1}), composted cane residue (35 Mg ha^{-1}), and an untreated control without organic amendments. Organic amendment treatments (composted and un-composted cane residue) were imposed within each cultivation level (cultivated and minimum cultivated) (Fig. 3.1). Each combination of amendment and cultivation level was replicated six times. Each replicate plot was 15 m in length and 0.75 m width.

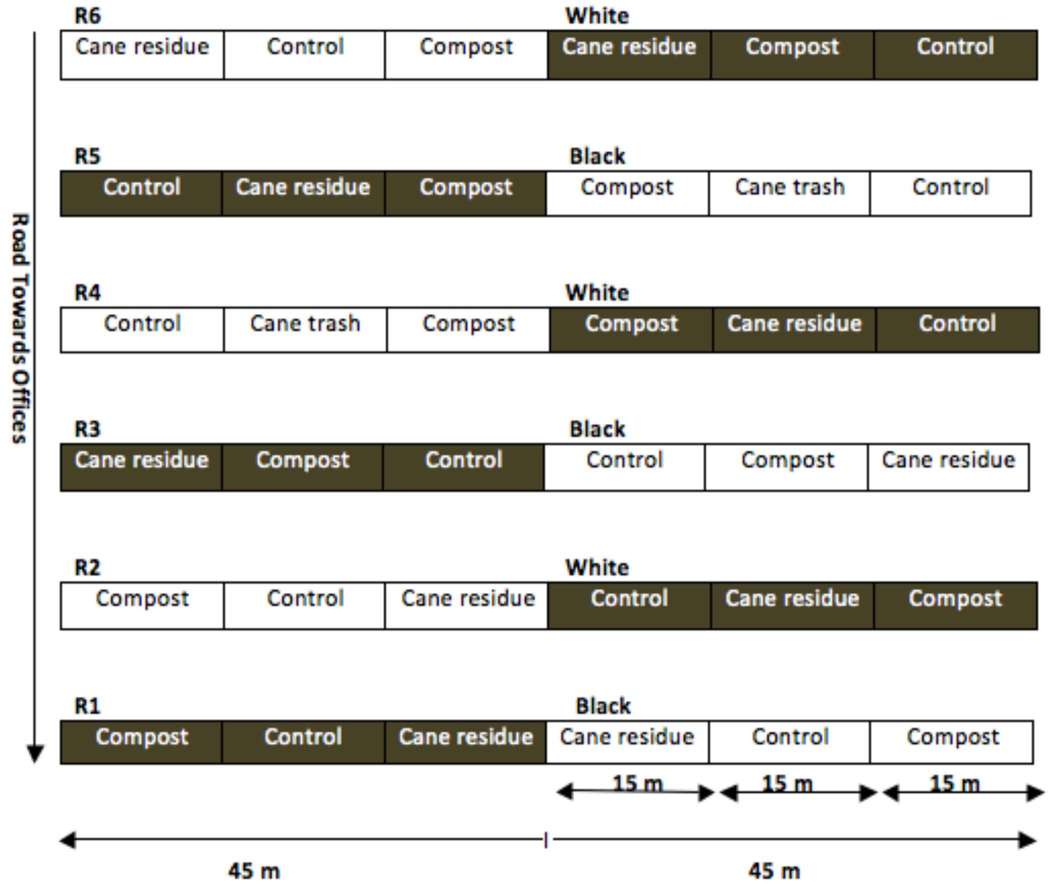

Figure 3.1 Layout plan of 2012 field experiment. In the layout; black shaded area is minimum cultivated, white area is cultivated and Rn represents row number.

Site preparation and crop management practices were as described in chapter 2 (General Materials and Methods). Capsicum (var. Warlock) and chilli (var. Caysan) were transplanted in rows in 75 cm width raised beds at 25 cm spacing. Each replication contained 40 capsicum plants and 20 chilli plants.

Root morphology in 2012 was studied at vegetative, flowering, early fruiting and harvesting stages in capsicum and at harvesting time in chilli from May to November. Soil samples for root morphology assessment at the vegetative stage were taken one month after transplanting the capsicum seedlings. Flowering stage was defined as when 5-7 open flowers appeared on more than 50% of the plants. The early fruiting stage was defined as when the capsicum fruits are still growing, but physiologically at an immature stage. The root samples at harvesting time were collected when 50% of the surface area of fruit had turned red in both capsicum and chilli. Roots were collected by whole root system excavation and soil coring (Bohm, 1979) for capsicum and chilli, respectively. The collected soils with roots were washed and the clean root systems scanned to assess morphological features.

In the 2013 field trial, the effects of rate of composted cane residue addition on soil respiration, root development and chilli fruit yield were assessed. Three composted cane residue application rates (7.5, 15 and 22.5 Mg ha^{-1}) were applied in rows within the commercial chilli crop. The compost was incorporated into the soil 6 weeks before planting, formed into beds and covered with white plastic mulch. Double lines of chilli were planted with 35 cm between lines spacing and 15 cm between plant spacing in a 75 cm width row. Pseudo-replication was obtained by assessing plants at different positions along the 100 m length of each of the three treated beds, with greater variability noted along the length of neighbouring beds in the field than between beds that were only 1.5 m apart. The trial designs also included the buffer sections at the end of the row to remove the end of row effect. It was considered unnecessary to include the multiple rows for each treatment as the rows were widely spaced to minimise any difference between row effects. In the year 2013 and 2014, the objective was to investigate whether incorporating very high rate of compost affect the root development, thus control was not included. Due to the lack of control plants, compost-incorporated soil was collected immediately after chilli transplanting time from the treatment and control plots and then capsicum seeds were sown in the collected soil, in a pot experiment as given in 3.2.2 section to check the root morphology and root health.

In 2014, the composted cane residue application rates were repeated in a second commercial chilli crop, with seedlings transplanted in double lines at the same spacing as used in the 2013 trial. In the both trials, root system assessment was undertaken on plants using the soil coring methodology for sampling. Soil cores were collected from 0 to 30 cm soil depth, from the side of each sampled plant at the vegetative, flowering and harvesting stages in 2013, and at the vegetative stage in 2014. A total of 15 plants in 2013 and 15 plants in 2014 were sampled at each growth stage, per treatment. Roots were separated from the soil core by washing and sieving, and cleaned roots were scanned to assess morphological features.

3.2.2 Pot experiment

A pot trial was undertaken in 2013 to assess the effects of the compost rate additions on root development in capsicum. This experiment was conducted to assess the root health in capsicum at the same rate of compost application in chilli crop in the commercial field using Hydrosol soil. Soil from the 2013 chilli trial site was collected at the early vegetative stage, and sieved through a 2-mm sieve to remove gravel, dead plant roots and large organic debris. Six replications (n=6) for each composted cane residue addition rate treatment were prepared by adding 380 g soil into 237 ml styrofoam cups. In each pot, three seeds of capsicum (var. California Wonder) were sown at 5 mm depth and thinned to a single plant per pot when cotyledons emerged. Treatments were arranged in a complete random design. Water content at field capacity was pre-determined before the establishment of the experiment. During the experiment, soil water content in each pot was maintained at field capacity. Field capacity was maintained as mentioned in chapter 2. Pots were held at room where the daily temperature ranges from 22 to 26^{o}C. Seedlings were harvested 3 weeks after emergence and root health assessments and root morphological measurements were completed.

3.2.2.1 Soil nutrient analysis

Available soil nitrogen (NO_3-N) was measured six weeks after transplanting capsicum seedling in the field in 2012 and at the flowering stage in the chilli field in 2013. In 2012, soil samples were collected from two different places from each replication for each treatment and mixed together. In 2013, 10 soil samples (n=10) were collected from each treatment and bulked and then sub-samples were prepared. Each year soil samples were collected from the 0-10 cm soil depth by using soil auger, mixed thoroughly by hands, and a sub-sample was prepared for each treatment. The prepared soil samples were sent to the commercial laboratory for the NO_3-N and other basic soil nutrients analysis. From each treatment, only one sample was sent for the soil analysis such as pH, EC, organic carbon etc. Soil nutrients were analysed using methods described in chapter 2.

3.2.2.2 Root sampling and root health assessment

Root extraction/sampling and root health assessment were carried out for the field and the pot grown crops. In 2012, whole root systems were excavated at each sampling date for capsicums. A 9.12 cm internal diameter soil core auger was used to collect the chilli root samples in the 2012 trial. At each growth stage at least one average grown capsicum whole root system was excavated (n=12) from each replication, but when two or more plants were excavated they were averaged for single replication. In chilli, three plants were excavated from three replication plots and then averaged for each replication (n=3). In 2013, samples were only collected using the core auger. Cores were collected from one side of the plant to 20 cm soil depth at the vegetative stage, and to 30 cm soil depth at flowering, early fruiting and harvesting time. Each sample was collected at 10 cm depth increments. In 2013, root samples were collected from 15 randomly selected, average grown chilli plants (n=15). In 2014, soil core samples were collected using a 6.1 cm internal diameter auger. Cores were collected to 18 cm depth from 15 randomly selected individual plants (n=15). Immediately after root systems were excavated or soil cores were collected, roots were separated from the soil by gently washing samples through a 1-mm sieve. Extraneous organic matter debris and stones were removed by hand from samples before the roots were scanned. In the pot experiment, root health assessments and root morphological measurements of capsicum seedlings were completed immediately post-excavation at the end of three-week trial in 2013. Root health assessment in chilli in 2014 was carried at the vegetative stage, from the plants used for the root morphology assessment (n=15).

Before scanning, root samples were laid on black paper to assess root health. Root health was assessed using a 1-9 scale as described by Gugino et al. (2009). In short: 1= clear white and healthy root, 3= light discoloration and lesions covering maximum 10% root surface, 5= 25% of root tissue have lesions and little decay, 7-9= more than 75% root discoloured and decayed or at advanced stage of decay.

3.2.2.3 Root morphology

Cleaned roots were scanned using a scanner (Epson Perfection V700, Digital ICE Tech.) and root morphological parameters were measured using WinRHIZO software as described in chapter 2. Numbers of visible lateral roots present in capsicum seedling from the pot trial were manually recorded. Lateral roots present in each core sample in chilli plants, in 2014, were counted after washing soil and removing other debris.

3.2.2.4 Soil respiration, crop biomass and yield

Soil respiration in the field was measured using an EGM3 (PP Systems, UK) soil respiration chamber. As the soil temperature varies with time of day, every time measurements were taken one hour after the irrigation with the assumption that water content in the field and/or pot will be at field capacity and causes lesser temperature fluctuation between treatments and control. Measurement was taken in between two plants in a row. Measurements were collected just before taking root samples. One measurement was taken per replicate plot in the 2012 capsicum trial (n=12). In the 2013 field trial, 15 measurements (n=15) were taken at the vegetative stage and 10 (n=10) at the flowering and harvesting stages from each treatment plot.

Plant aboveground biomass and root biomass were recorded from the same capsicum and chilli plants used for the root morphology study at different crop growth stages; vegetative, flowering and harvesting. The chilli fruit yield was recorded from the same plants used for the root sampling at the harvesting stage. The dry weights were obtained by drying to a constant weight at 70^{o}C.

3.2.2.5 Data analysis

The effect of organic matter type and rate of compost application on root system development and crop yield in capsicum and chilli was determined using the Generalised Linear Model (GLM). Each year a separate GLM was used to analyse the data, as the main crops and soil environments were different. The effect of composted and uncomposted organic materials on root parameters such as root length, root surface area and root volume were determined by using GLM. Root health among different rates of compost application for chilli in the field, and capsicum in the pot experiment were separately analysed using the GLM. Pearson's correlation coefficient (r) was used to identify the strength of relationship between soil respiration and root length. Anderson–Darling and Bartlett's test were performed for normality and homogeneity of variance test, respectively. Data transformation was used for all data that did not display normality, but original data were presented. Data analysis was conducted with MINITAB-16 (Minitab, 2009). The mean separation was carried by using Tukey's test. All determinations of significance in the data were presented at $p < 0.05$.

3.3 Results

3.3.1 2012-Field experiment

3.3.1.1 Soil respiration

Soil respiration increased with the addition of organicmaterials in 2012 trial. Soil respiration rate was greatest at all crop growth stages in the uncomposted cane residue treatment followed by composted cane residue and least in the control in the 2012 trial. In uncomposted cane residue, it was significantly higher at the vegetative, flowering and early fruiting stages compared to the control but statistically same as the composted treatments. The differences were not significant at fruit harvesting time (Table 3.1).

Table 3.1 Mean soil respiration rate at different growth stages in 2012 in capsicum field. Values are means of n=12 and df=2. Means are separated by Tukey's test. Means with different letters in a row are significantly different at p < 0.05.

Crop stages	Soil respiration (g CO_2 $m^{-2}hr^{-1}$)		
	Control	Composted	Uncomposted
Vegetative	0.29^{b}	0.39^{ab}	0.58^{a}
Flowering	0.26^{b}	0.39^{ab}	0.60^{a}
Early fruiting	0.47^{b}	0.93^{ab}	1.34^{a}
Harvesting	0.66^{a}	0.92^{a}	0.97^{a}

Note- application rate of composted and uncomposted cane residue are 35 and 30 Mg ha^{-1}.

In the years 2012, the total root length was analysed to investigate if any correlation existed with the soil respiration. The results revealed that the strength of the correlation was very low and not significant (data not presented). Effect of organic amendment type on root growth

3.3.1.2 Effect of organic amendment types on root growth

The effect of application of uncomposted cane was significantly higher to root length, root surface area, total root volume at the vegetative, flowering and early fruiting stages compared to the composted cane residue application and the control. The effect was not statistically different between treatments at the harvesting stage (Table 3.2).

Table 3.2 Mean total root length, total surface area and total root volume per plant at different crop growth stages in capsicum and chilli in 2012 trial. Values are means of n=12 and df=2 for capsicum and n=3 and df=2 for chilli. Means are separated by Tukey's test. Means with different letters in a row are significantly different at $p < 0.05$.

Crop stages	Root parameters	Treatments		
		Control	Composted	Uncomposted
Capsicum				
Vegetative	Length (cm)	1001.5[a]	873.8[a]	595.0[b]
	Area (cm^2)	178.0[a]	151.8[a]	103.2[b]
	Volume (cm^3)	2.5[a]	2.1[a]	1.4[b]
Flowering	Length (cm)	2118.7[a]	2004.9[a]	1152.4[b]
	Area (cm^2)	453.7[a]	393.4[a]	186.4[b]
	Volume (cm^3)	7.9[a]	6.3[a]	2.4[b]
Early fruiting	Length (cm)	6178.4[a]	5743.8[a]	2907.3[b]
	Area (cm^2)	1367.4[a]	1223.9[a]	680.2[b]
	Volume (cm^3)	24.9[a]	21.5[a]	13.1[b]
Harvesting	Length (cm)	9039.0[a]	8532.0[a]	8818.0[a]
	Area (cm^2)	1448.7[a]	1335.8[a]	1377.3[a]
	Volume (cm^3)	18.6[a]	16.8[a]	17.3[a]
Chilli				
Harvesting	Length (cm)	311.7[a]	233.3[a]	276.5[a]
	Area (cm^2)	39.9[a]	32.5[a]	31.9[a]
	Volume (cm^3)	0.5[a]	0.4[a]	0.4[a]

Note- application rate of composted and uncomposted cane residue are 35 and 30 Mg ha^{-1}.

Mean root length in the uncomposted cane residue treatment was 40.6%, 45.6% and 53.0% lower compared to that of the control treatment and 32.0% 42.5% and 49.4% lower compared to the composted cane treatment at vegetative, flowering and early fruiting stages in capsicum respectively. The difference between control and composted cane residue treatments for total root length varied from 2 to 13%, but were not significant. A similar trend was found for root volume in capsicum. Root surface area in the control and composted cane residue was double that of the un-composted cane residue treatments. No significant differences between treatments were found at the harvesting stage in capsicum (Table 3.2). Similarly, no significant differences between treatments were found in chilli at the harvesting stage (Table 3.2).

3.3.1.3 Biomass and crop yield

There were significant differences between treatments in yield, but not in plant biomass were found in the 2012 trials. Capsicum yield at harvest was significantly higher in control than in the uncomposed cane residue treatment, but similar to the composted cane residue treatment (Table 3.3). Chilli fruit yield, in 2012, at harvest in control was 16% and 18% higher than in the composted and uncomposted cane residue treatments respectively, but the yields were not statistically different. The aboveground biomass in chilli in the 2012 trial did not vary significantly due to high plant-to-plant variations. Plants from the control had on average nearly double the biomass of plants from the uncomposted cane residue treatment.

Table 3.3 Mean aboveground biomass, root biomass and fruit yield per plant at different crop growth stages in capsicum and chilli crop in the year 2012. Values are means of n=12 and df=2 for capsicum and n=3 and df=2 for chilli. Means are separated by Tukey's test. Different letter in a column are significantly different at p < 0.05.

	Treatment	Biomass (g plant^{-1})				Yield (g plant^{-1})
		Aboveground		Root		
		Flower	Harvest	Flower	Harvest	
Capsicum	Control	27.7^{a}	70.2^{a}	7.2^{a}	10.0^{a}	1407^{a}
	Composted	23.3^{a}	68.6^{a}	6.8^{a}	9.2^{a}	1317^{ab}
	Uncomposted	25.3^{a}	63.4^{a}	7.2^{a}	9.4^{a}	1294^{b}
Chilli	Control	-	100.3^{a}	-	-	210^{a}
	Composted	-	78.2^{a}	-	-	134^{a}
	Uncomposted	-	67.2^{a}	-	-	125^{a}

***Yield is presented from bulk harvest. Application rate of composted and uncomposted cane residue are 35 and 30 Mg ha-1.**

3.3.1.4 Soil nutrient content

The 2012 research trial showed that the available NO_3-N in control (38 mgkg^{-1}) was much similar to the composted (30 mg kg^{-1}) treatment but much higher than in the uncomposted (3.5 mg kg^{-1}) cane residue applied treatment. As the nutrient analysis was carried only for one bulk composit sample from each treatment, no statistical comparision was conducted.

The field trial in 2012 showed a significant difference in the capsicum root and shoot growth at different time of the crop life cycle was due to the type of incorporated organic amendments, therefore; the later study focused on the effect of organic amendments on root growth. However, a brief results on the effect of cultivation levels are presented here.

3.3.1.5 Effect of cultivation level

The effect of soil cultivation level on root morphological parameters varied with crop growth stages. The total root length and root surface area at the vegetative stage was 901.3 vs 745.6 cm and 160.7 vs 127.9 cm^2 in cultivated and minimally cultivated soil, respectively, and were significantly different ($p < 0.05$, n=18, df=1). The root surface area was 1212.5 and 968.5 cm^2 in cultivated and minimally cultivated soil at the early fruiting stage was significant at $p < 0.05$ (n=18, df=1). At other times, the root length, surface area and root volume were not statistically different between cultivations.

3.3.2 2013-Field experiment

3.3.2.1 Soil respiration

Soil respiration increased with increasing rates of compost application in 2013 trial. In 2013, soil respiration rate was higher in the highest rate of composted cane residue treatments in the chilli field. In the 22.5 Mg ha^{-1} application rate treatments, soil respiration was significantly higher than in the 7.5 Mg ha^{-1} treatments at flowering and harvesting stages but was the same to the 15 Mg ha^{-1}treatment at the harvesting stage. No statistically significant differences in soil respiration rates were found between the treatments at the vegetative stage in the chilli crop (Table 3.4).

Table 3.4 Mean soil respiration rate at different crop growth stages in 2013 in chilli field. Values are means of n=15 and df=2 at the vegetative stage and n=10 and df=2 in other crop stages. Means are separated by Tukey's test. Means with different letters within a crop in a row are significantly different at $p < 0.05$.

Crop stages	Soil respiration (g CO_2 m^{-2} hr^{-1})		
	7.5 Mg ha^{-1}	15 Mg ha^{-1}	22 Mg ha^{-1}
Vegetative	0.66^{a}	0.76^{a}	0.79^{a}
Flowering	0.88^{b}	1.19^{b}	1.97^{a}
Harvesting	0.76^{b}	0.80^{ab}	1.20^{a}

3.3.2.2 Effect of compost application rate on root growth

Total root length, surface area and volume in chilli plants in the vegetative stage were significantly effected by composted cane residue application rate, with greater root system development in the 22.5 Mg ha^{-1} application rate treatment followed by 15 Mg $ha^{-1}$and least in 7.5 Mg ha^{-1} (Table 3.5). The treatment differences were not statistically significant at the flowering and harvesting stages. Surface area and volume were significantly higher at 22.5 Mg ha^{-1} compared to 7.5 Mg ha^{-1} ($p < 0.05$), but same as 15 Mg ha^{-1} ($p > 0.05$) at the vegetative stage. At the later growth stages all root morphological parameters were statistically the same. Table 3.5 shows a trend that chilli root length, surface area and root volume at very high compost application rate is reducing from flowering stage.

Table 3.5 Mean total root length, total surface area and total root volume per plant at different crop growth stages in chilli in 2013 field trial. Values are means of n=15 and df=2. Means are separated by Tukey's test. Means with different letters in a row are significantly different at $p < 0.05$.

Crop stages	Root parameters	Compost application rate		
		7.5 Mg ha^{-1}	15 Mg ha^{-1}	22.5 Mg ha^{-1}
Vegetative	Length (cm)	210.1^{b}	230.5^{a}	297.9^{a}
	Area (cm^2)	30.5^{b}	33.7^{ab}	44.6^{a}
	Volume (cm^3)	0.4^{b}	0.4^{ab}	0.5^{a}
Flowering	Length (cm)	488.8^{a}	558.6^{a}	472.7^{a}
	Area (cm^2)	79.9^{a}	87.3^{a}	80.0^{a}
	Volume (cm^3)	1.1^{a}	1.1^{a}	1.1^{a}
Harvesting	Length (cm)	336.0^{a}	350.6^{a}	281.7^{a}
	Area (cm^2)	59.0^{a}	65.1^{a}	49.0^{a}
	Volume (cm^3)	0.8^{a}	1.0^{a}	0.7^{a}

3.3.2.3 Biomass and crop yield

There were significant differences between treatments in yield but not in plant biomass were found in chilli in 2013. The chilli yield in the 2013 trial was significantly higher in the 15 compared to 7.5 Mg ha^{-1} treatment (Table 3.6)

Table 3.6 Mean aboveground biomass, root biomass and fruit yield per plant at different crop growth stages in chilli crop in the year 2013. Values are means of n=15 and df=2. Means are separated by Tukey's test. Different letter in a column are significantly different at $p < 0.05$.

	Treatment	Biomass (g plant^{-1})				Yield (g plant^{-1})
		Aboveground		Root		
		Flower	Harvest	Flower	Harvest	
Chilli	7.5 Mg ha^{-1}	64.7^{a}	102.3^{a}	0.19^{a}	0.13^{a}	102^{b}
	15 Mg ha^{-1}	77.7^{a}	124.5^{a}	0.22^{a}	0.16^{a}	131^{a}
	22.5 Mg ha^{-1}	68.1^{a}	127.8^{a}	0.17^{a}	0.13^{a}	117^{ab}

Chilli yield is from the plants taken for aboveground and root biomass measurement.

Chilli yield at harvest in 2013 was not significantly different between 15 and 22.5 Mg ha^{-1} rate of compost application, but 7.5 Mg ha^{-1} produced significantly lower fruit yield compared to 15 Mg ha^{-1} compost application rate. Compost application rate at

15 and 22.5 Mgha^{-1}produced 28% and 14.7% higher crop yield compared to 7.5 Mg ha^{-1} compost application rate (Table 3.6).

3.3.2.4 Lateral roots and root health assessment

In the year 2013, the soil collected from the chilli field after compost incorporation and then sown with capsicum seeds in the pot experiment. The number of lateral roots was significantly higher in the 15 Mg ha^{-1} treatments than the 22.5 Mg ha^{-1} treatment (Table 3.7). Root health assessment in the same experiment showed better root health in the 15 Mg ha^{-1} treatment, with less than 10% root area displaying lesions or discolouration, than in the other treatments which had more than 10% root area with lesions in 22.5 Mg ha^{-1} treatment, and more than 75% root area discoloured or with necrotic lesions in the control and 7.5 Mg ha^{-1}treatments (Table 3.7).

Table 3.7 Effects of different rate of compost application on mean number of lateral roots and root health assessment in 2013 pot trial. Values are means of n=6 and df=3. Means are separated by Tukey's test. Means that do not share the letters in a column are significantly different at p < 0.05.

Crops	Compost application rate	No. of lateral roots	Root-health
Capsicum	Control	4.5^{a}	6.3^{ab}
	7.5 Mg ha^{-1}	3.5^{ab}	7.3^{a}
	15 Mg ha^{-1}	5.7^{a}	3.0^{bc}
	22.5 Mg ha^{-1}	1.5^{b}	3.2^{c}

An assessment of plant health, in the treatment row, at harvest in the 2013 trial revealed that the number of wilted plants at mid-day in a 100 m row length was highest (n=57) in the 22.5 Mg ha^{-1}treatment followed by the 7.5 Mg ha^{-1} (n=42) and 15 Mg ha^{-1} (n=28) treatments. When roots were excavated from 5 freshly wilted plants in each treatment and assessed for root damage, the average percentage of the total root system that was discoloured was 42%, 39% and 27% in the 22.5, 7.5 and 15 Mg ha^{-1} treatments respectively.

3.3.2.5 Soil nutrient content

The 2013 research trial showed that the available NO_3-N in 22.5, 15 and 7.5 Mg ha^{-1} treatments were 219, 54 and 30 mg kg^{-1}, respectively. Nitrate nitrogen analysis was carried only for one bulk composit sample from each treatment, thus no statistical comparision was conducted.

3.3.3 2014- Field experiment

3.3.3.1 Lateral roots and root health assessment

In the 2014 trial, the 15 Mg ha^{-1} composted cane residue application rate treatment produced plants with 10% and 13% higher number of lateral roots compared to the 7.5 and 22.5 Mg ha^{-1} treatments, respectively. In terms of root system health assessment, the 15 Mg ha^{-1} treatment resulted in less than 10% of the entire root system being damaged while in the 22.5 Mg ha^{-1} treatment displayed more than 10% of the entire root system discoloured or necrotic tissue (Table 3.8).

Table 3.8 Effects of different rate of compost application on mean number of lateral roots and root health assessment in chilli crop in 2014 field trial. Values are means of n=15 and df=2. Means are separated by Tukey's test. Means that do not share the letters in a column are significantly different at p < 0.05.

Crop	Compost application rate	No. of lateral roots	Root-health
Chilli	7.5 Mg ha^{-1}	7.7^{ab}	2.7^{a}
	15 Mg ha^{-1}	10.4^{a}	2.5^{a}
	22.5 Mg ha^{-1}	6.9^{b}	3.3^{a}

3.4 Discussion

Soil respiration increased with organic material application to the soil as supported by Araújo et al. (2009). Microbes utilize the soil organic carbon for growth and activity (Garcia-Gil et al., 2000). However, the results do not support that the soil with increased microbial activity (presented as soil respiration) associated with the addition of organics supports greater root system development. Clearly the soil system is a complex interplay of several abiotic and biotic components that interact with the plant root system to influence crop growth and development. Soil respiration in 2012 capsicum trial shows that soil respiration is constantly higher in organic material incorporated treatments than in control soil, but at the harvest time, the soil respiration becomes similar in treatments and control. The similarity in soil respiration among treatments and control could be due to towards the end of crop growth, higher proportion of plant root died and decays thus increased the soil respiration in control. On the other hand, with time the soil organic carbon available for microbes reduces and decreases soil respiration in compost and uncomposted treatment. The increased soil respiration in control and decreased soil respiration in treatment could have contributed in no difference in soil respiration between treatments and control at the harvesting stage.

The significantly shorter total root length and smaller size root surface area and volume in the cane residue treatment in 2012 may be explained by the higher soil respiration in the cane residue treatments compared with the control. Fresh cane residue into soil increased the carbon source available to microbes, but may have reduced plant available nitrogen via immobilisation. Both soil microbes and plant compete for the same available soil nitrogen (Hodge et al., 2000), and nitrogen availability to the plant therefore depends on the net balance of mineralization and immobilization (Mary et al., 1996). But, nitrogen availability from the added organic materials through mineralization is determined by carbon to nitrogen ratio (C:N) of incorporated organic materials (Kaye & Hart, 1997) and by the soil nitrogen content. Organic materials with C:N ratio greater than 30 does not have enough nitrogen to support rapid microbial growth and results in competition for nitrogen with the plant in the same soil pool (Hodge et al., 2000; Kaye & Hart, 1997).

In this experiment, the undecomposed organic materials applied as cane residue had a very high C:N ratio (> 90) (Thorburn et al., 2001) containing at least 45% total carbon and 0.5% total nitrogen (Robertson & Thorburn, 2007b). The high soil respiration rate in the uncomposted cane residue treatment compared to the control at all growth stages demonstrates a high rate of microbial growth with little available nitrogen from the added organic material, suggesting utilisation of the nitrogen pool available in the soil solution. In this experiment, the cane residue was applied at higher rate but the nitrogen was applied at the recommendation rate, which is utilized by the soil microbes for its growth, thus causing nitrogen deficiency to the plant. Nitrogen deficiency symptoms including yellowish leaf colour, reduced root growth and stunted plant growth by nitrogen immobilization for the first month of crop growth were consistent with a smaller root growth and lower photosynthetic rate with low or no nitrogen application in groundnut (Hossain & Hamid, 2007). In contrast, the control was supplied with sufficient fertilizer nitrogen, and the composted treatment had a C:N ratio less than 30 that may have reduced the nitrogen immobilization rate and therefore did not reduce plant growth. The reduced root growth and development in uncomposted cane residue treatment could be the indirect effect of nitrogen immobilization due to the increased microbial activity. In contrast, a laboratory experiment by Malamy & Ryan (2001) shows that the number and length of lateral roots in *Arabidopsis* was lower in high sucrose and low nitrogen (high C:N ratio) media, showing the reduced root growth is a nutritional response.

At the harvesting stage in capsicum and chilli in 2012, the similar root morphological characteristics in all treatments could be due to sufficient nutrient supply from the decomposing cane residue that support better root growth at later growth stages. Robertson and Thorburn (2007a) shows that only 50-70%, depending on weather and soil type), cane residue decompose in seven month time, and this may be a bit higher when incorporated as like in this experiment. That means, the sufficient nitrogen is available for the uncomposted cane residue applied treatment for the root growth around the harvesting stage, thus additive nutrient supply from composting cane residue and external supply supports longer root growth and thus become similar to compost applied treatment and the control.

The shorter root length in early growth stage in uncomposted cane residue applied treatment despite external nitrogen application also indicates a different mechanism such as allelopathic effect of uncomposted cane residue that controls the root growth in capsicum. Crop residue is known to have water soluble phenolic compounds such as vanillic, caffeic, ferulic, chlorogenic, gallic, benzoic and syringic acids (Hurney & Ridge, 1992) releases into the soil by washing the residue by irrigation water or at the time of decomposition that may have affected the root growth. Thus, this experiment suggest that not only the high C:N ratio of the uncomposted cane residue, the totoxic chemicals released from the residue by washing at the time of irrigation and decomposition may affect the crop root growth.

The longer root length at the vegetative stage in capsicum plants grown in cultivated compared to minimally cultivated soil may be due to the higher soil penetration resistance at early stage in minimum cultivation compared to the cultivated treatment (Karamanos et al., 2004). With adequate irrigation through the drip irrigation system in a plastic mulch system, maintaining soil moisture near field capacity would minimize differences in soil penetration resistance, which may explain the lack of a cultivation treatment response across the duration of the experiment.

The increase in compost application rate increased the chilli root growth at the vegetative stage. The increased crop growth could be associated with the increase in microbial activity (presented as soil respiration) and increase in microbial population. Rashad et al. (2011) presented that compost application in planting soil increases microbial population of bacteria, actinomycetes and fungi and, increases plant growth. The increase in plant growth is due to the functional benefit of bacterial populations that are antagonistic to root rot pathogens such as *Fusarium*, *Pythium*, *Rhizoctonia*, *Phythophthora* spp., presence of siderophores and IAA producing bacteria or phosphate solubilizing bacteria (Alvarez et al., 1995). After vegetative stage, root and shoot growth at very high rate of compost application (22.5 Mg ha^{-1}) trends to decrease compared with lower compost application rate (15 and 7.5 Mg ha^{-1}). This decrease in trend in chilli root and shoot growth at very high rate of compost could be due to the changes in the soil environment. The 22.5 Mg ha^{-1} compost (C:N ratio < 30) applied soil had higher microbial respiration and caused higher nitrogen mineralization, thereby increasing nitrogen availability to the plants . The reduced root growth at the very high rate of compost application could be due to the high level of mineralized nitrogen or higher moisture absorbing capacity of compost, both of them promotes crop root damage by pathogenic fungi (Abiodun et al., 2015; Café-Filho & Duniway, 1995).

The very high rate of compost-applied treatment also had a higher number of wilted plants and a higher proportion of root damage compared to the 15 Mg ha^{-1} compost applied treatment. The increase in root rot severity with increased nitrate nitrogen availability in soil is also supported by Walkneh et al., (1993) and Workneh & Bruggen (1993) that the corky root rot severity caused by *Phytophthora parasitica* in tomato is positively correlated with the nitrate concentration in soil. In the chilli-growing period there was very low rainfall recorded (compared to the long-term average, see chapter 2), but soil was regularly irrigated through drip irrigation, thus effect of rainfall or drought are not counted for other possible reason of reduced root growth at very high rate of compost application treatment.

In the same compost application rate treatments as in 2013, the 2014 field experiment presented no evidence of wilting in any treatment at the harvesting stage. Reduced root development was however recorded at the higher application rate. The lower number of first order lateral roots in the highest compost application rate treatment in the 2014 field experiment, and also in the pot trial, was supported with nutrient availability affecting both the number and location of lateral root initiation (Drew & Saker, 1978). Zhang & Forde (2000) presented evidence that the detrimental effect of high nitrate availability was through inhibition of immature lateral root growth just after their emergence.

This research shows that the treatments vary in the total root length, surface area and root volume, but did not differ statistically for their root and shoot biomass between the treatments. The treatments with longer roots producing higher fruit yield could be related to the longer roots with larger root surface area translocated more nutrients thus could have higher rate of photosynthesis thus more photosynthate accumulated in fruit producing higher crop yield. Alameda & Villar (2012) presented an indirect relationship between root growth and yield, showing plants with longer root systems having increased yield.

3.5 Conclusions

This research concludes that applying higher amount of uncomposted or composted organic amendments into the crop field increases the soil respiration, but soil respiration has no relation with the root development. Applying high rates of un-composted and composted organic material reduced the plant root system development and demonstrates that high rate of organic material addition in soil is not beneficial for the plant root system development and plant performance. High rate of uncomposted cane residue produced shorter root length compared to the composted organic matter, thus fresh organic materials should not be used at the planting without sufficient nitrogen application. Similarly higher rate of compost application produced shorter root length with lower crop yield, showing high rate of nitrogen availability is also detrimental for crop growth. This result has suggested that the high C:N ratio of the uncomposted organic matter as one of the possible effect on root development. This finding has significant implications for growers aiming to increase soil organic matter levels through un-composted organic matter additions.

4 Effect of carbon to nitrogen ratio (C:N ratio) on plant root growth and soil microbial diversity in fresh organic material incorporated soil

Abstract

Organic residue incorporation is considered beneficial for improving soil biology, supplying plant nutrients and supporting higher crop yield. This study examined the capsicum plant growth and soil microbial activity at different carbon to nitrogen ratio's (C:N ratio) by varying organic carbon and nitrogen application in soil. Use of fresh cane residue as organic matter and calcium nitrate as a nitrogen source, at rates adjusted to deliver different C:N ratio's, the experiments showed that a shorter root system developed and plant growth was reduced at both higher and lower C:N ratios. While the soil microbial activity was reduced when C:N ratio was very low and increased with increasing C:N ratio. Significant root development and microbial activity were measured at a medium C:N ratio. Both lower and higher C:N ratio treatments produced significantly smaller root systems with shorter total root length and, smaller surface area and root volume compared to the medium C:N ratio. The reduction in root development at very low C:N ratio could be attributed to the reduced microbial activity, which may have inactivated the functional role of specific microbes. The reduced root morphological parameters at high C:N ratio was explained by the increased microbial activity at high C:N ratio that reduced the nitrogen availability to plant growth. A increase in AWCD and evenness value at higher C:N ratio suggest that the change in the soil microbial community structure may have contributed to the functional response in plant growth.

Key words: bacteria, fungi, microbial activity, microbial diversity, root volume, soil respiration, total root length.

4.1 Introduction

Organic matter addition in soil increases organic carbon. SOC is beneficial for the soil biology through increased microbial activity and microbial diversity (Chaoui et al., 2003; Zhen et al., 2014). The increased microbial activity is responsible for the release of nitrogen required for the plant growth, from the organic matter, through mineralization.

Nitrogen is one of the essential elements for the plant growth, impacting on, amongst other aspects, root growth and development. Studies have shown that organic matter through mineralization provides nitrogen to the plant and produces crop growth similar to the synthetic fertilizer or better than control without synthetic fertilizer (Chaoui et al., 2003). In the same experiment, compost applied treatment produced higher shoot biomass compared to the control without the compost. Similarly,higher shoot and grain yield in maize (*Zea mays* L.) was obtained with the incorporation of crop residue of pigeon pea (*Cajanas cajan*L.) compared to the haricot beans (*Phaseolus vulgaris* L.) or maize residue incorporation (Abera et al., 2013). The increased response to maize growth to crop residue or compost application is due to the readily available nitrogen supply from the organic matter (Abera et al., 2013; Chaoui et al., 2003).

Nitrogen supply from the organic amendments in the soil varies on the type of the organic matter especially their composition. In an experiment monitoring the net nitrogen mineralization from soils amended with different organic matter showed highest nitrogen mineralization from chicken manure followed by mungbean stover (*Vigna mungo*L.) and wheat (*Triticum aestivum* L.) residue amended soil (Khalil et al., 2005). The differences in the mineralization was due to the differences in the composition of the organic matter, in which the easily decomposing organic matter supplies more nutrients available to the plants than by the slow decomposing organic matter (Carvalho et al., 2013).

Previous research have ascertained that the organic materials with different C:N ratio affect on crop growth and yield, presenting higher maize (*Zea mays* L.) yield when crop residue with lower C:N ratio was incorporated in to the soil (Abera et al., 2013). The reduced maize growth and yield at higher C:N ratio is claimed due to reduced nitrogen mineralization from the crop residue with high C:N ratio (Abera et al., 2013). At higher C:N ratio, the microorganism competes with plant for the same nitrogen pool (Hodge et al., 2000). At higher C:N ratio, the increased carbon supply increases the soil microbial activity, and soil microorganisms being better competitors compared to the plant that locked up the available soil nitrogen preventing plant to access nitrogen for its growth.

The difference in C:N ratio of the individual organic matter varies in the decomposition rate and therefore differ in the nutrient supply to the plant (Carvalho et al., 2013). Difference in decomposition of organic matter favours the variation in the microbial abundance and composition among different organic matter and their compost. The microbes present in organic matter such as compost are also known to have positive role in the growth of maize, pearl millet (*Pennisetum glaucum* L.) and canola (*Brassica napus* cv. Hyola 401) through different mechanisms such as nutrient acquisition (Hameeda et al., 2008; Hayat et al., 2013), or hormonal influences (Hameeda et al., 2006; Patten & Glick, 2002). Therefore, the effect of different organic materials on plants could not be generalized as the sole effect of C:N ratio.

The effect of C:N ratio on plant root development in laboratory shows that the number and length of the lateral roots in *Arabidopsis* reduce with the increase in C:N ratio of growth media containing sucrose and nitrate (Malamy & Ryan, 2001). Similarly *Arabidopsis* seedlings grown in half strength artificial media supplemented with sucrose and nitrate to vary carbon and nitrogen levels showed reduced root length at both high and low C:N ratio (Gao et al., 2008). The reduction in *Arabidopsis* root development at high C:N ratio may be explained by nitrogen starvation (Zhang et al., 2007), but it has also been postulated that high C:N ratio conditions blocks auxin, a plant growth regulator considered responsible for root initiation, movement from shoot to the root system in the hypocotyl region (Malamy & Ryan, 2001). Zhang & Forde (2000) found that *Arabidopsis* grown in high nitrate to low sucrose (low C:N) ratio concentration exhibited a significantly lower number of lateral roots, with the response described as a systemic inhibitory effect involving a signal from the plant shoot. This may be involved transcriptional change in a regulatory gene (Gao et al., 2008).

The theories presented above to describe the effect of C:N ratio in artificial substrates on root development involve either nutritional, metabolic or on the basis of regulatory gene expression mechanisms. Yet, plant growth assessed *in vitro* with different substrates may respond differently in soil system where presence of biotic community, more importantly soil microbes, play a critical role in nutrient availability, root health and crop growth. Understanding the effect of C:N ratio on crop root growth becomes important for the cane growing area where large amount of cane residue, which has high C:N ratio (> 90), is used as organic material. This research, therefore, aimed to investigate how the varying C:N ratio in soil impact on the root growth and soil microbial community. Therefore, this research tested the following research hypothesis; i) very high and low C:N ratio negatively affect the crop root length and ii) the root development at very high and low C:N ratio in field crops is microbially mediated. This study used a single type of organic material, cane residue left after the sugarcane harvest, adjusted to different C:N ratios and examine its effect on the soil microorganismal activity and diversity, and plant root development.

4.2 Materials and methods

4.2.1 Experiment one - effect of variable carbon on root development

As the field trial from chapter 3 suggested, the C:N ratio could be a possible mechanism that affected on the root system development and crop growth when capsicum seedlings were grown in soil incorporated with uncomposted cane residue. A pot trial was conducted at CQUniversity partially shaded glasshouse. Although direct incorporation of cane residue into soil at the time of land preparation is the farmers practice, to ensure uniform mixing in soil cane residue was prepared by hammer milling to fine powder. The effect of different C:N ratio’s with variable rates of soil organic carbon was investigated by incorporating powdered cane residue with 1 g of calcium nitrate (15.5% N and 18% Ca, 25 Kg pack, Redox, China) as nitrogen source and 260 g sandy-loam, Hydrosol soil by hands, adjusting the C:N ratio of 8.8, 13.7, 19.7, 25.4. C:N ratio was calculated from the known weight of mass and percentage of moisture, carbon and nitrogen content in each material using a online program developed by the Kernel University (CWMI, 1996). Carbon and nitrogen content in soil and cane residue was analysed by sending samples in the commercial laboratory as describes in chapter 2. To match with the farmers practices calcium nitrate fertilizer was used in this and in the following experiments. The mixture with known C:N ratio was kept in plastic cups of 70 mm top and 50 mm bottom diameter with 80 mm height. Each treatment was replicated three times (n=3) and each pot was sown with 4 capsicum seeds (var. California Wonder). Then number of capsicum seedling emergence was recorded for 10 days. The moisture in each pot was maintained at field capacity as mentioned in chapter 2. In this experiment the moisture content was 94 mL at 26^{o}C.

In this experiment, there was not sufficient capsicum seedlings germinated to investigate the early seedling root growth at varying soil carbon rate. Thus following experiments were conducted using capsicum seedlings.

4.2.2 Experiment two - effect of variable nitrogen on root development

A pot trial with different rate of nitrogen application was conducted in a polyhouse at DAF, Bundaberg (24^{o}51'00.61"S, 152^{o}24'03.89"E). The experimental treatment involved varying nitrogen application rate in a fixed amount of soil amended with organic matter (cane residue). Powdered cane residue was prepared as described in section 4.2.1 and soaked in water for 24 hours to leach nutrients and any water-soluble allelochemicals that may have been present, and then air-dried at room temperature. Calcium nitrate (15.5% N and 18% Ca, 25 Kg pack, Redox, China) fertilizer was used as nitrogen source in the experiment. The other components in this fertilizer may also have influenced root growth response, but not considered in this experiment. Calcium nitrate fertilizer at 0.2, 3, 6, 10 and 15 g was mixed thoroughly with 1250 g Red Ferrosol soil amended with 21 g of powdered cane residue at the rate of 30 Mg ha^{-1}. Calcium nitrate fertilizer was used as nitrogen source to match with the farmer's practice of nitrogen source. These treatments corresponds to C:N ratio's of 14.3, 12.3, 10.6, 8.8 and 7.4 respectively. C:N ratio of the treatments were adjusted as described in section 4.2.1. A control was also applied with no nitrogen added to the soil and with C:N ratio of 15. Each treatment was replicated in 18 times, to excavate 6 plants (n=6) each time at 2, 4 and 6 weeks after transplanting, in plastic pots of 140 mm top, 110 mm at the bottom diameter and 145 mm height.

Capsicum seedlings (var. California Wonder) were grown in the polyhouse in a seedling raising mixture. Since the experiment focused on transplanted capsicum root system development in the soil condition, individual seedlings were transplanted 42 days after emergence in the pot prepared above and then pots were randomly arranged on a polyhouse metallic table. Water content of the pots was kept at field capacity throughout the growing period, checking in every two days. Chapter 3 showed that the difference in root growth at early vegetative stage in transplanted capsicum seedling is crucial for the difference in the root growth and crop yield, this experiment will therefore investigate the capsicum root growth at different time (two, four and six weeks) after transplanting. Two, four and six weeks after transplanting the seedlings, six plants from each treatment (n=6) were destructively sampled for root system assessment. Soil from each root system was removed using tap water and then root morphology, and root and shoot biomass were measured.

4.2.3 Experiment three - variable carbon and nitrogen application

This experiment aims how the root development in capsicum varies with small change in the soil C:N ratio up to which it is considered beneficial for the plant growth. C:N ratio was adjusted by varying both carbon and nitrogen inputs in Red Ferrosol soil. Calcium nitrate application rates of 1, 2, 3, 4 and 5 g and cane residue at 10, 20, 30, 40 and 50 g were incorporated with 800 g Red Ferrosol soil in all possible combinations, resulting in C:N ratio's between 9.9 to 31. The nitrogen and cane residue were thoroughly incorporated by hand in 800 g same Red Ferrosol soil used in section 4.2.2 and put the mixture in 140 mm top and 110 mm bottom diameter and 145 mm height plastic pots. Each treatment was replicated six times. The treatments were arranged in three blocks with a randomized complete block design, and two replications of each treatment in each block (n=6). Forty-two day old capsicum seedlings (var. California Wonder) were transplanted into each pot. Soil water content was managed to field capacity as described in chapter 2. Intact capsicum plants and their root systems were taken and a four weeks exposure effect of soil at varying C:N ratio was tested. Experiment in section 4.2.2 showed capsicum root systems were not sufficiently explored in the pot soil at two weeks after transplanting, while at six weeks time the root systems were over-croweded and difficult to assess without any damage. Thus, capsicum root system at four weeks after transplanting seedlings was selected for further root growth study. This provides information on the effect of partially decomposed cane residue on root system development about the middle of the vegetative stage, which can represent the vegetative stage. In this experiment, 3 plants (n=3) from each treatment with C:N ratios of 9.9, 14.0, 18.8, 24.5 and 31.3 were also selected for detailed root morphological study. The cleaned roots were transported immediately to the Central Queensland University laboratory (24°54'03.93"S, 152°18'44.45"E) to measure root morphology. Soils from selected treatments were collected for microbial activity and diversity measurement.

4.2.3.1 Root cleaning and root morphology measurement

The cleaned roots from each plant were transferred to a tray with 1cm water for root scanning. Root was scanned using a WinRHIZO Pro 2012a (Regents Instr. Inc., Canada) connected to the scanner (Epson Perfection V700, Digital ICE Tech.) equipped with transparency unit (TPU) light systems. Root parameters (total root length, total surface area, total root volume and root average diameter) were measured at 400 dpi resolutions for the plants collected from the experiment 2 and 3 presented above.

4.2.3.2 Root, shoot biomass and soil nutrient analysis

Plant roots used for the scanning and the shoot from corresponding plants was collected and oven dried at 70°C until constant weight. The dried root and shoot weight were taken with analytical balance (AG 204 DeltaRange, Switzerland).

Soil available nitrogen (NO_3-N) was measured in experiment two in the second excavation. From each replication pot, soil samples were collected from the root zone and mixed together for each treatment and a representative soil sample was prepared as described in chapter 2. The sample was then sent to the commercial analytical laboratory to measure NO_3-N. No other soil chemical properties were measured for the treatments. Soil nitrate nitrogen was measured as described in chapter 2.

4.2.3.3 Soil respiration and microbial diversity

At four and six weeks, soil respiration was measured from six individual pots (n=6) with capsicum seedling. Soil respiration was measured on the side of each plant immediately before the plant harvest, using an EGM3 (PP System, UK). Thirty-minutes before measuring soil respiration, each pot was irrigated to its field capacity.

Soil microbial communities were analysed by a plate count method and community level physiological profiling (CLPP) using Biolog plates (Biolog, Hayward, USA). Details about the plate count and CLPP method is presented in chapter 1. Although both of these methods can only culture limited microbes present in the soil environment, this is widely used and accepted for the microbial community analysis in different environments as already described in chapter 1. In CLPP eco-plates were used for bacteria and FF plates for fungi. A 10 g of fresh soil sample collected from the pot trial were mixed with 100 ml one quarter strength ringer solution and shaken for 10 min. Procedure was followed as modified from Graystone et al. (2001). In short, a 10-fold dilution was prepared for each soil sample. The diluted sample was further diluted by taking 1 mL from the previously diluted sample added to 9 mL of ringer solution and make total of 7 dilution levels. Aliquots (100 microliter) from each dilution level were spread on plate count agar for the enumeration of bacteria and Sabourad dextrose agar (SDA) for the fungi. To make the media more selective for fungi, chloramphenicol and gentamicin (Sigma, China) were added as 50 mg L^{-1} media. The plates were incubated at 25°C and bacteria and fungi colonies were enumerated 48 hrs and 72 hrs after incubation, respectively. The 10^{-4} dilution level of the same soil sample was used for the community level physiological profiling (CLPP). The dilution level was chosen at 50-300 colonies of microbes by using plate count method. Fifty ml of the same dilution extract was centrifuged at 750 g for 10 min and 150 and 100 micro litre of supernatant from each sample were injected to each well of ecoplates and FF plates, respectively. Each treatment for both ecoplate and FF plates was replicated three times (n=3). Plates were incubated at 25°C for 7 days and colour development was measured as absorbance using an automated plate reader at A590 nm. The readings were taken using micro plate reader (ELX808 BLG, USA) and Gen5 2.0 software (BioTek Instruments Inc., USA). Plates were read once in 24 hrs and values were expressed as average well colour development (AWCD) for individual carbon sources over the incubated period. AWCD for individual carbon substrate was calculated as the average of the absorbance value of carbon sources after subtracting the individual absorbance value from the absorbance value at of zero carbon (Garland & Mills 1991).

$$AWCD = \sum (Wi-A)/31$$

Where Wi is the absorbance value within each well, and A is the absorbance value with zero carbon sources in the plate.

Microbial diversity among treatments was expressed in term of Shannon-Wiener diversity index and evenness index (Hill et al., 2003).

Shannon-Wiener Index (H) = $-\sum Pi\ (\ln Pi)$ (Hill et al., 2003)

Where, *Pi* represented the ratio of activity on a particular substrate to the sum of activities on all substrates.

Evenness Index (E) = $H/\ln S$ (Hill et al., 2003)

Where, *H* represented the diversity index, and *S* was the number of carbon source used for the growth of bacteria or fungi in the same plate.

4.2.3.4 Data analysis

In order to determine the effect of supplementary nitrogen on root root length, surface area, root volume, number of root tips, and root and shoot biomass in experiment two, one-way analysis of variance (ANOVA) was performed. Similarly, effect of treatments with different C:N ratio on soil respiration was determined by using one-way ANOVA. Effect of added organic matter and nitrogen, and the interaction between them on root length, two-way ANOVA was performed. Anderson–Darling and Bartlett's test were performed for normality and homogeneity of variance test, respectively. Non-normal data were transformed and results were presented for the original data. The mean separation was carried by using Tukey's test. All the data analysis was conducted using MINITAB-16 statistical program (MINITAB, 2009). All determinations of significance in the data were presented at $p < 0.05$.

4.3 Results

4.3.1 Effect of varying carbon levels

In this experiment, there was not sufficient seedling germination at higher rate of cane residue application. The seedling germination percentage in treatments with no cane residue, 5, 12 and 20 g cane residue application produced 100, 83.4, 50 and

33.34% capsicum seedling germination. While measuring the field capacity of the different treatments, the higher rate of cane residue treatment shows a dark-brown liquid flowing from the bottom of the pots. As the seedlings were not properly established at varying rate of cane residue application, crop root developments with varying nitrogen levels were investigated in the following experiment.

4.3.2 Effect of varying nitrogen levels

Analysis of root morphologyat two weeks after transplanting showed that the total root length at lower and higher nitrogen application rates was reduced significantly compared to the moderate nitrogen application rate (Table 4.1). Total root length was longest at 10.6 C:N ratio and similar to 12.3 C:N ratio but much less at higher and lower C:N ratios. Total root length at 10.6 C:N was at least 63% longer than in control, and 17.3% longer than 14.3, 10 and 7.4 C:N ratios. Surface area and root volume at 10.6 C:N ratio was double than in the control but statistically similar to 12.3 C:N ratio. Root surface area at 14.3, 12.3, 8.8 and 7.4 are statistically similar but lower than at 10.6 C:N ratio. Total root volume and root branching also follow similar trend as root length and surface area showing largest root volume and branching at 10.6 C:N ratio (Table 4.1).

Table 4.1 Effects of C:N ratio on root development two weeks after transplanting capsicum seedlings presented as per plant. Values are means of n=6 and df=5. Means are separated by Tukey's test. Means in the same column followed by the different letters are significantly different at p < 0.05.

C:N ratio	Length (cm)	Surface area (cm^2)	Volume (cm^3)	No of tips (no.)
15	49.1^{b}	5.6^{c}	0.05^{c}	60.3^{c}
14.3	54.1^{b}	6.6^{bc}	0.06^{bc}	69.5^{bc}
12.3	69.3^{ab}	9.1^{ab}	0.09^{ab}	90.5^{ab}
10.6	81.4^{a}	11.2^{a}	0.12^{a}	107.7^{a}
8.8	55.9^{b}	7.3^{bc}	0.07^{bc}	65.3^{bc}
7.4	54.6^{b}	6.7^{bc}	0.06^{bc}	67.3^{bc}

The root morphology at four weeks after transplanting showed similar results as those reported for the plants at two weeks after transplanting. In four weeks time no plants survive in the control treatment. A significantly longer total root length, surface area, root volume were recorded at 12.3 C:N ratio compared to 14.3, 8.8 and 7.4 C:N ratio ($p < 0.05$) but no significant difference was noted between 12.3 and 10.6 C:N ratio ($p > 0.05$) (Table 4.2). Root length at 12.3 C:N ratio was 4, 3 and 5 times longer than in 14.3, 8.8 and 7.4 C:N ratio respectively. Surface area and root volume at 12.3 C:N ratio was 5, 4 and 7 times larger for surface area and 6, 4 and 8 times larger for root volume, which was statistically significant for 14.3, 8.8 and 7.4 C:N ratio respectively (Table 4.2). In a similar trend to root length, 12.3 and 10.6 C:N ratio produced root surface area and root volume results that did not differ significantly ($p > 0.05$). Total number of root branches was statistically higher at 12.3 C:N ratios producing 3, 2, 3 and 3 times more root tips than 14.3, 10.6, 8.8 and 7.4 C:N ratio ($p < 0.05$). Total number of root tips in other treatments were at least 50% lesser than at 12.3 C:N ratio.Visual observation showed that at high and low C:N ratio the lateral root length were also shorter compared to the 12.3 and 10.6 C:N ratios.

The 12.3 C:N ratio treatment produced significantly taller plants compared to 14.3, 8.8 and 7.4 C:N ratio but similar to 10.6 C:N ratio. Treatments with longer total root length, surface area and volume also produced statistically higher shoot and root biomass compared to others (Table 4.2).

Table 4.2 Effects of C:N ratio on root development four weeks after transplanting capsicum seedlings presented as per plant. Values are means of n=6 and df=4. Means are separated by Tukey's test. Means in the same column followed by the different letters are significantly different at p < 0.05.

C:N ratio	Length (cm)	Surface area (cm^2)	Volume (cm^3)	No of tips (no.)	Plant height (cm)	Shoot biomass (g)	Root biomass (g)
14.3	58.9^{b}	7.1^{b}	0.1^{b}	45.5^{c}	3.9^{b}	0.008^{c}	0.005^{b}
12.3	280.8^{a}	40.5^{a}	0.5^{a}	169.0^{a}	4.6^{a}	0.106^{a}	0.033^{a}
10.6	181.6^{a}	26.3^{a}	0.3^{a}	83.5^{b}	4.3^{ab}	0.086^{a}	0.025^{a}
8.8	72.3^{b}	9.9^{b}	0.1^{b}	55.2^{bc}	3.8^{b}	0.045^{b}	0.008^{b}
7.4	42.5^{b}	5.3^{b}	0.1^{b}	45.2^{c}	3.7^{b}	0.026^{b}	0.004^{b}

Root excavation at six weeks after transplanting was performed for all treatments except the lowest and the highest nitrogen applied treatments (very high and no nitrogen applied treatments) because plants in these treatments failed to survive. The roots were visually different between the treatments in term of root density and proliferation. The 12.3 C:N ratio treatment had much branched and heavy root system then followed by 10.6, 8.8 and 14.3 C:N ratio. As the visual assessment suggested the same trend as observed at 2 and 4 weeks after transplanting, and the loss of plants reduce replication, only root and shoot biomass was assessed. The root dry weight in 12.3 C:N ratio was at least 2 and up to 9 times higher ($p < 0.05$) than in 10.6 and 8.8 C:N ratio respectively. Similarly, shoot biomass was also 2 and 6 times higher in 12.3 C:N ratio compared to 10.6 and 8.8 C:N ratio (Table 4.3).

Table 4.3 Effects of C:N ratio on root and shoot biomass in capsicum plant at six weeks after transplanting presented as per plant. Values are means of n=6 and df=3. Means are separated by Tukey's test. Means in the same row followed by the different letters are significantly different at $p < 0.05$.

Plant parameters	C:N ratio			
	14.3	12.3	10.6	8.8
Root biomass (g)	0.005^{b}	0.196^{a}	0.070^{b}	0.020^{b}
Shoot biomass (g)	0.006^{b}	0.573^{a}	0.216^{b}	0.097^{b}

Measurement of soil respiration in the different C:N ratio treatment showed that the respiration was inhibited in soil with high rates of nitrogen application. Soil respiration at 12.3 C:N ratio is 2 times higher than at 8.8 and 7.4 C:N ratio ($p < 0.05$), but statistically no different with 15, 14.3 or 10.6 C:N ratios ($p > 0.05$) (Table 4.4).

Table 4.4 Effects of different C:N ratio on soil respiration at 4 and 6 weeks after transplanting capsicum seedlings. Values are means of n=6 and df=5 at 4 weeks and n=6 and df=3 at 6 weeks. Means are separated by Tukey's test. Means in the same row followed by the different letters are significantly different at $p < 0.05$.

Time		Soil respiration (g CO_2 m^{-2} hr^{-1})				
		C:N ratio				
	15	14.3	12.3	10.6	8.8	7.4
4 weeks	0.26ab	0.19ab	0.33a	0.25ab	0.17b	0.17b
6 weeks	-	0.30ab	0.39a	0.25b	0.21b	-

4.3.3 Effect of varying carbon and nitrogen at the same time

Two-way ANOVA showed that the carbon application treatments did not affect the root length but the effect of nitrogen and its interaction with carbon were significant ($p < 0.05$). Result showed that the effect of nitrogen application on root length varied with the amount of organic matter applied. Total root length decreased at lower and higher nitrogen application rate when a low amount of organic matter was used, but the total root length increased with the increase in organic matter and nitrogen application rate. Root length also decreased when organic matter was increased but nitrogen supply was limited, which was clearly seen in the lowest nitrogen application treatments in the experiment. The interaction presents that root length along the similar C:N ratio is statistically similar (Table 4.5). Total root length was significantly lower in the treatments with high carbon and low nitrogen or low carbon and high nitrogen, which therefore had either high C:N ratio or very low C:N ratio respectively (Table 4.5). The decreasing carbon with increasing nitrogen across the diagonal, from high carbon to high nitrogen in Table 4.5, the root length was significantly shorter at the extreme ends and longer at the centre. Other treatment combinations of increasing carbon with increasing nitrogen produce statistically similar root length (Table 4.5).

Table 4.5 Effects of carbon and nitrogen interaction at four weeks after transplanting on total root length in the capsicum plants presented as per plant. Values are means of n=6 and df=16. Means are separated by Tukey's test. Means with different letters are significantly different at $p < 0.05$. Value within a parenthesis is C:N ratio. OM is organic material amendment.

OM (g)	Nitrogen applied as calcium nitrate (g)				
	1	2	3	4	5
10	1892^{ab} (17.29)	1671^{abcd} (14.58)	1452^{bcdef} (12.61)	1500^{bcdef} (11.10)	1117^{efg} (9.92)
20	1368^{bcdefg} (21.40)	1722^{abc} (18.21)	1689^{abcd} (15.84)	1278^{cdefg} (14.02)	1263^{cdefg} (12.58)
30	1666^{abcd} (25.06)	$1849.^{ab}$ (21.52)	1657^{abcde} (18.82)	1704^{abc} (16.74)	1088^{fg} (15.07)
40	1154^{defg} (28.36)	1761^{abc} (24.50)	1500^{bcdef} (21.57)	1890^{ab} (19.26)	1437^{bcdef} (17.40)
50	851^{g} (31.34)	1851^{ab} (27.26)	2085^{a} (24.11)	1738^{abc} (21.62)	1628^{abcdef} (19.60)

The total root length, surface area, root volume and total number of root tips were significantly lower at high or low C:N ratio compared to the medium C:N ratio used in the experiment (Table 4.6).

Table 4.6 Effect of different C:N ratio on capsicum root morphological parameters presented as per plant. Values are means of n=3 and df=4. Means are separated by Tukey's test. Means with different letters in a same column are significantly different at p < 0.05.

C:N ratio	Total root length (cm)	Surface area (cm^2)	Root volume (cm^3)	No of tips (no.)	Root biomass (g)	Shoot biomass (g)
9.9	754^{bc}	$132.^{bc}$	1.8^{b}	586.70^{c}	0.09^{ab}	0.39^{a}
14.0	993^{b}	172^{b}	2.4^{b}	899.30^{bc}	0.10^{ab}	0.41^{a}
18.8	1340^{a}	268^{a}	4.3^{a}	1370.70^{ab}	0.17^{a}	0.53^{a}
24.5	1430^{a}	289^{a}	4.7^{a}	1541.00^{a}	0.17^{a}	0.54^{a}
31.3	546^{c}	86^{c}	1.1^{b}	420.30^{c}	0.06^{b}	0.08^{b}

4.3.4 Soil microbial activity and diversity

Soil microbial activity at different C:N ratios, presented as an average well colour development (AWCD) in CLPP tests, varied with different combinations of carbon and nitrogen. Microbial activity increased with time and was higher in soil with higher cane residue application and lower nitrogen application. The microbial activity was lower in soil with low cane residue and high nitrogen application (Fig. 4.1 and 4.2). For bacteria, the AWCD at 24.5 and 31.3 C:N ratio are significantly higher than any other lower C:N ratio (Fig. 4.1). Fungi AWCD is also significantly higher at 31.3 CN ratio, which is more distinct after 120 hrs of incubation time (Fig. 4.2).

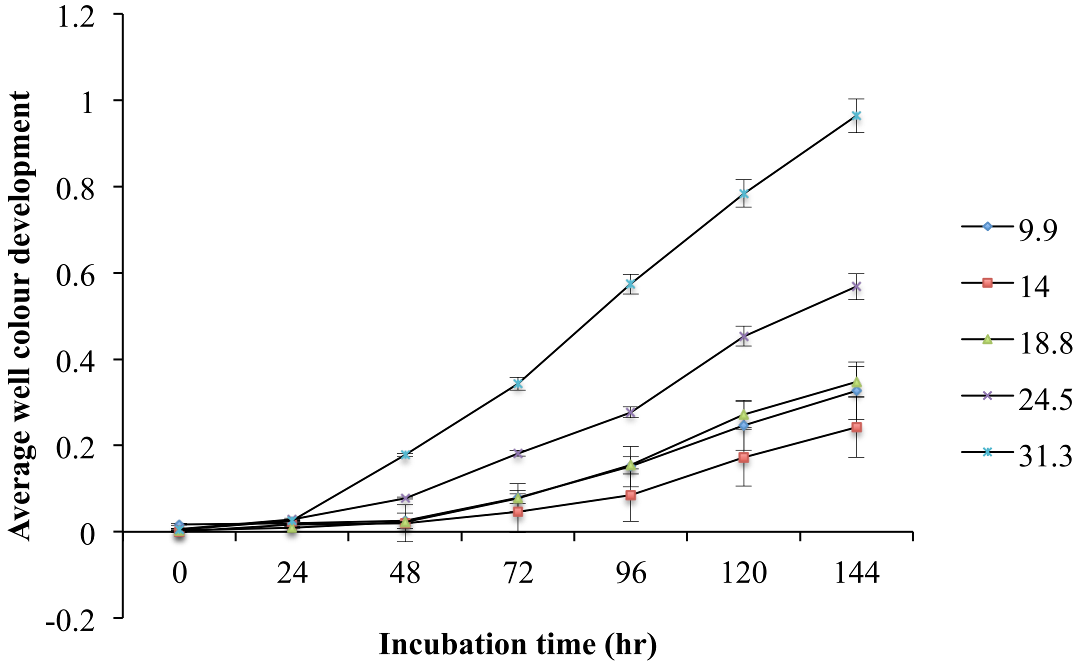

Figure 4.1 Bacterial activity at different C:N ratios with n=3 and df=4, presented as average well colour development (AWCD) at different incubation times (hours). The vertical bars are standard errors. The numbers in the legend are C:N ratios.

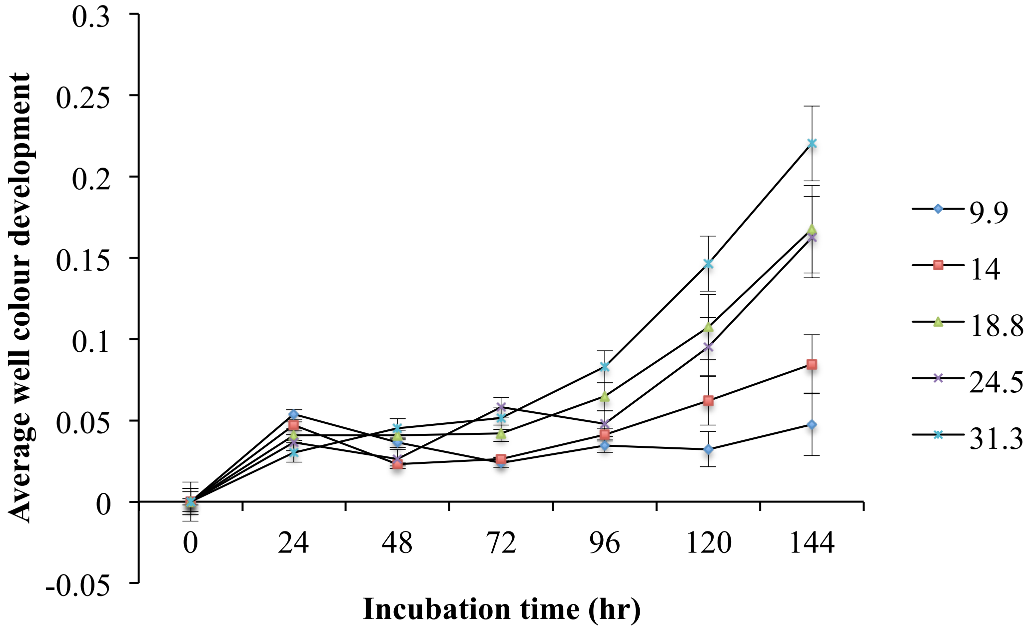

Figure 4.2 Fungi activity at different C:N ratio with n=3 and df=4, presented as average well colour development (AWCD) at different incubation times (hours). The vertical bars are standard errors. The numbers in the legend are the C:N ratios.

The analysis of variance for bacteria and fungi, 120 hours after incubation, showed that microbial activity was significantly higher in high C:N ratio compared to the low C:N ratio treatments (Table 4.7).

Bacterial diversity at different level of C:N ratio was not statistically different ($p > 0.05$) (Table 4.7). The fungus diversity index showed the 14.02 C:N ratio produced a significantly lower diversity value compared to other carbon levels but similar to 9.9 C:N ratio (Table 4.7). The microbial evenness showed that increased C:N ratio produced higher evenness value, and evenness value for both bacterial and fungi is statistically higher at 31.3 C:N ratio compared to 14 and lower C:N ratio (Table 4.7). The results showed that microbial diversity does not vary with the C:N ratio, but there is less variation among the microbes present in the soil sample at higher C:N ratio compared to the lower C:N ratio.

Table 4.7 Effects of different C:N ratios on the activity and diversity of bacteria and fungi.Values are means of n=3 and df=4. Means were separated by Tukey's test. Different letters within a column are significantly different at $p < 0.05$.

C:N ratio	Bacteria			Fungi		
	AWCD	Shannon diversity	Evenness	AWCD	Shannon diversity	Evenness
9.9	0.24^{c}	2.7^{a}	0.84^{bc}	0.03^{d}	3.1^{ab}	0.70^{b}
14.0	0.17^{c}	2.5^{a}	0.77^{c}	0.06^{cd}	2.7^{b}	0.66^{b}
18.8	0.27^{c}	2.8^{a}	0.89^{ab}	0.10^{ab}	3.5^{a}	0.77^{ab}
24.5	0.45^{b}	2.7^{a}	0.89^{ab}	0.09^{bc}	3.4^{a}	0.77^{ab}
31.3	0.78^{a}	3.1^{a}	0.91^{a}	0.14^{a}	3.6^{a}	0.82^{a}

4.3.5 Soil nutrient content

The soil nutrient analysis (in experiment 2) showed that nitrate nitrogen (NO_3-N) in soil was much lower when no nitrogen supplement was provided, or at the lower nitrogen application rate. Nitrogen availability increased at high rate of calcium nitrate application. The nitrate nitrogen was 0.77 mg kg^{-1} in 15 C:N ratio, while the value were 23, 52, 58, 137 and 308 mg kg^{-1} for 14.3, 12.3, 10.6, 8.8 and 7.4 C:N ratio treatments, respectively. Nitrate content was measured from a single sample from each treatment so that no statistical analysis is presented in this result.

4.4 Discussion

The results show that capsicum seed germination reduced with the increased cane residue application rate when same amount of nitrogen was used in each treatment. Although this experiment did not check the allelopathic chemicals present in the cane residue leachate, the dark-brown water flowing from the bottom of the pots (while measuring the field capacity of the treatments) indicates that reduction in capsicum seed germination could be due to allelopathic effect of cane residue present in the cane residue. The negative effect of cane residue in capsicum seed germination in this result is supported by the resualt that cane residue leachate at 100% concentration reduces 20% and 17% in oat (*Avena nuda* L.) and rye (*Secale cereal* L.) seed germination, respectively, compared to control in water without leachate application (Viator et al., 2006).

Result from second experiment presents that the root development was restricted at relatively higher or lower C:N ratio of the substrate. Root development was better at the optimum C:N ratio but the range varied with the amount of the substrate used. When nitrogen application rate was varied in soil, a optimum range of 10 to 12.5 C:N ratio was found best value for soil for the better root and shoot growth. A shift in optimum C:N ratio in the second experiment may be due to the influence of the amount of substrate used for the transplanted crop growth. The higher amount of substrate used in experiment three compared to the experiment two could have provided more carbon to microbes for its growth that could have supplied more nutrients to the plant thus plant produced longer roots. The greatest root development was observed in the middle range of the C:N ratios used compared to the very high or very low C:N ratio in both second and third experiments.

The reduced root development at the lower rate of nitrogen application in the second experiment was associated with reduced available nitrogen. The root growth reduced with limited nitrogen availability, but when nitrogen becomes non-limiting with the increasing levels of nitrogen application, the root growth response becomes evident, which in turn has impact on total root surface area and root volume as well. Researches show that the increase in nitrogen application rate increases the root biomass, root length and shoot growth in corn (*Zea mays*) and pepper (*Capsicum annuum* L.) (Asghar & Kanehiro, 1977; Leskovar et al, 1989). However, at the very high rate of nitrogen application, the total root growth was negatively affected (Wang et al., 2008). The reduced total root length in capsicum at very low C:N ratio could also be due to the high rate of nitrogen available in the soil. The increased nitrogen availability reduces the need of longer root system in search of nutrients in the soil system (Robinson & Rorison, 1983). Not only the total root length, very low C:N ratio also affect the total number of root tips, which was used as a proxy of number of lateral root in the experiments. The reduced number of lateral roots reduces the total root surface area and root volume. The reduced root length, surface area and root volume in capsicum are also supported with the evidence that the low C:N ratio in the growth media at early growth stages inhibited lateral root development in Arabidopsis (Zhang & Forde, 2000).

In the second experiment, the lower nitrogen availability at lower nitrogen application rate was combined with higher soil microbial respiration. The increased soil respiration shows the increase in microbial activity. The increasing microbial activity utilizes the available soil nitrogen for its own growth, which further aggravated the reduced nitrogen availability to plants. As observed in the previous experiment, the higher nitrogen application rate also negatively affected root development and soil microbial activity (Al-Kaisi et al., 2008). The reduced soil microbial activity at higher nitrogen application is due to the reduced cumulative soil CO_2 emissions at high rates of nitrogen application (Al-Kaisi et al., 2008; Wilson & Al-Kaisi, 2008).

In the third experiment (Table 4.6), capsicum total root length decreased with the decrease in C:N ratio at the low carbon levels and high nitrogen application e.g. 10 g of cane residue with 5 g of calcium nitrate application. As previously discussed, the potential higher nitrogen availability could have direct impact reducing the root elongation and lateral root formation (Zhang & Forde, 2000). The result shows that at lower C:N ratio in both experiment two and three the microbial activity (measured as soil respiration or AWCD) was reduced. This result is consistant with the other study that soil microbial activity decreased with nitrogen fertilization in soil (Ramirez et al., 2010).. The lower fungal diversity at lower C:N ratio possibly explains that functional activity of certain fungal groups are inhibited that may have affected the nitrogen dynamics and plant root development.

When cane residue was incorporated at very high rate and increases the nitrogen application rate, the total root length also increased, which supports the theory of a non-limiting response of nitrogen in the treatment that continuously supports both microbial activity and also the root growth. Root development was suppressed at very high C:N ratio (31.8) with high rates of uncomposted cane residue incorporation and small amount of nitrogen (1 g $CaNO_3$). High C:N ratio also found with very high microbial activity measured in term of soil respiration or AWCD in both experiment two and three. The higher microbial activity in organic matter amendment soil has been widely reported (Araújoet al. 2009; Goyal et al. 1999; Scherer et al., 2011). The higher microbial activity (AWCD) at very high C:N ratio possibly led to scavenging of available nitrogen, resulting in nitrogen becoming limiting for the root growth.

Although available nitrogen was not analysed at the crop root excavation time in experiment three, the result from experiment two presents that available nitrogen was very low at high C:N ratio. The reduced nitrogen availability in soil causes poor crop root growth (Gharakhand et al., 2012). The root growth response increases with nitrogen availability at higher cane residue application due to optimum available nitrogen for root growth.

The microbial activity presented as either soil respiration or AWCD was a response to the organic residue applications. Increase in the application rate of carbon substrate increases the soil microbial activity (Vinhal-Freitas et al., 2010). Although Shannon diversity index for bacteria was similar at all level of C:N ratio, the rate of microbial development varied between bacteria and fungi. The activity of soil bacteria was less affected at early growth stages in cane residue decomposition. This was reflected in the no significant difference in the Shannon diversity index among treatments. Fungi are the early colonizers in the organic residue decomposition processes, thus at low C:N ratio fungal activities could have significantly affected, probably due to abundant nitrogen availability. The results shows that the Shannon diversity index among treatments were similar for both bacteria and fungi, which may be due to the same organic material used across all treatments. But, the AWCD and evenness value increased with the increase in C:N ratio indicates the potential interaction between soil microbes and nitrogen, thus affecting abundance and evenness. A difference in microbial evenness value in both bacteria and fungi could be due to the increased amount of nitrogen supplied with cane residue. Wilson & Al-Kaisi (2008) and Al-Kaisi et al. (2008) showed reduced cumulative soil CO_2 emissions at high rate of nitrogen application in soil. The high rate of nitrogen application could have decreased the soil pH, which reduces the soil respiration (Ladd et al., 1994).

4.5 Conclusions

This research showed that varying C:N ratio either by varying the nitrogen application rate or the organic carbon application rate affected the plant root system development and shoot growth. In both experiments, reducing C:N ratio either by increasing nitrogen application rate at the same organic matter application rate or reducing organic matter rate at constant nitrogen application rate reduced root growth. Root growth was increased at the medium C:N ratio, a more balanced C:N ratio, in both experiments and again decreased at very high C:N ratio (31) when increased soil organic carbon application or when nitrogen application rate was decreased. The result from the first experiment with reduced capsicum seed germination at higher cane residue application treatment suggest that along with the effect of C:N ratio there is need to explore the allelopathic effect of cane residue on seed germination and root growth.

5 Effect of seedlings growth media on Chilli (*Capsicum annuum*) seedling root growth and crop performance in the field

Abstract

The increasing cost of potting media components used in vegetable cell-raised seedling production has focussed attention on the uses of different sources of organic media. Performance of seedlings following transplanting is an important quality parameter to be assessed when new media components are used. This study examined chilli (*Capsicum annuum* L. var. Caysan) seedlings of the same age grown in different media and assessed root morphological parameters and plant performance following transplanting into the field. Following transplanting, seedlings grown in commercially formulated compost produced significantly longer total root length, larger root surface area and total root volume than seedlings grown in conventional peat based potting media. The root and shoot dry weight as well as harvestable fruit yield for plants grown from compost-based seedlings were also significantly higher ($p < 0.05$). At transplanting, compost-based seedlings had statistically similar sized root system with the conventional seedlings, but were statistically different after transplanting in the field, suggesting the enhanced root system development in the compost based seedling plants may have been due to the biological effect of the microbes present in the seedling raised in compost based media. Photosynthetic quantum yield and leaf stomata conductance were both significantly higher in plants from compost-based seedlings than conventional peat-based seedlings, and it was concluded that the enhanced root system development provided greater capacity to maintain plant water status. This research indicates that greater attention to growth media effects on root system development during cell raised seedling production provides strategies to improve vegetable transplant performance in the field.

Key words: root length, root surface area, root volume, seedlings, stomata conductance, quantum yield, yield.

5.1 Introduction

Roots are responsible for providing anchorage to the plant, and for the water and mineral uptake from the soil. Longer roots with a larger surface area can exploit a larger soil volume, and can therefore provide better moisture and nutrient supply to the plant. Longer roots have also been shown to increase tolerance to environmental stresses (Nahar & Gretzmacher, 2011; Rauf & Sadaqat, 2007). Root development is thus a critical determinant of success in the establishment of crops in field conditions. This is particularly the case for horticultural crops where seedlings are transplanted into the field from a controlled nursery environment.

In mechanised vegetable production, seedling growers commonly use peat-based seedling growing media in a controlled environment (e.g. temperature, humidity) so that higher quality and uniform seedlings are attained. Peats have been used as growing media because of their desired seedling growing characters such as porosity and high cation exchange capacity (Li et al., 2009). Peat has been a cost effective media option for seedling growers, but costs are increasing due to limited availability, so there is a need to identify alternative media that are able to support production of quality seedlings in the nursery.

Compost prepared from different sources of organic materials (Díaz-Pérezet al., 2010; Medina et al., 2009; Sanchez-Monedero et al., 2004) or from vermicompost (Lazcano et al., 2009; Manh & Wang, 2014) has been used for growing seedlings for horticulture crops. Organic matter used for making compost includes urban green waste, sewage sludge, and spent mushroom. Researchers have shown that the use of compost in combination with peat produces seedlings of similar or better quality than only peat-based media. For example, Lazcano et al. (2009) substituted peat substrate with low doses of compost (10% and 20%) prepared from cow manure or high doses of vermicompost (50%, 75% or 100%) prepared from pig manure, both of which produced tomato seedlings with higher shoot and root biomass compared to the commercial peat-based media. Similarly, Atiyeh et al. (2000) showed that tomato, pepper and lettuce seedlings grown in coir/perlite or peat/perlite amended with 10 and 20% vermicompost prepared from the pig manure or food waste, with or without fertilizer, produced similar or higher shoot, root biomass. Similarly, peat-based media combined with compost produced vegetable seedlings with increased height and stem diameter compared to those grown in peat and/or perlite-based media (Grazia et al., 2007; Herrera et al., 2008).

The quality of the nursery-raised seedlings is ultimately determined by their performance in open field conditions after transplanting. One of the factors that affects crop growth, yield and yield attributes in the transplanted crop is the transplanted seedling age (Leskovar et al., 1991; Shukla et al., 2011). Another factor that affects seedling establishment in field is the root system development.

Seedlings with optimum age are required for transplanting in many crops to get the highest crop yield (Vevrina, 1998). For example; summer squash transplants produced higher shoot and root growth at 21 days old seedling, and dry root and shoot weight was reduced with younger and older seedling age although total crop yield did not vary significantly with the seedling transplanting age (NeSmith, 1993). The reduced shoot growth in older seedlings was potentially due to earlier flowering with reproductive sinks competing with vegetative shoot growth (NeSmith, 1993). Similarly, 4-5 week old tomato seedling transplants produced higher root dry weight, relative growth rate and total fruit yield compared to 3 or 6 week old seedlings (Leskovar et al., 1991), while capsicum seedlings transplanted at 33-37 days old outperformed younger and older seedlings for the number of fruit and fruit yield per plant (Shukla et al., 2011). In both cases, it was concluded that the lower biomass with less accumulated resources contributed to the lower yield in younger seedlings, or that the younger plant was not able to respond as rapidly to early transplanting stress because of a poorly developed root system.

A vigorous root system is a characteristic of seedlings at planting that is important for better crop growth and yield under field condition. A well-developed root system in the seedling stage provides better connection between the soil and root systems. Seedlings with capacity to rapidly develop a larger root system can better withstand early transplanting shock, biotic and abiotic stresses compared to seedlings with lesser root systems. Hence, a robust root system at the seedling stage for the transplanted vegetable crop is highly desirable for the increased crop growth and yield.

The addition of compost to the nursery media can affect seedling root development and root characteristics. In vegetable crops, Lazcano et al. (2009) demonstrated that addition of pig manure vermicompost at a rate of 50% and above of total media composition produced tomato seedlings with significantly larger root volume and degree of root branching compared to peat-based media. Organic matter amendments including compost contain a consortium of different bacteria (Boulter et al., 2002; Hayat et al., 2013). Plants root in the compost-applied treatments are colonized with the heterotrophic bacteria, and the bacterial colonization is associated with the increased plant biomass but the result may vary with soil type (Iverson and Maier, 2009). Jack et al. (2011) show that bacterial community diversity is significantly higher in compost-based and manure-based tomato seedling growth media compared to compost unamended peat-based tomato seedling growing media, but did not explain in what-way. Use of bacteria isolated from compost materials significantly increased the root volume and plant growth (Hameeda et al., 2007). It is considered that plant growth promoting rhizobacteria such as *Pseudomonas* spp. bacteria increase root branching (Gamarelo et al., 2004). The increases in plant root and shoot growth are associated with the functional role of rhizobacteria (Ahemad & Kibret, 2014). Several mechanisms have been suggested to explain the influence of compost on the plant growth. These include physical effects such as reduced bulk density, increased soil aeration and porosity for the root growth (Atiyah et al., 2001; Hashemimajd et al., 2004), increased nutrition for plant growth (Atiyah et al., 2000, 2001), suppression of pathogen population (Sabet et al., 2013) or increasing the specific defence mechanism in the plant system (Vallad et al., 2003).

Although the benefits of composts and microorganisms present in them are increasingly accepted in promoting the nursery performance of vegetable seedling, demonstration of performance in the open field condition would be useful for the growers. Therefore, this research aimed to assess performance of vegetable seedlings of same age, grown in different media, in open field conditions. The hypotheses tested were; i) chilli seedlings grown with compost media produce longer root length compared to seedlings grown in conventional peat-based seedling growing media, and ii) Compost based transplants produces a larger root system and higher crop yield when transplanted into open field conditions compared to peat-based transplants under similar field management practices.

5.2 Materials and methods

5.2.1 Nursery seedling production

Chilli (*Capsicum annuum* L. var. Caysan) seeds were sown on June 15, 2014 in a commercial seedling nursery with controlled environment of temperature and humidity. Seeds were sown in cell plug trays with 18 mm^3 plug volume. The research utilised two different seedling growth media, one conventional peat-based media and the other was an industrial compost media (hereafter referred as compost) with composted poultry manure as one of the major components of the compost. Compost was not sanitized at the time of application. Compost used as seedling-growing media was not analysed for its nutritional content as well. The compost being formulated industrially, its composition was also not disclosed to the researchers. At the six-leaf stage, 42 days after sowing, seedlings were dispatched from the nursery for field planting. From the supplied seedlings, 20 average grown seedlings (n=20) were selected from each treatment for the shoot and root characteristic measurements.

5.2.2 Field transplantation phase

Chilli seedlings were transplanted in the field with sandy-loam soil (Hydrosol) at the AustChilli commercial farm at Bundaberg, Australia (24^o56’10.01”S, 152^o24’10.93”E). Seedlings were transplanted in double line on a raised row of 0.75 m width, and 1.5 m spacing between the rows. As the rows were sufficiently spaced (1.5 m), the row effects were minimal. Also, the trial design included the buffer section at the end of each row to remove the end of row or edge effect. It was considered unnecessary to use multiple rows to reduce any between rows effects as the wide row spacing reduces the likelihood of any significant effect would exist. In each row, seedlings were spaced 0.15 m between lines and 0.35 m spacing between the plants. Plants in the two rows were arranged alternatively. In the field, drip irrigation was provided through lines located between the two chilli rows. Standard insect and disease control practices used in commercial chilli production were applied when required.

5.2.2.1 Root morphology measurement

Root morphology was measured at the time of transplanting. Twenty chilli seedlings (n=20) were selected at transplanting from the seedling tray for root assessment. A further 20 plants (n=20) were excavated at the vegetative growth stage for the root and shoot assessment. In this study, the vegetative stage was defined as 30 days after transplanting seedlings in the field. Chilli in the field starts flowering 2 months after transplanting, hence taking sample at 1 month represents the vegetative growth stage. At the harvesting stage, 12 plants (n=12) were assessed for the root and shoot characteristic. The harvesting stage was defined as the date that the farmer commenced commercial harvesting. Chilli fruits were harvested when the fruits were still green. The statistical analysis of the previous field experiment showed that a reduced sample size also provided similar level of differences, thus the sample size was reduced to a smaller size at harvesting stage compared to the vegetative stage. Root morphology parameters measured were total root length, surface area, average root diameter and root volume. In the field, root samples were collected from 20 cm soil depth at the vegetative stage, and to 30 cm depth at the harvesting stage. A 10 cm diameter root-sampling auger was used to collect root samples, with soil cores divided into 10 cm depth increments. Samples were cleaned using low-pressure tap water, with care taken to retain all root sections. Cleaned roots were scanned in a tray with 1 cm water depth using a scanner (Epson perfection V700 PHOTO) with a dual lens system. Root morphology was measured from the scanned root image using a WinRHIZO Pro 2012a (Regents Instruments, Toronto, Canada) program at 400 dpi resolutions.

5.2.2.2 Plant height, stem diameter, and root and shoot biomass

Plant height was measured at the seedling transplanting stage (n=20), from the base of the hypocotyl to the apical bud on the plant. At the vegetative stage, in the field, plant height was measured from the soil surface to the tip of the vertical axis of the plant (n=20). Stem diameter at the vegetative stage was measured at the middle of the first internode using an analogue vernier calliper. Plant height, stem diameter, root, shoot biomasses were measured for the same plants collected for root morphology assessment. Shoot material, and roots after scanning, were dried at 70°C until a constant weight was attained, and dry weight was recorded.

5.2.2.3 Quantum yield and stomatal conductance

Photosynthetic quantum yield was measured to assess the photosynthetic efficiency of the plants. Quantum yield was expressed as moles of CO_2 fixed per moles of photon absorbed (Singsaas et al., 2001) and in this research was used as proxy measure of photosynthetic rate. Quantum yield was measured in the youngest fully expanded leaf from each of 20 plants (n=20) from each treatment. All measurements were taken at pre-dawn stage using (Photon System Instrument, FP100, Checz Republic), provided with an internal light source for constant illumination. Stomata conductance was measured using a leaf porometer (Decagon Devices, Inc). Stomata conductance was measured at the vegetative stage, at mid-day, from the same plant used for quantum yield assessment.

5.2.2.4 Crop yield

Fruit yield was measured at the harvesting stage, when the first commercial pick of the crop was undertaken, for each of the plants selected for the root sampling (n=12). As the crop is an indeterminate flowering type fifteen plants were selected from each treatment for the yield assessment. Fruits were harvested in sequential harvest whenever commercial harvesting operations were conducted. Fruit yield from individual harvest were added to calculate the total yield.

5.2.2.5 Data analysis

The effects of different seedling growing media on root and shoot characteristics were described by using an unpaired 2-sample t-test. The treatment effects on shoot and root dry weight and crop yield were also analysed by using a 2-sample t-test. Data were tested for the homogeneity of variance and normality using Bartlett's and Anderson-Darling test, respectively. Non-normal data were transformed and results were presented for the original data. Pearson's correlation (r) was used to test if any linear correlation existed between the root biomass, shoot biomass and crop yield. All statistics were calculated using Minitab-16 software (Minitab, 2009). All determinations of significance in the data were presented at $p < 0.05$.

5.3 Results

5.3.1 Nursery seedling production stage

Plant heights of the chilli seedlings from the 2 treatments at transplanting were statistically different ($p < 0.05$), with seedling grown in conventional media being taller than those grown in the compost-based media (Table 5.1). All root morphology parameters such as root length, avg. root diameter, root surface area and root volume were not statistically different ($p > 0.05$), but there was a trend towards increased root system size in the compost media than in the conventional media. The compost-based seedlings were 11, 11 and 11% greater in total root length, surface area and volume compared to the conventional, respectively. Chilli seedlings grown in conventional growth media were significantly taller ($p < 0.05$) than the seedlings grown in the compost media, but the root and shoot dry weight did not differ significantly between the two seedling groups (Table 5.1).

Table 5.1 Effect of growth media on chilli seedling characteristics (per plant) at the time of transplant (42 days). The values are means of n=20 and df=38. Different letters in a row represents significant difference at p < 0.05.

Root/shoot characteristics	**Seedling growing media**	
	Compost	**Conventional**
Total root length (cm)	271.70^{a}	244.80^{a}
Avg. root diameter (mm)	0.65^{a}	0.65^{a}
Root surface area (cm^2)	55.90^{a}	50.20^{a}
Root volume (cm^3)	0.91^{a}	0.82^{a}
Root dry weight (g)	0.07^{a}	0.07^{a}
Plant height (cm)	10.53^{a}	11.79^{b}
Shoot dry weight (g)	0.25^{a}	0.27^{a}
Root:shoot ratio	0.30^{a}	0.29^{a}

5.3.2 Field transplanting phase

One month after transplanting (at the vegetative stage), root length, root surface area and root volume were significantly larger for plants grown from the compost-based seedlings (Table 5.2). No statistical difference in average root diameter was measured between the plant groups at the vegetative stage. Plants grown with compost-based seedlings were taller, higher in shoot dry mass and larger in stem diameter, with these differences being statistically significant at $p < 0.05$ (Table 5.2).

Table 5.2 Chilli plant characteristics (per plant) at the vegetative and harvesting stage in the commercial field for the seedling grown with compost and conventional media. The values are means of n=20 and df=38 at vegetative stage, and n=12 and df=22 at harvesting stage. Different letters in a row represent significant differences at p < 0.05.

Characters/plant	Vegetative stage		Harvesting stage	
	Compost	Conventional	Compost	Conventional
Root length (cm)	299[a]	243[b]	1962[a]	1355[b]
Avg. root diameter (mm)	0.65[a]	0.64[a]	0.54[a]	0.54[a]
Root surface area (cm^2)	61[a]	49[b]	341[a]	220[b]
Root volume (cm^3)	1.01[a]	0.80[b]	4.8[a]	2.9[b]
Root dry weight (g)	0.06[a]	0.04[b]	0.87[a]	0.43[b]
Plant height (mm)	190[a]	169[b]	-	-
Shoot diameter (mm)	0.58[a]	0.51[b]	-	-
Shoot dry weight (g)	2.6[a]	1.9[b]	325[a]	267[b]
Root:shoot ratio	0.02[a]	0.02[a]	0.01[a]	0.00[b]
First harvest fruit yield (g)	-	-	340[a]	325[a]
Total yield (g)$	-	-	830[a]	640[b]

$ Fresh weight

At the first harvesting stage the root excavation revealed significant differences between treatments in root morphological characteristics and shoot dry weight. In general terms, the plants grown from seedlings raised in conventional media were smaller than those raised in the compost-based media (Table 5.2). A major change in the root to shoot ratio was also measured at the harvesting stage ($p < 0.05$), with a higher ratio for the compost-based seedlings. The bulk fruit yield at first harvest was relatively higher for plants grown from compost-based transplants compared to those from the conventional transplants. Total fruit yield from the sequential harvest was

29.6% higher in the plot with seedlings transplanted from the compost-based media compared to conventional ($p < 0.05$) (Table 5.2).

5.3.2.1 Photosynthesis and stomatal conductance

Significant differences between treatments in plant physiology assessments were recorded after transplanting. The average photosynthetic quantum yield at the vegetative stage in the field was significantly higher in plants grown from compost-based seedlings compared to the conventionally grown seedlings (0.42 vs. 0.36) (n=20, df=2, $p < 0.05$). Similarly, stomata conductance at mid-day was 8.9% lower in plants grown with conventionally grown seedlings compared to the plants grown with compost-based seedlings (372.8 vs. 339.6 mmol $m^{-2}s^{-1}$) (n=20, df=2, $p < 0.05$).

5.3.2.2 Relationships between root length, plant biomass and crop yield

Correlation analysis for the plant characteristics at the first fruit harvesting stage in the field showed that shoot biomass was positively correlated with the crop yield for both compost and conventional, r=0.47 and 0.75 respectively, showing the relationship between the variables are stronger in the plants grown with conventionally grown seedlings compared to the compost-based seedlings. The relationship between those variables were statistically not significant for the crops that uses compost-based seedlings ($p > 0.05$) and significant ($p < 0.05$) for the conventional seedling used crop (Fig. 5.1). The two outliers in the figure present the high variation in plants. The uniformly grown plants were selected at the early vegetative stage, and with time few plants shows very dense or less foliage with variation in fruit yield. For other variables such as root and shoot biomass, and root length and fruit yield, the strength of relation was very weak (data not presented).

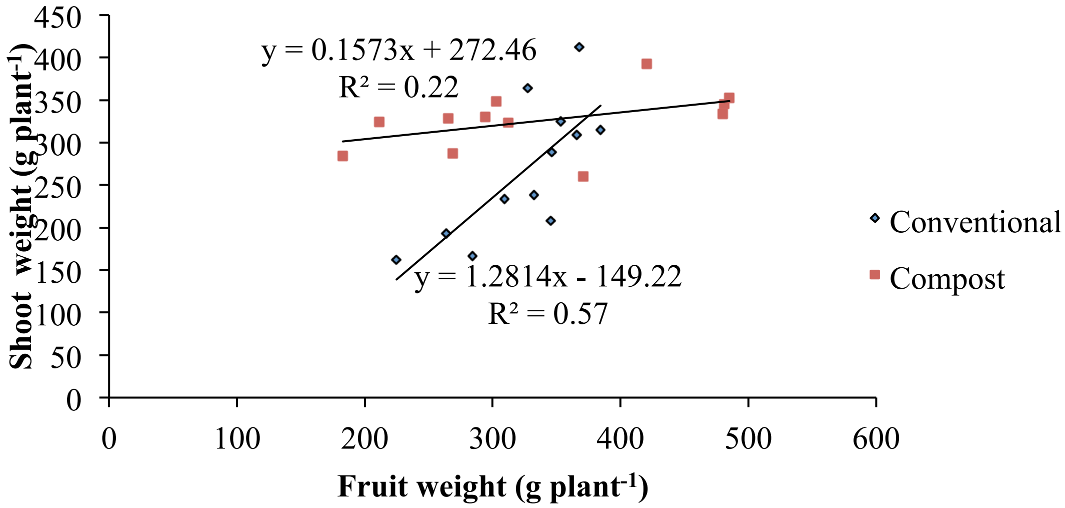

Figure 5.1 Linear relation between shoot biomass and fruit yield at the harvesting stage for conventional and compost-based seedlings **(n=12)**

5.4 Discussion

The results show that the nursery media had a significant effect ($p < 0.05$) on root development and crop yield after transplanting. The root growth rates in compost-based seedlings were higher than in the conventional based seedlings, and the shoot biomass response was positively correlated to fruit yield in conventional seedlings. These responses in chilli is consistent with previous reports of increased root and shoot growth in bell pepper and tomato seedling transplant grown with the compost compared to the commercial peat-based substrate (Díaz-Pérezet al., 2008a, 2008b). In the field, the compost-based seedling has increasing capacity to initiate rapid root growth after transplanting. These results suggest that the use of compost as substrate benefits the root and shoot growth. As both treatments were supplied with the sufficient soluble fertilizer solutions, the vigorous root system development measured in the compost-based seedlings in the field could be due to the difference in the physical characteristics such as aeration, nutritional effect or biological components such as beneficial microbes present in compost or through the nutritional or hormonal influences. Alternatively, small differences at transplanting between treatments in seedling size may have contributed to differences in growth after transplanting, with substrate physical features influencing seedling size (Kerbiriou et al., 2013).

In the nursery, with adequate nutrient supply, the benefits of compost on substrate physical characteristics such as aeration and bulk density could have contributed to the trends in seedling root characteristics in the compost compared to those grown in the conventional substrate. Although physical soil characteristics were not measured for the seedling growing media, a previous study shows that compost applied plant-growing substrate has lowered bulk density and increased soil aeration compared to control without compost application (Atiyah et al., 2001).

Results show a similarity in root characteristics between the seedling-transplanting phase and the vegetative stage in conventional-based seedling, this could be due to mortality of some of the seedling root and lack of root establishment between the root system and soil at early growth stage in the field. The compost-based seedling showed very high rate of root and plant height growth at vegetative stage compared to the seedling stage. This could have been supported by the increased root system development and it's increasing efficiency of supplying nutrients to the crop.

The vigorous root system development in compost-based seedlings compared to the peat-based seedlings in the field is potentially explains by the biologically mediated activities of microorganisms. For example, the application of composted plant residue in pineapple increased the root and shoot growth, root length and number of roots, plant height and root and shoot biomass compared to the control without compost (Liu et al., 2013). In the same experiment, they concluded that compost application increases the soil microbial abundance and their activity in term of bacteria, fungi and actinomycetes population and their enzymatic activities that stimulates increasing availability of soil nitrogen and phosphorus to the plants and for it's growth. Jack et al. (2011) showed that the use of plant-based and manure-based compost in tomato (*Lycopersicum esculentum*) seedling production in nursery has significantly different and higher bacterial community compared to the peat-based growth media. Jack et al. (2011) also showed that the higher microbial community structure in compost-based seedling continues in field transplanted seedling, and the difference in microbial community and their role is recognized as the reason to higher crop growth and yield in compost-based seedlings. Further, a study shows that the field use of compost prepared from different organic sources (green waste, urban organic waste, manure waste and sewage sludge) increases microbial diversity compared to the soil with no compost (Ros et al., 2006). On the other hand, the present of slow decomposing peat in the field, when conventional based seedlings were used, might not support enough microbial growth to support the plant growth.

This research did not study on the microbial community either in the growth media or in the field, but compost is rich in beneficial microbes such as *Bacillus* sp., *Pseudomonas* sp, *Azospirillum* sp. and many others (Boulter et al., 2002). The wider array of beneficial microbes present in the compost used in growing seedling transfer to the field along with the seedling at the time of seedling transplanting, which in turn could have positively affected the root growth. The conventional growth media that contains peat may have changes the microbial community and composition in the field, but not to the extent that influence the plant response. The beneficial effect of compost-based microbes on plant root growth can be justified from the researches that compost isolated bacteria such as *Enterobacter asburaie* PS 13, *E. cloache* EB 27, *Serratia marcesense* EB 67 and others like *Pseudomonas* sp. CDB 35 and *P.* sp BWB 21 increased maize (*Zea mays*) root length at least by 42.8% compared to control without any of those bacteria (Hameeda, et al., 2008). Similarly, *Bacillus* and *Pseudomonas* spp. isolated from the compost and inoculated in sorghum (ICSV 93046) in a glasshouse experiment produced 463% and 583.3% higher root volume compared to the uninoculated control (Hameeda et al., 2007). In another study, inoculating pearl millet (*Pennisetum glaucum* L.) seed with different bacteria such as *B. circulans* EB 35, *Serratia* sp. EB 75 and many other *Pseudomonas* spp., isolated from the compost significantly increased shoot length, root length density and plant dry weight (Hameeda et al., 2006).

The benefits of plant growth promoting bacteria present in the compost-based seedling in the field could be associated directly or indirectly for the increased plant growth. This research did not analyse the microbial population and available soil nutrients, but past studies show that the beneficial bacteria present in the compost are also associated with enhancing plant growth either by nutrient acquisition and/or increased uptake of nitrogen, phosphorous and potassium (Egamberdiyeva, 2007; Hameeda et al., 2008). For instance, *Bacillus*, *Pseudomonas*, *Lysibactor*, *Serratia* and many other compost isolated bacteria spp. were capable of solubilising tri-calcium phosphate so that phosphorus becomes available to the plant (Hayat et al., 2013).

Increased root and shoot growth in the compost-based seedlings may also be associated with the auxin mediated plant growth promotion in the field through the ACC deaminase and IAA activity of different microbes present in the compost (Hameeda et al., 2006). For example, one of the common bacteria present in compost, *Pseudomonas putida*, when applied to seeds of IAA deficient canola mutants, produced 35 to 50% longer roots than IAA deficient mutants or control with uninoculated seed (Patten and Glick, 2002).

After transplanting, the longer total root length and increased branching present in plants from compost-based seedlings might be associated with the better soil foraging capacity. The longer vertical roots present in the compost grown seedling acquire more soil moisture from the subsoil layer, and the longer lateral roots on topsoil layer are efficient for the acquisition of phosphorus (Ho et al., 2005). The significantly longer total root length supports acquiring sufficient moisture, hence; regulation of stomata conductance in plants with longer root system is less important compared to the treatment with shorter root length. Therefore, stomata conductance was relatively higher in treatment with longer root system.

The linear correlation between aboveground biomass and yield presents variation in plant performance at harvest between the chilli plants grown with compost-based and conventional seedlings. The aboveground biomass was linearly correlated with fruit yield in both compost and conventional peat based seedlings, but the relationship is not significant in compost-based seedlings. The small shoot biomass with shorter root length explains lower fruit yield in conventional seedlings. In compost based seedlings, the non significant relationship of shoot biomass with fruit yield and root length with biomass or yield support the claim that the microbes present around the root system and in the rhizosphere in compost-based seedling should be responsible for the higher aboveground biomass and crop yield, may be through the increased acquisition and uptake of nutrients.

This research shows compost-based seedling growth media is superior in the field crop performance compared to the conventional peat-based growth media. This research did not analyse the chemical property of the seedling growth media, but the peat used as growth media is known with high carbon to nitrogen ratio (C:N > 30) (Jack et al., 2011; Vavrina et al., 2002), compared to the compost, and takes longer time for composting. Presence of slow decomposing material in the rhizosphere may not supply sufficient nutrients to the plants. The slow decomposing material might not have sufficient microbes to support the plant growth. Similarly, chapter 4 presents that a small change in the microbial activity in the growth media do not bring a wider variation in the microbial diversity and capsicum root growth. Linking to this, farmers in the sugarcane-growing region use cane residue as blanket or incorporated into the soil, but cane residue also has very high C:N ratio and takes considerable time for complete decomposition and to get benefit from compost. Thus, a reduced root, shoot growth and crop yield with high C:N ratio cane residue applied treatment, when nitrogen was applied, suggest that the cane residue may have affected the crop growth in a different mechanism such as allelopathic effect.

5.5 Conclusions

The results of this research suggest that the use of compost as a nursery substrate media improves seedling root development and performance in open field conditions when compared with conventionally grown seedlings. The research opens up a new study area of why the seedling root system varies in the compost-applied media when compared to conventional growth media in the field condition. The increase root system in the field in compost-based seedling could be due to better physical environment or supplying higher nutrition by compost present in the root systems compard to the slow decomposing peat-based media available in the peat-based seedlings. This research also hints that compost could have influenced the root development through microbial mediated process (e.g. supplying nutrition, hormonal influence or biological effect), but farmers in the sugarcane growing region use large quantity of cane residue in the field, which may affect the vegetable root system development in a different mechanism such as allelopathic effect as discussed in the discussion.

6 Effect of microbes in reducing the toxic effect of crop residue leachate on crop seed germination, seedling emergence, and root development in transplanted crops

Abstract

The use of organic materials in cropping systems is increasing, but in addition to the many beneficial effects associated with this practice, negative impacts on crop establishment can occur depending on the organic material application time. This study examined the effects of water-soluble allelochemicals leached from uncomposted organic material on seed germination and seedling root growth, and the use of microbial solutions applied to seeds and seedlings to mitigate deleterious effects of exposure to allelochemicals. Seedling root growth rate of capsicum, cabbage and barley decreased following exposure to leachate from cane residue, lucerne or sorghum straw. Capsicum and cabbage seed germination declined with increasing leachate concentration while barley germination remained unaffected irrespective of leachate concentration. Inoculating commercially formulated microbes in capsicum seeds at the time of planting was shown to improve the emergence rate of seedlings compared to untreated seeds when irrigated with cane residue leachate in pot experiments. Under field conditions, capsicum plant growth and root development in seedlings dipped in microbial solutions of *Bacillus* and *Pseudomonas* spp. prior to planting in soil amended with cane residue was increased significantly compared with seedlings dipped in water. Treated seedling displayed significantly higher root and shoot biomass, longer total root length, larger surface area, root volume, and total fruit yield at harvest. This research concludes that biological additives have the potential to overcome the deleterious effects on seedling growth that occur when transplanting occurs soon after incorporation of uncomposted crop residue into the soil.

Key words: *Bacillus* sp., biomass, cane residue, capsicum, crop yield, leachate, root length, root volume, *Pseudomonas* sp.

6.1 Introduction

The use of organic materials in cropping systems increases soil organic matter content (Hou et al., 2012). The benefits of organic materials on soil physical, chemical and biological activities are well documented (Bouajila & Sanaa, 2011; Hou et al., 2012; Wei et al., 2015). Organic materials are usually incorporated into the soil to gain benefits for crops. Uncomposted organic materials such as crop residue at higher application rate can lock-up the available soil nitrogen and become deficit to the crop. Limited nitrogen can be solved with the external nitrogen supply, but water-soluble allelochemicals can; however, be extracted from organic materials and their presence in the soil solution can negatively affect plant root development and crop growth. Therefore, when uncomposted organic material is incorporated into soils, it is common practice to delay sowing of a crop until the material has been partially decomposed down by soil microbes. This decomposition process decreases the risk of negative effects on plants from allelochemicals released from the organic materials, and delaying sowing may also allow leaching or binding of allelochemicals to soil particles, thus supporting crop seed germination and plant growth.

When composted organic material is used, the benefits of organic material incorporation in to the soil includes reducing bulk density and compaction, conserving soil water, buffering soil pH and increasing the cation exchange capacity (CEC), increasing nutrient availability and microbial activity and biomass (Aggelides & Londra, 2000; Chen et al., 2001; Garcia-Gil et al., 2000). Organic material is also known to supress detrimental effect of pests and pathogens, thus reduce the root disease incidence (Stirling et al., 2005; Stirling & Eden 2008). Addition of organic materials such as crop residue or compost increases the soil microbial activity, increases the population of beneficial bacteria and saprophytic fungi that outcompetes plant pathogens through the mechanism of competition, antibiosis, hyperparasitism or through inducing resistance to the host plant (Hoitink et al., 1997). These benefits have promoted the increased use of organic amendments as a potential to reduce the soil borne diseases, and for the nutrient and water management strategy in crop production. Many different approaches to achieve this are used, and while benefits are generally reported, the results vary with many factors including type of organic material, application timing and method of incorporation into topsoil depth.

Organic materials are generally either incorporated into the soil or left as a mulch layer or 'blanket' in the field, depending on the cropping systems. In vegetable production, unlike a row crop field, organic materials are most often incorporated into soil before seedling transplanting. In row crops, crop residues are either incorporated or left as a mulch on the soil surface after crop harvest. The time between the organic material application and planting affects the crop responses. For example, when fresh organic materials such as crop residues are used in vegetable cropping, the residues need to be well decomposed to obtain a benefit (Roy et al., 2010). Depending on its composition and growth stage, crop residues such as cane residue can take a substantial time (>1 year) to decompose (Robertson & Thorburn, 2007a). Therefore, farmers' delays sowing or planting crops in fresh or uncomposted organic material applied field to obtain the benefit of organic materials such as crop residue.

In sugarcane growing regions, vegetable and fruit crops such as zucchini (*Cucurbita pepo*), pumpkin (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), soybean (*Glycine max*) are grown as complementary crops to fit in the rotation with the sugarcane crop to assist in weed and disease management (DAF, 2011). Also, sometimes farmers convert sugarcane field to vegetable growing, permanently. After cane harvest, managing the cane residue before vegetable growing is important. In the past, cane residue were used to burn after cane harvest, but now a days retention of residue in the field after sugarcane harvest is standard practice in Australian sugarcane farming systems (Robertson & Thorburn, 2007b). The residues from sugarcane harvest, commonly referred as the cane residue, builds up soil organic matter during the 3 to 6 year duration of a sugarcane crop (Robertson & Thorburn, 2007b).

Growing vegetable crops by incorporating large quantity of fresh cane residue in soil in chapter 3 and a pot experiment in chapter 4 shows that the uncomposted cane residue decreases vegetable seed germination, root growth and capsicum yield. The shorter root system of capsicum in large quantity of cane residue applied treatment was concluded to be due to the allelopathic chemicals released from the cane residue during irrigation or cane residue decomposition. To reduce the allelopathic effect, residue needs to be decomposed. The extended period required for decomposition of the cane residue is, however, problematic for growers wishing to follow the sugarcane crop with a vegetable crop in a rotation as it restricts timing of planting and therefore limits the range of vegetable crops that can be grown. Removing the cane residue blanket and cultivating the soil for vegetable crop planting decreases soil organic matter content and therefore destroys the benefits of cane residue blanket retention in the sugarcane cropping system. Strategies are required to retain the cane residue blanket and also allow planting of vegetable crops before the cane residue blanket has decomposed.

The uncomposted or partially composted organic material releases allelochemicals into the soil system through solubilisation or volatilization of toxic compounds, or by degradation and decaying of the organic materials (De Albuquerque et al., 2011). Allelochemicals are the phytochemicals released from the plant materials into the environment that can positively or negatively affect plant growth and development (Rice, 1984). Water extracts or leachates from crop residues have been shown to negatively affect crop seed germination, root and shoot growth (El-Darier et al., 2014; Turk & Tawaha, 2002), with the effect attributed to allelochemicals in the leachate. The effect of organic leachates on crop seed germination and root development has been examined under laboratory (Turk & Tawaha, 2002; Viator et al., 2006) and greenhouse pot culture conditions (Sampietro & Vattuone, 2006a; Sampietro & Vattuone, 2006b; Turk & Tawaha, 2003), but there are limited evidences on how does the crop residue allelochemicals impact on transplanted vegetable crops root growth in the field condition. Observations of poor capsicum crop performance in the field experiment in chapter 3 in which uncomposted cane trash was incorporated into the soil before seedling transplanting, and also in a previous study such as, when fresh rice straw was used in soil the growth and yield of maize and brinjal was reduced compared with the use of composted straw (Roy et al., 2010). Those examples may reflect the allelopathic response.

Reducing the effect of toxic allelochemicals is essential for the benefit of crop growth and yield, and a biological solution using microbes is a potential strategy. Microbes play a functional role of increased nutrient uptake and remediation of toxic chemicals from contaminated soil (Hariprasad & Niranjana, 2009; He et al., 2009). *Bacillus* and *Pseudomanas* spp. are beneficial in phosphate solubilization and uptake (Hariprasad & Niranjana, 2009; Wang et al., 2014; Zaidi et al., 2003). *Bacillus* and *Pseudomonas* spp. may reduce soil borne root disease severity and pathogen density (Akhtar & Siddiqui, 2008; Liu et al., 2009). Evidence also exists that adding beneficial bacteria to soil, or to seeds or seedlings, can increase crop growth and yield (Akhtar & Siddiqui, 2008; Dey et al., 2004; Orhan et al., 2006). Different commercial microbial products are now available as soil additives with the claimed benefits of increase crop yield (Banerjee & Yesmin, 2004), reduced soil borne diseases (Roberti et al., 2012; Punja & Yip, 2003) and improve soil biology (BioAg, 2015).

Besides commercial microbial products, uses of compost tea for the improved crop growth are also getting popular. Compost tea is water extract from the composted material (Radovich et al., 2011), which is described as to benefit the crop growth and to control the plant diseases. For example, an aerated compost tea prepared from mixture of different compost (medicinal herb compost, vermicompost and rice straw compost) produced longer root length and shoot weight in both sweet corn and soybean compared to untreated control, when drenched on the base and foliar spray (Kim et al., 2015). Similarly, use of both aerated and non-aerated compost tea prepared from crop residue compost, vermicompost, spent mushroom compost or grape marc compost significantly reduced the disease severity caused by *Phytophthora capsisi* and *P. parasitica* and increased plant growth in chilli (*Capsicum annuum*) (Marin et al., 2014). The increased plant growth and reduced disease severity by compost tea is associated with the increased nutrient availability and increased beneficial bacteria and fungi population which may be responsible in controlling pathogen population (Kim et al., 2015; Kone et al., 2010).

Considering the potential benefits of microbes to plant growth, this research chapter aimed to examine whether microbes can be utilized to minimize the allelopathic effect of organic material on seedling emergence, root development, crop growth and yield. If the allelopathic effect of toxic compounds can be minimized biologically, farmers may be able to reduce the time-gap between organic matter application and crop establishment without compromising the soil quality. Therefore, this research tested the following research hypotheses; i) Increased concentration of organic residue leachate reduces crop seed germination and root development ii) Application of microbes reduces the allelopathic effect of organic material extract on seed emergence iii) Field application of microbes reduces the allelopathic effect of organic material thereby enhance plant growth and root development.

6.2 Materials and methods

6.2.1 Effects of cane residue leachates on seed germination

The effects of organic matter leachates on seed germination and seedling root morphology were assessed in laboratory germination assays at CQUniversity, Bundaberg (24°54'03.93"S, 152°18'44.45"E). Leachate samples were generated from dried and powdered sugarcane (*Saccharum officinarum*), lucerne (*Medicago sativa*) and sorghum (*Sorghum halepense*) stem and leaf samples as well as from two composts, one formed from a cane trash and sawdust base and the second from leaf litter. Leachates were prepared by adding 20 g of organic matter samples to 150 mL of deionised water for 24 hrs, stirring occasionally, and filtered using Whatman No. 1 filter paper. The crop residue leachate was not analysed for its carbon, nitrogen or any other chemical properties. The main objective of this experiment was to determine if the crop residue leachate affect seed germination and root growth in different crops chemically or not. The chemical influence of leachate was determined by using sterile leachate produced by passing the leachate through the syringe filter, so it was not considered necessary to analyse the chemical elements in leachate. The stock leachate was prepared from the same powdered cane residue every time. The cane leachate was prepared as one extraction for each research at the Central Queensland University laboratory. To maintain the uniformity of extraction the ratio between amount of powdered cane residue and deionised water was maintained same in every experiment. The stock leachate solutions were then used to generate a range of leachate treatments. Different concentrations were prepared by diluting the stock solution (denoted 100% concentration) with deionised water. A sterile leachate was prepared by passing the stock solution through a 0.22 μm syringe filter (Millex GP, IRE) to remove any microbes that may have been present. Deionised water was used as a control treatment.

Germination testing was performed using barley (*Hordeum vulgare*), cabbage (*Brassica oleracia* var. capitata) and capsicum/bell-pepper (*Capsicum annuum*). Twenty-five seeds were placed between two filter papers moistened with leachates and placed in 9 cm diameter petri dishes. The petri dishes were incubated at a constant temperature of 25^{o}C in the dark. Moisture was maintained for by putting 1 mL deionised water on the top filter paper when required. Triplicate samples were used for each treatment in all germination tests.

The numbers of germinated seeds were recorded in every second day. A seed was considered germinated when the radicle had emerged from the seed coat. At the end of each eight-day germination test, the radicle from each germinated seed was excised and morphological features (root length, avg. diameter, root surface area and root volume) were measured. Value for seed germination and root characteristics from each petri dish was averaged for statistical analysis considering all seedlings within a petri dish is pseudo-replication (n=3).

6.2.2 Effect of cane residue leachate on seedling emergence

The effects of cane residue leachate concentration on seedling emergence, and capacity of microbial solutions to ameliorate the allelopathic effects of the leachate on seedling emergence, were investigated in pot trials. Leachate was prepared as described above. Assays were performed in 237 mL styrofoam pots (70 mm top and 50 mm bottom diameter with 80 mm height) with 380 g of sieved and air-dried sandy-loam soil added to each pot. Field capacity of the soil in the pots was calculated as 84.42 mL of water per pot. Cane residue leachate concentrations of 0%, 20%, 40%, 60%, 80% and 100%, as prepared in the previous experiment, were applied by adding differing ratios of stock leachate and deionised water to pots to achieve field capacity. Five capsicum seeds were sown in each pot at 5 mm depth. Each treatment was replicated 5 times (n=5). The pots were kept in a partially shaded room with complete random design. From the 8th day after sowing, the number of emerged seedlings was counted everyday for 15 days.

The effect of microbial solution amendments on seedling emergence in styrofoam pots containing sandy-loam soil and 80% cane residue leachate was examined. The microbial products used in this experiment were bought from the commercial microbial supplier (New Edge Microbials Pty Ltd, Australia). The microbes were verified by the laboratory tests by the supplier before used in the experiment. The microbes used the experiment are beneficial live microbial cultures recommended to use as seed inoculums. Microbes used in the experiment were chosen as they were claimed to improve the plant growth.

Capsicum seeds were sown at 5 mm soil depth. After sowing seeds, 500 μL microbial solution was injected on each capsicum seed before being covered with soil. Microbes used as treatments were *Bacillus* sp. both in freeze-dried and liquid formulations, *Pseudomonas* sp., *Glomus* sp., *Azotobacter* sp. and the mixture of different microbial seed inoclums. The mixed treatments include *Pseudomonas* spp. + *Bacillus* spp., *Pseudomonas* spp. + *Streptomyces* spp., *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. Microbial solutions were prepared by mixing one vial of freeze-dried microbes (20 g vial size, 5×10^{11} CFU bacteria) in 2 L deionised water for the freeze dried product, and for the powdered and liquid formulations the equivalent amount were added to 2 L deionised water. Microbial population is the total microbes present in a vial, which was later mixed with water and poured in seedling transplanting hole. It is unrealistic to present microbial population in term of per gram soil in this case. Compost leachate was also included as one of the treatments. Compost leachate was prepared by washing the mature compost in deionised water (1:100 L). In this experiment, a positive control with the cane residue leachate application, a negative control without leachate and microbial solutions were also maintained. Styrofoam cups of same size filled with similar amount and types of soil used in the previous experiment were used in this experiment as well. For each treatment and control, five capsicum seeds were sown in each cup. For each treatment and control, 20 replications (20 cups) were maintained in the experiment (n=20). The experiment was arranged in completely randomized design. Seedling emergence was recorded every day for 26 days.

Each pot experiment was regularly monitored for the soil water and weed seedlings. Soil water was maintained at field capacity as described in chapter 2. Weed seedlings emerged at any time in the cups were hand pulled.

6.2.3 Effect of cane residue on crop growth and yield

A field experiment to examine the capacity of microbial solutions to overcome allelopathic effects of cane residue was conducted at the Department of Agriculture and Fisheries (DAF) Research Facility, Bundaberg, Queensland (24°51'00.61"S, 152°24'03.89"E). Capsicum seedlings (*Capsicum annuum* var. Warlock) were transplanted into 70 cm width raised soil beds without sterilizing the soil, in August 2014. Before capsicum seedling transplanting, cane residue was applied at the rate of 16 Mg ha^{-1} and incorporated in the soil using a rotary hoe. Beds were prepared using a bed former. Drip irrigation lines and plastic mulch were laid as in commercial cultivation. The experiment was arranged in a latin square design, with each of the four treatments replicated six times (n=6). Treatments were; cane trash but no microbes (positive control), cane trash + *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp., cane trash + *Pseudomonas* spp. + *Bacillus* spp., and no cane trash and no microbes (negative control).

Microbial treatments were applied by first dipping transplants into the microbial solution, and then adding 100 mL microbial solution into the planting hole just before planting of the seedling. For the control, the seedlings were dipped in tap water and 100 mL water was added in planting hole. Microbial solutions were prepared by mixing one-vial freeze-dried microbes with 10 L water. Microbial solution was poured in planting hole so it was not realistic to calculate/mention how much inoculant was used per gram soil.

Insect pests and diseases were controlled following standard commercial practices. Five weeks after transplanting the seedlings, when plants were still at the vegetative stage, whole root systems of four plants from each replication were excavated as described by Bohm (1979) to study root morphology. For this experiment, the shoot and root measurements taken for four plants from each replication were averaged afterwards for data analysis.

6.2.3.1 Root scanning and morphology measurement

The root system from each plant was carefully washed to remove soil and extra debris and root morphology was measured using WinRHIZO Pro 2012a (Regents Instr. Inc. Canada) software as described in chapter 2.

6.2.3.2 Plant height, biomass and crop yield

Plant height and stem node number was recorded for each excavated plant from the base to the top of the plant (n=6). Root and shoot dry weight were recorded following drying in an oven at 70^{o}C till a constant weight was attained. At crop maturity, fruits were harvested when 90% or more surface area had turned red. Fruits were harvested in sequence of one-week interval. The number of fruit and individual fruit fresh weight were recorded for 10 plants from each replication (n=6).

6.2.3.3 Data analysis

To determine the effect of organic matter leachate solution on crop seed germination, and root development in laboratory Generalized Linear Model (GLM) was performed. The effect of microbial solution on plant growth, root morphology and fruit yield in transplanted capsicum seedlings in cane trash incorporated field were analysed by using GLM. Anderson–Darling and Bartlett's test were performed to test the normality and homogeneity of variance respectively. Non-normal data were transformed and results were presented for the original data. Data analysis for the normalized data was conducted with MINITAB-16 (Minitab, 2009). The mean separation was carried by using Tukey's test in parametric test. Data from the seed germination and seedling root development in the laboratory experiment were analysed separately for individual crops at different leachate concentrations. The seedling emergence data for the leachate concentrations and the use of different microbes did not follow the parametric test. MINITAB-16 was not able to separate the treatment difference for the non-parametric test; therefore, data were analysed by using Kruskal-Wallis test with SPSS program (IBM SPSS, 2013). In Kruskal-Wallis test, treatments were separated by using a post-hoc test in SPSS. All data were presented at significance level of $p < 0.05$.

6.3 Results

6.3.1 Seed germination

Seed germination percentage was significantly reduced when seeds were exposed to crop residue leachates, with the effect increasing at higher percentage of leachate concentrations. Capsicum and cabbage seed germination was reduced by 50% or greater when exposed to sugarcane, lucerne or sorghum leachate at 100%. Barley seed germination was affected to a lesser extent. In all cases, except in 50% sugarcane extract, barley seed germination remained above 86% and was least affected ($p > 0.05$) by organic matter extract concentration (Table 6.1).

The effect of filtered (sterile) and unfiltered 100% leachate on seed germination was statistically similar except for cabbage seed germination where sterile sugarcane and lucerne extract reduced seed germination by a further 10 and 30% compared to the corresponding unfiltered 100% extract ($p < 0.05$).

No capsicum seeds germinated at 100% concentration of lucerne and sorghum leachate (Table 6.1). Capsicum and cabbage germination at 50% sugarcane and lucerne leachate, respectively, did not vary from the control ($p > 0.05$). Use of different organic matter leachate at 50% concentration reduced capsicum seed germination from 10 to 20% while this value increased to above 60% compared to the control when extract concentration was doubled.

Capsicum seed germination percentage at different extract concentrations of both mature compost leachates did not differ ($p > 0.05$) when compared with the control. Seed germination percentage in these treatments was very high, varying from 98.7 to 100% (Table 6.1).

Table 6.1 Concentration effect of cane residue, lucerne and sorghum cold-water leachate on capsicum, cabbage and barley seed germination. Values are means of n=3 and df=3 for uncomposted organic material leachate and n=3 and df=2 for compost leachate. Means are separated by Tukey's test. Different letters in a row represents significant different at p < 0.05.

Organic matter Source	Crop type	Germination %			
		Control	50%	100%	100% sterile
Cane residue					
	Capsicum	98.7^a	88.0^a	34.7^b	48.0^b
	Cabbage	96.0^a	54.7^b	46.7^b	34.7^c
	Barley	98.7^a	72.0^b	94.7^a	97.3^a
Lucerne					
	Capsicum	98.7^a	81.3^b	0.0^c	0.0^c
	Cabbage	96.0^a	88.0^a	52.0^b	20.0^c
	Barley	98.0^a	92.0^a	93.3^a	86.7^a
Sorghum					
	Capsicum	98.7^a	77.3^b	0.0^c	0.0^c
	Cabbage	96.0^a	42.7^b	2.7^c	10.7^c
	Barley	98.7^a	96.0^a	92.0^a	90.7^a
Composted organic matter leachate					
Leaf litter	Capsicum	98.7^a	98.7^a	98.7^a	-
Cane residue + sawdust	Capsicum	98.7^a	98.7^a	100.0^a	-

Root length decreased with exposure to increasing leachate concentration ($p < 0.05$) (Table 6.2). At 50% leachate concentration of sugarcane, lucerne and sorghum, capsicum root length was reduced by 74, 32 and 80% respectively when compared with the control. Cabbage also showed a significant reduction of 57, 37 and 92% in root length at 50% extract concentration of sugarcane, lucerne and sorghum, respectively. Doubling leachate concentration reduced cabbage root development by at least four fold. Barley root length with all residue leachates was significantly reduced ($p < 0.05$) at 50% concentration when compared to the control. Barley root length did not differ between 50 and 100% sugarcane leachate treatments ($p > 0.05$), but was reducedtwo and four fold in lucerne and sorghum leachate ($p < 0.05$), respectively. The root length at 100% sterile leachate was similar to 100% leachate, but was significantly shorter ($p < 0.05$) than the control and the 50% concentration for all the crop types (Table 6.2). Root length between the treatments and control did not vary significantly for the compost leachate ($p > 0.05$) (Table 6.2).

Table 6.2 Concentration effect of cane residue, lucerne and sorghum cold-water leachate on capsicum, barley and cabbage root length. Values are means of n=3 and df=3 for un-composted organic material leachate and n=3 and df=2 for compost leachate. Means are separated by Tukey's test. Different letters in a row represents significant different at p < 0.05.

Organic matter Source	Crop type	Root length (cm plant^{-1})			
		Control	50%	100%	100% sterile
Cane residue					
	Capsicum	1.7^{a}	0.4^{b}	0.1^{bc}	0.0^{c}
	Cabbage	2.7^{a}	1.1^{b}	0.2^{c}	0.1^{c}
	Barley	7.1^{a}	3.3^{b}	3.2^{b}	2.4^{b}
Lucerne					
	Capsicum	1.7^{a}	1.0^{b}	0.0^{c}	0.0^{c}
	Cabbage	2.7^{a}	1.69^{b}	0.37^{c}	0.13^{c}
	Barley	7.1^{a}	4.56^{b}	2.06^{c}	0.51^{c}
Sorghum					
	Capsicum	1.7^{a}	0.3^{b}	0.0^{c}	0.0^{c}
	Cabbage	2.7^{a}	0.1^{b}	0.0^{bc}	0.0^{c}
	Barley	7.1^{a}	3.1^{b}	0.8^{c}	0.6^{c}
Composted organic matter leachate					
Leaf litter	Capsicum	1.64^{a}	1.86^{a}	2.03^{a}	-
Cane residue + sawdust	Capsicum	1.64^{a}	1.74^{a}	1.82^{a}	-

6.3.2 Seedling emergence

Cane residue leachate inhibited seedling emergence when applied to soil at the highest concentrations (Fig. 6.1). Significantly lower ($p < 0.05$, Kruskal-Wallis test) seedling emergence was recorded at 100% leachate concentration compared to other concentrations and the control. The cumulative seedling emergence was faster in the control than in other treatments (Fig. 6.2). Ten days after sowing, the cumulative seedling emergence in the 40% concentration treatment exceeded emergence percentage in the control and other treatments, but was lower than the control at the end of the experiment. Cumulative emergence of capsicum seedling shows that emergence at 100% cane residue leachate concentration level is significantly lower from 12 days after sowing compared to all other treatments (Fig.6.2).

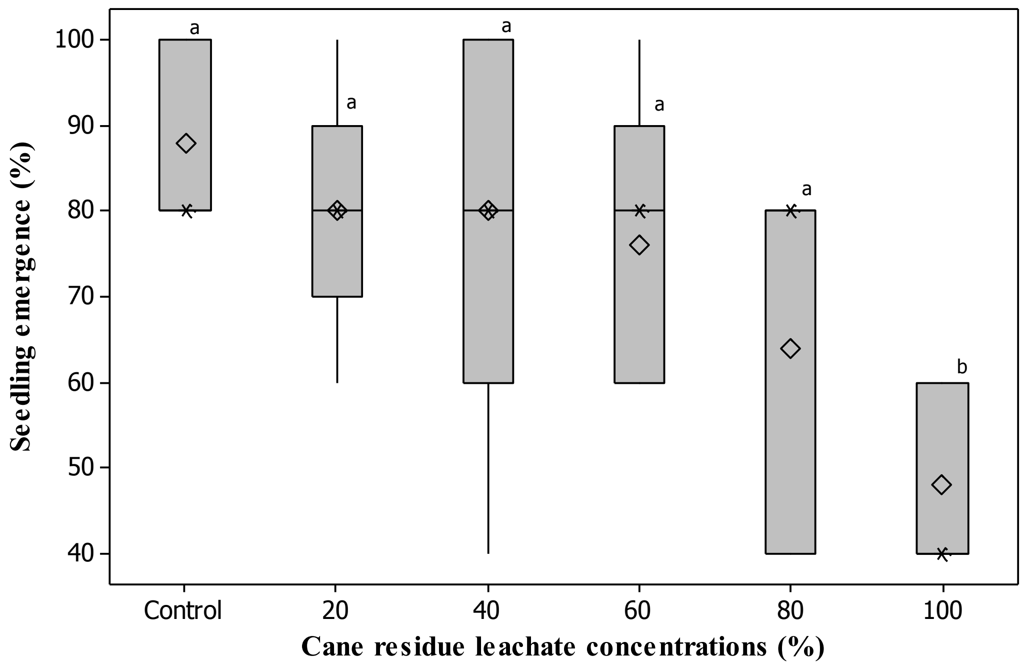

Figure 6.1 Effect of cane residue leachate at different concentrations on capsicum seedling emergence (%) (n=5 and df=5). The boxes show median (crosses), mean (diamonds) and upper and lower quartiles (25 to 75 quartiles) of the data. The whisker indicates minimum and maximum values. Different letters between the bars indicate significant different at $p < 0.05$ by the Kruskal-Wallis test.

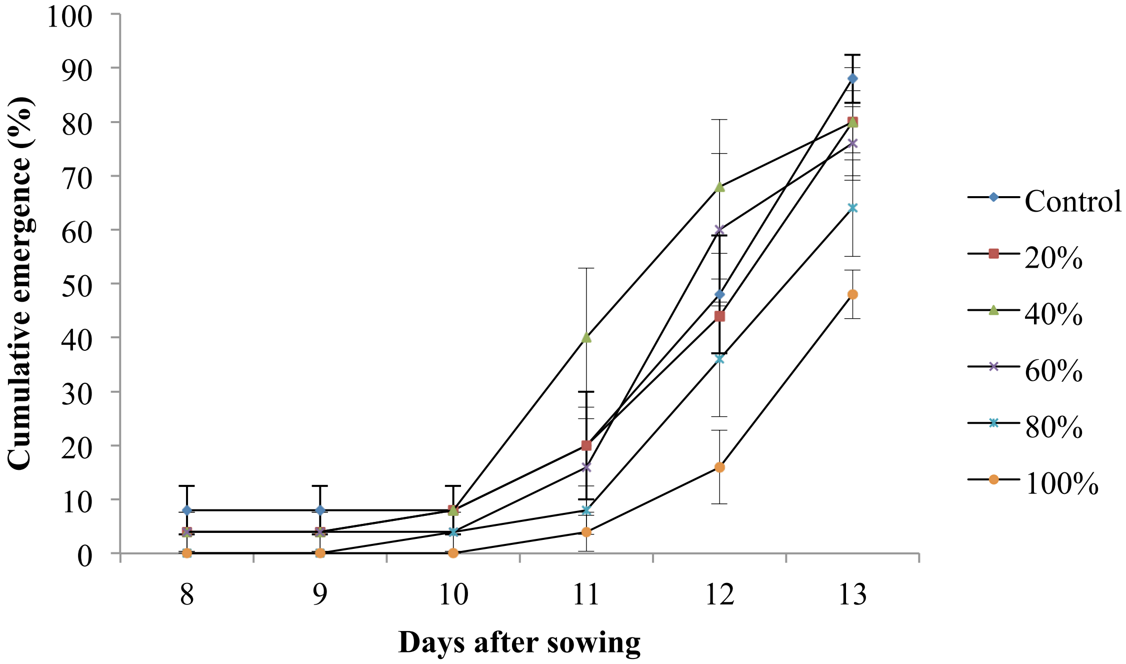

Figure 6.2 Cumulative capsicum seedling emergence (%) at different cane residue leachate concentrations (n=5). Vertical bars represent standard errors.

The addition of some of the microbial solutions to seeds increased capsicum seedling emergence compared to the positive control. Significant treatment differences were recorded. Treatments with microbial solutions containing *Bacillus* spp. powdered formulation (E), *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. (FOG) and *Pseudomonas* spp. + *Bacillus* spp. (ERP) produced a similar emergence percentage response as the negative control (NC) (Fig. 6.3) and significantly higher emergence rate than the positive control (Control) (Fig. 6.3). Treatment with *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. (FOG) produced a significantly higher seedling emergence percentage compared to *Glomus* spp. (M), *Pseudomonas* spp. + *Streptomyces* spp. (C), compost leachate (O) and *Azatobacter* spp. (A) with the highest mean rank and mean germination percentage. Treatment with *Pseudomonas* spp. + *Bacillus* spp. (ERP) produced a similar seedling emergence response to the other treatments.

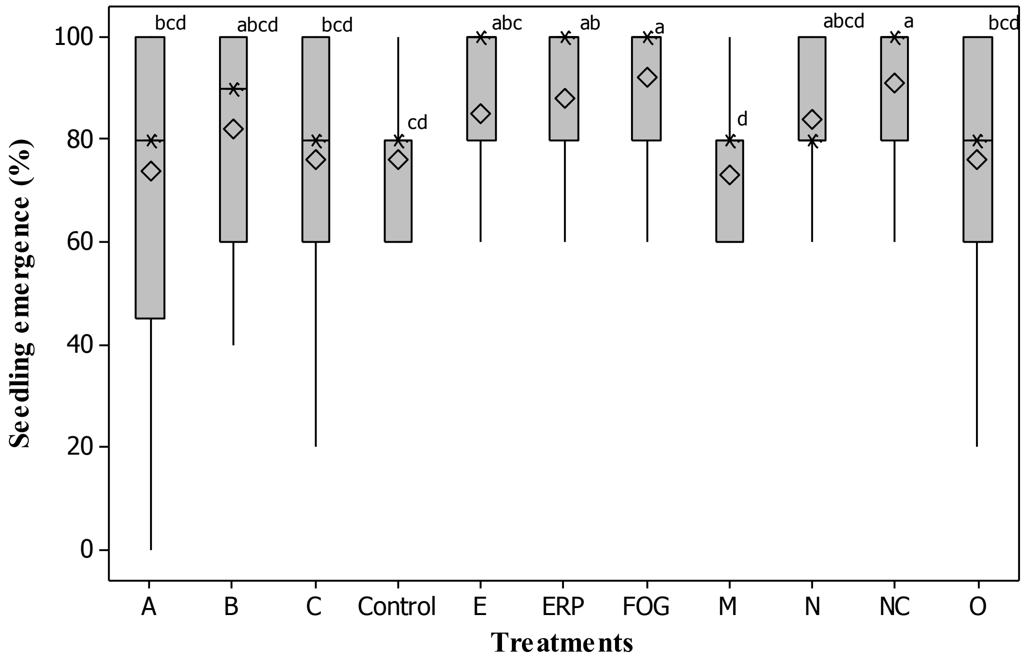

Figure 6.3 Effect of microbes on capsicum seedling emergence (%). The boxes show median (crosses), mean (diamonds) and upper and lower quartiles (25 to 75 quartiles) of the data. The whisker indicates minimum and maximum values. Different letters between the bars indicate significant different at $p < 0.05$ for the Kruskal-Wallis test. In the figure, A- *Azatobacter* spp., B- *Bacillussubtilis* (liquid product), C- *Pseudomonas* spp. + *Streptomyces* spp., E- *Bacillus* spp. (freeze dried product), ERP- *Pseudomonas* spp. + *Bacillus* spp., FOG- *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp., M- *Glomus* spp., N- *Pseudomonas* spp., O- Compost leachate, Control- +ve control, NC- -ve control.

Seedling emergence in the positive control attained 50% emergence at 17 days after sowing, with the seedling emergencerate declining after this time (Fig. 6.4). Treatment with *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. (FOG), *Pseudomonas* spp. + *Bacillus* spp. (ERP) and *Bacillus* spp. (powdered freeze dried) (E) also resulted in 50% emergence in the same period but remained higher than other treatments including the positive control (Control) over the entire experimental period. Other treatments reached 50% emergence in 19 days but cumulative emergence was lower than *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp. (FOG), *Pseudomonas* spp. + *Bacillus* spp. (ERP) and *Bacillus* spp. (freeze dried) (E) treatments. The negative control (NC) achieved 50% emergence at a later date but emergence kept increasing until the end of the experiment (Fig. 6.4).

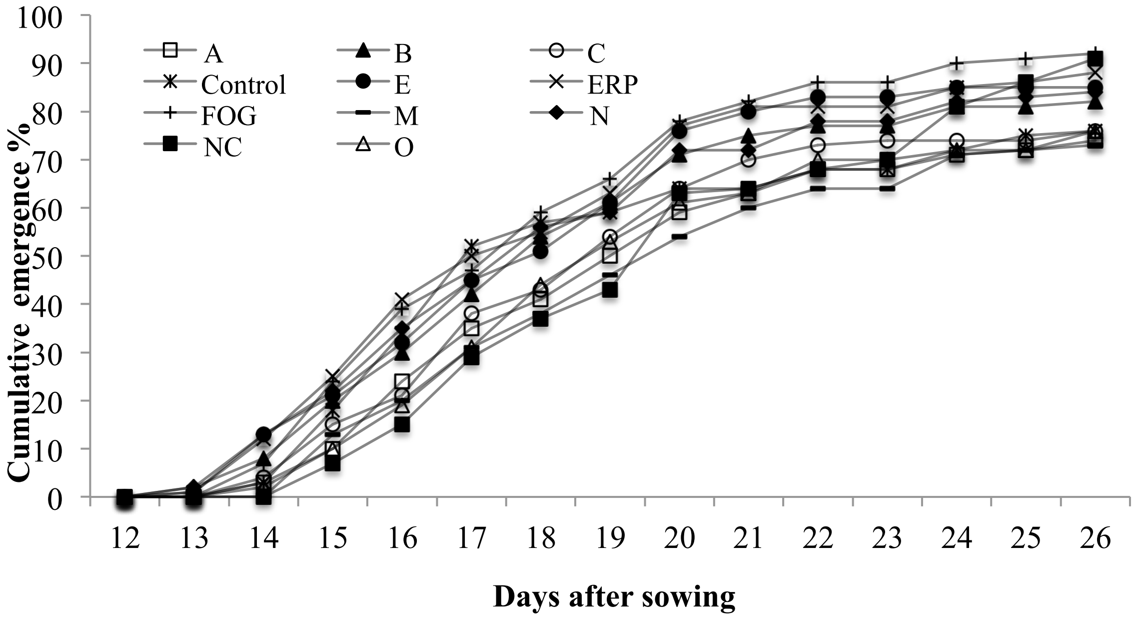

Figure 6.4 Cumulative capsicum seedling emergences for different microbes applied treatments including positive and negative control. In the figure, A- *Azatobacter* spp., B-*Bacillussubtilis* (liquid formulations), C- *Pseudomonas* spp. + *Streptomyces* spp., E- *Bacillus* spp. (freeze dried product), ERP- *Pseudomonas* spp. + *Bacillus* spp., FOG- *Pseudomonas* spp. + *Bacillus* spp. + *Serratia* spp., M- *Glomus* spp., N- *Pseudomonas* spp., O- Compost leachate, Control- +ve control, NC- -ve control.

6.3.3 Field crop growth and yield

The two microbial solutions that performed best in the pot trial were used in the field assessment. The aboveground plant growth and root growth in capsicum was reduced by exposure to cane residue incorporated in the soil (Table 6.3). Inoculating plant roots with microbes before transplanting was able to offset that effect and resulted in increased plant height, number of nodes, and root and shoot biomass compared to the positive control ($p < 0.05$). Fruit number at harvest for microbial treatments was not significantly different from either control treatment but the total fresh fruit weight was significantly higher in microbial treatments compared to the positive control (Table 6.3). The individual fruit size was also significantly smaller in the positive control compared to the microbial treatments (Table 6.3).

Table 6.3 Effect of microbes on capsicum plant height, number of nodes, root and shoot biomass at vegetative stage per plant and fruit yield at harvest. Values are means of n=6 and df=3. Means are separated by Tukey's test. Different letters in a column are significantly different at p < 0.05.

Treatments	Plant height (mm)	No of nodes (no)	Root dry wt. (g)	Shoot dry wt. (g)	Total no of fruits (no)	Total fresh fruit wt. (kg)	Per fruit wt. (g)
Control (negative)	173.0^{a}	8.1^{a}	0.36^{a}	2.42^{a}	2.9^{a}	0.38^{a}	141.5^{a}
Pseudomonas+Bacillus+ Serratia spp.	143.7^{b}	6.6^{b}	0.14^{b}	0.62^{b}	2.3^{ab}	0.33^{a}	141.4^{a}
Pseudomonas+Bacillus spp.	139.8^{b}	6.7^{b}	0.14^{b}	0.58^{b}	2.6^{ab}	0.35^{a}	137.2^{a}
Control (positive)	118.1^{c}	4.5^{c}	0.10^{c}	0.30^{c}	2.1^{b}	0.24^{b}	114.2^{b}

Microbial inoculation of seedlings transplanted into soil with incorporated cane residue (CR-soil) produced larger plant root systems than untreated plants (Table 6.4). Plants from all treatments grown in CR-soil produced significantly shorter total root length, surface area and total root volume than the negative control ($p < 0.05$). Positive control plants also had lower root length, area and volume than the microbial treatment plants (Table 6.4). The two microbially-treated produced similar root systems ($p > 0.05$). Microbially treated plants grown in CR-soil also produced thicker roots than with the positive control ($p < 0.05$) (Table 6.4).

Table 6.4 Effect of microbes on capsicum root morphological features; total root length, avg. root diameter, surface area and total root volume per plant in 2014. Values are means of n=6 and df=3. Means are separated by Tukey's test. Different letters in a column represents significant difference at p < 0.05.

Treatments	Length (cm)	Avg. diameter (mm)	Surface area (cm^2)	Root volume (cm^3)
Control (negative)	1629^{a}	0.56^{a}	288.6^{a}	4.19^{a}
Pseudomonas+Bacillus+Serratia spp.	874^{b}	0.51^{a}	142.8^{b}	2.00^{b}
Pseudomonas+Bacillus spp.	774^{b}	0.47^{ab}	119.1^{b}	1.48^{b}
Control (positive)	598^{c}	0.43^{b}	82.2^{c}	0.93^{c}

6.4 Discussion

Leachate from different organic matter sources all inhibited crop seed germination. Other studies support the recorded inhibitory effect of water extractable allelochemicals from organic matter residues on seed germination (Viator et al., 2006; Singh et al., 2003). The inhibitory effect of organic matter leachate has also been noted to increase with concentration, but the response varies with the types of crop seed (El-Darier et al., 2014, Viator et al., 2006).

The phytotoxic effect on seeds of uncomposed organic materials contrasts with the response to compost leachate where no influence on germination or seedling root morphology were recorded. It is evident that composting is able to lower the inhibiting effect of uncomposted organic material, as noted in a previous study (Rajbanshi & Inubushi, 1998). In their working germination index of chinese cabbage (*Brassica rapa*) seed in composted lantana (*Lantana camara*) leaf extract (prepared by shaking composted material with deionised water at 1:10 L ratio, centrifuged and filtered) significantly increased compared with the raw, uncomposted leaf extract, indicating the inhibiting potential of lantana raw extract was destroyed after composting (Rajbanshi & Inubushi, 1998). However, the response varies with types of plant residue, for example; chinese cabbage (*Brassica rapa*) seed germination did not increase when compared between the eupatorium (*Eupatorium adenophorum*) raw leaf extract and it's compost extract, due to the increased electrical conductivity of the extract after composting (Rajbanshi & Inubushi, 1998). Another research by Tiquia & Tam (1998) also presented that phytotoxic chemical compounds were eliminated in the mature compost compared to the compost at early stage, and the crop seed germination and root growth was significantly higher for the mature compost than in the early stage compost or un-composted materials.

Similar to germination, average total root length was reduced with the increase in leachate concentration and the effect varied with crop type. For example, barley (*Hordeum vulagre*) root development was unaffected irrespective of the cane residue leachate concentration. The reduced root length with exposure to increased water soluble organic material leachate concentration is consistent with other studies. Increased organic material leachate concentration reduced root growth and elongation in several crop species including lentil (*Lens esculenta*), wheat (*Triticum aestivum*), alfalfa (*Medicago sativa*) and weed species including beggerticks (*Bidens subalternans* L.) and wild mustard (*Brassica campestris* L.) (Chon et al., 2002; Sampietro and Vattuone, 2006b; Singh et al., 2003; Viator, et al., 2006).

Increased leachate concentration also inhibited seedling emergence, with the lowest emergence at the highest leachate concentration in the pot experiment. The higher total seedling emergence following microbes additions compared to the positive control demonstrated the beneficial effect of microbes on seedling emergence. The difference in total seedling emergence in cane residue leachate applied soil (positive control) and with the deionised water (negative control) was the result of the allelopathic effect of the cane trash leachate reducing total cumulative seedling emergence compared with the positive control. The lower seedling emergence in the negative control at the beginning of the experiment compared to the other treatments could be the result of imbibition differences. In negative control seeds were sown in the soil at field capacity, while in other treatments microbial solution was directly injected on the seed potentially increasing water availability around the seed compared to the seeds in the negative control.

Organic amendments including crop residues have been identified as producing allelochemicals that are responsible for deleterious effects to crops (Singh et al., 2003). Different phytotoxic chemicals such as benzoic and phenolic acids are present in the water extract of crop residues, and may be responsible for the inhibition of seed germination (Sampietro et al., 2006; Viator et al., 2006). The reduced root length and dry weight in the cane leachate applied treatment compared to the control is supported by the evidence that cane residue leachate contains hydroxamic acids (2,4-dihydroxy-1, 4-benzoxazin-3-one (DIBOA) and benzoxazolin-2-one (BOA)) that have been shown to inhibit lentil root growth at least by 50% (Singh et al., 2003). Likewise phenolic compounds including ferulic, vanillic and syringic acids were isolated from the cane residue leachates and were phytotoxic to root elongation of lettuce, wild mustard and pigweed (Sampietro et al., 2006). Lucerne (*Medicago sativa*) and sorghum (*Sorghum halepense*), as well acane residue, contain phenolic acids and their derivatives coumarin, cumaric, cinnamic, vanillic and ferulic acids, which have been shown to be allelopathic to root length and hypocotyl length (Chon et al., 2002; Chon & Kim, 2002).

Benzoic acids, phenolic compounds and their derivatives such as vanillic, ferulic and coumaric acids significantly affect transpiration, net photosynthesis and stomata conductance (Yu et al., 2003). Benzoic acid has also been shown to cause reduced nutrient uptake and increase cell damage (Baziramakinga et al., 1995). These responses may contribute to the reduced root and plant growth in cane residue applied treatments recorded both in the laboratory and in the field.

In the pot experiment, microbes effectively reduced the inhibiting effect of cane residue leachate on seedling emergence and produced similar or improved emergence to the negative control. Use of *Pseudomonas*, *Bacillus* and *Serratia* species produced higher rate of seedling emergence compared to others. This result shows that not all microbes are equally beneficial for reducing the allelopathic effect of cane residue leachate in the capsicum seedling emergence. The lower rate of seedling emergence in compost leachate could be due to the lack of sufficient number of microbes to reduce the negative effect of leachate in seedling emergence. One potential mechanism that microbial inoculum increase seedling emergence could be that the microbes provide a protective covering to the seed against the allelopathic effect of cane residue leachate.

In the field experiment, *Pseudomonas* + *Bacillus* + *Serratia* spp. applied to the seedling at the transplanting time may colonize the root surface and form a biofilm to protect the root (Bais et al, 2004; Barahona et al., 2010). Previous research shows that beneficial bacteria form a protective biofilm and produce lower root disease incidence (Haggag & Timmusk, 2007). Although several mechanisms have been discussed on how bacteria may protect the root system (Compant et al., 2005), one of the potential mechanisms might be that microbes provide a physical barrier on the outer root surface against the deleterious effect of allelochemicals released from the cane residue, which is supported by an example that the biofilms are impermeable to the toxic chemicals because of it's repellent nature (Epstein et al., 2011). An alternative mechanism could be microbial degradation of complex hydrocarbons of allelochamicals, for example the contribution of *Bacillus* sp. on degradation of vanillic acid (Alvarez-Rodriguez et al., 2003) and capacity of *Bacillus* and *Pseudomonas* spp. to degrade complex hydrocarbons has previously been demonstrated (Ghazali et al., 2004).

The ability of microbes to reduce the allelopathic effect of cane residue resulted in increased plant growth including dry shoot weight, plant height, and number of nodes. These results were supported by previous studies that plants treated with beneficial microbes such as *Pseudomonas* sp. displayed increased dry weight, root length, shoot length per plant in *Pisum sativum* L, and increased root elongation in *Zea mays* L. (Shararoona et al., 2006; Zahir et al., 2008). The increase in root length support acquisition of water and nutrients from the soil system supports greater shoot growth. Further, the role of microbes in nutrient acquisition and influence on phyto-hormone production may contribute to an increase in plant growth in microbial inoculations (Canbolat et al., 2006; Shahab et al., 2009).

6.5 Conclusions

This research shows that applying/building a higher amount of uncomposed organic material in the crop field produces an allelopathic effect on crop seed germination and root system development. However, the germination response varied with the organic matter source and crop type. This research demonstrated that microbes were beneficial in reducing the negative effect of organic material leachate on crop seedling emergence and crop growth under field conditions. This finding has significant implications for growers aiming to increase soil organic matter levels through uncomposted organic material additions but with limited flexibility in crop rotation timing and therefore risk of allelopathic effects occurring. Microbes may be utilized as dipping the seedlings root before transplanting, and pouring the microbial solution in the transplanting holes, which support to maximize the microbial population in the transplanting bed. The microbes present in the root system support to reduce the allelopathic effect of fresh cane residue incorporated in soil just few weeks before transplanting capsicum seedling. Use of beneficial microbes thus, supporting increased shoot and root growth and therefore total crop yield of capsicum. This will help the farmers to reduce the period between organic residue incorporation and seed/seedling planting.

7 Effect of microbial solutions inoculation on root damage in leachate applied capsicum seedling and plant growth in residue incorporated transplanted crop in field

Abstract

The use of microbes as soil additives and seed or seedling inoculants as a management strategy to reduce plant root disease incidence and severity is increasing. This study examined whether microbial inoculation could be effective in protecting plant roots from damage caused by organic matter leachate. Capsicum seedling root damage increased with the cane residue leachate exposure time, and with increased leachate concentrations. Microbial inoculation of the seedling root system before exposure to leachate reduced the level of root damage. Inoculating the seedling root system with microbial solutions for longer times and exposing seedlings to cane residue leachate for shorter times produced less root damage compared to shorter inoculation time and longer leachate exposure time. The reduced root damage in long time microbe inoculation treatment could be due to thicker microbial biofilm formation around the plant root that protected the root from the allellopathic effect of cane residue leachate. The root damage caused by cane residue leachate was also significantly lower when microbial inoculation was completed at high temperature compared to the lower temperature. Inoculating seedlings for longer times at higher temperature was most beneficial in reducing allellopathic effects of water-soluble leachate released from the organic matter when incorporated in to the soil.

Keywords: inoculation, leachate, microbes, root length, root health, surface area, temperature.

7.1 Introduction

Commercial crop production practices in developed countries are based on the routine use of chemical fertilizer, pesticide and heavy farm machines that cause serious soil health related problems. Growers are often advised to reduce potentially negative consequences of intensive crop production practices by adding organic amendments to soils to increase organic carbon content. Organic amendments added into the soil increases the soil organic carbon content, providing a food source and energy supply to the soil microbes. The increased microbial growth can enhance nutrient supply and disease suppression (Craft & Nelson, 1996; Zaman et al., 1999).

While organic amendments have been a practice used in agriculture for centuries to improve the biological and physical status of the soil, more recently additives and specific microbial solutions have been promoted as having beneficial effects associated with their biological activity in the soil. Studies have shown that soil additives can have a positive effect on crop growth and performance. For instance, use of compost tea increased the shoot dry weight, root dry weight and total root length in pak choi (*Brassica rapa* cv. Bonsai) compared to the control without tea, with the size of the response varying with the source of compost (Pant et al., 2012). The increased growth response was concluded to be due to either increased nutrient uptake or to a response to the growth hormone-gibberellin present in the tea (Pant et al., 2012). The increased root and shoot growth in compost tea supplied treatments was also associated with the presence of a large number of active fungi and bacteria in the tea that enhance the soil organic carbon decomposition and nutrient availability to the plants through increased microbial activity (Pant et al., 2011).

Similarly, direct inoculation of plants with different microbes has also been shown to be beneficial in reducing the soil borne disease causing pathogen and disease severity in the plant. Inoculating *Bacillus subtilis* to wheat (*Triticum aestivum* cv. 97148) in a pot experiment reduced the take-all disease caused by *Gaeumannomyces graminis* var. *tritici* in the root system by 45% compared with the control only with the pathogen (Liu et al., 2009). Root knot nematode caused by *Meloidogyne incognita*and root rot disease caused by *Macrophomina phaseolina* in chick pea (*Cicer arietinum* L.) were significantly reduced when *Pseudomonas straita, Glomus intraradices,* and *Rhizobium* spp. where inoculated as individual or in combination in pot soil around the chick pea root zone (Akhtar &Siddiqui, 2008). Several other studies of bacterial species such as *Bacillus* and *Pseudomonas* spp. have shown beneficial effects in reducing root diseases in different crops (Liu et al., 2009; Prakob et al., 2009). The microbial inoculation treatments in these studies that reduced disease incidence and pathogen density were found to also increase root and shoot biomass and crop yield (Akhtar & Saddiqui, 2008; Liu et al., 2009).

The increasing understanding of the benefits of microbes in reducing the disease incidence and severity has resulted in several industrial formulated microbial products being released commercially (Gardener & Fravel, 2002). For instance, *Pseudomonas chlororaphis* (Cedomon), *Streptomyces* sp (Mycostop) and *Pseudomonas* sp (Proradix Agro) are commercial products that, when used to treat seeds or applied to transplant soil mixture, were effective in reducing root rot diseases in zucchini (*Cucurbita pepo* L) caused by *Fusarium solani* f.sp. *cucurbitae* race 1 (Roberti et al., 2012). Application of *Streptomyces griseoviridis* strain K61 (Mycostop) to substrate also inoculated with the pathogen *Pythium aphanidermatum* reduced the level of damping-off disease in cucumbers (*Cucumis sativus* L.) grown in the substrate in a greenhouse experiment (Punja and Yip, 2003).

Studies have shown that the beneficial microbes present in the soil additives or the inoculated microbes were responsible for reducing root disease incidence in crop plants. The beneficial microbes present in the additives or pure culture inoculation can form a microbial shield called as biofilm and protect the plant root systems. For instance, Bais et al. (2004) showed that *Arabidopsis* plant mortality was reduced following inoculation with the biofilm forming bacteria, *Bacillus subtilis* strain 6051 against *Pseudomonas syringae*. The *B. subtilis* was shown to form a biofilm around the plant root surface. Haggag (2007) showed the formation of biofilm by the polysaccharide producing *Paenibacillus polymyxa* (= *B. polymyxa*) on peanut (*Arachis hypogea* L.) roots reduce crown rot disease incidence caused by *Aspergillus niger* in the field. However, biofilm formation by bacteria is affected by several environmental factors such as salinity, pH and temperature (Puopolo et al., 2013; Qurashi & Sabri, 2012; Rinaudi et al., 2006), resulting in increased potential for variability in efficacy when used to reduce disease incidence.

The possible mechanisms by which the inoculating microbes benefit the plants have been presented in the literature review (chapter 1). In short, the possible mechanisms for the different bacteria used as bio-control agents in controlling root diseases include parasitism, competition, antibiosis, and secretion of lytic enzyme, promoting plant growth and induced systemic host resistance. Besides these mechanisms, the laboratory, pot and field experiments in chapter 6 presents that microbes around the root surface might have formed a protective layer of microbial community (biofilm) that function as a physical barrier against non-pathogenic factors such as the allelopathic effect of cane residue leachate. The physical barrier provided by the biofilm after inoculating seeds or seedlings with the microbes could explain the higher total percentage of capsicum seedlings emergence and higher root growth in the microbial solution inoculated treatments and grown in fresh uncomposted cane residue incorporated soil. This research aimed to investigate on how does the microbes reduce the root damage caused by the cane residue leachate. Therefore, this research tested the following hypothesis; i) inoculating casicum seedling with beneficial microbes reduces the root damage caused by the allelopathic effect of cane residue leachate, ii) increasing capsicum seedling inoculation time with beneficial microbes reduces root damage by allelopathic effect of cane residue leachate, and iii) microbes are more effective at higher temperature to reduce the root damage caused by allelopathic effect of cane residue leachate.

7.2 Materials and methods

7.2.1 Effect of cane residue leachate exposure time on root damage

Root damage following exposure of capsicum seedlings to cane residue leachate (*Capsicum annuum* var. California Wonder) in different time periods was assessed at Central Queensland University, Bundaberg laboratory (24^{o}54’03.93”S, 152^{o}18’44.45”E). The residue leachate was prepared in a similar way to that described in chapter 6. In short; 20 g powdered cane residue was soaked in 150 mL deionised water overnight with occasional stirring and leachate was separated from solids with Whatman’s No.1 filter paper. The stock leachate was prepared from the same powdered cane residue every time. The cane leachate was prepared as one extraction required for all experiments carried out at the same time. The extraction was done at the Central Queensland University laboratory room temperature. To maintain the uniformity of extraction, the ratio between amount of powdered cane residue and deionised water was maintained same in every extraction.

Pre-germinated capsicum seedlings were prepared by soaking capsicum seed in sodium hypochlorite 1% solution (v/v) for 5 min. then rinsing the seeds for 10 times with deionised water. The treated seeds were kept on moist filter paper in a petri dish and the petridish was sealed with parafilm and incubated at 25^{o}C for 5 days. The pre-germinated capsicum seedlings were placed on a filter paper moistened with 2 mL of extract in a 9 cm diameter petri dish. Seedlings were exposed to cane residue leachate for 1, 2, 3, 4 or 5 days. Each treatment was replicated in three petri dishes, and each petri dish contained 4 pre-germinated capsicum seedlings. Each petri dish was sealed with parafilm before being placed in an incubator held at a constant temperature (25^{o}C). The experiment was laid out in a complete random design. After receiving the required duration of exposure to cane residue leachate, seedlings were moved to filter paper moistened with deionised water. On the 5th day, the radicle was excised from each seedling and scanned for the root damage. For the root analysis, capsicum seedlings within a petri dish was considered pseudo-replication and values from 4 seedlings from a petri dish were averaged, thus individual petri dish was counted as one replication. This provided 3 replications for each treatment (n=3).

7.2.2 Effect of cane residue leachate concentration on root damage

The severity of root damage on capsicum seedling when exposed to different cane residue leachate concentrations was determined. The cane residue leachate was prepared as in the previous as mentioned in 7.2.1 section. Different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100% of raw leachate) solutions were prepared from the 100% stock solution. A control with deionised water, but no leachate solution was also included in the experiment. Pre-germinated capsicum seedlings were also prepared as in 7.2.1 section. Pre-germinated capsicum seedlings were placed in a petri dish and replicated as describes in section 7.2.1. Each petri dish was sealed and incubated as described in section 7.2.1, for 5 days. The experiment was laid out in a complete random design. On the 5th day radicle was excised from each seedling and scanned for root damage caused by the cane trash leachate. For data analysis, 3 replications (n=3) were considered as described in section 7.2.1.

7.2.3 Effect of seedling inoculation to bacteria on root damage

Microbial solutions were prepared by mixing 1 vial freeze-dried commercial microbial product (20 g vial, 5 x 10^{11}CFU bacteria) with 2 L deionised water. The microbes were verified by the laboratory tests by the supplier before used in the experiment. The microbes contained in the treatment were a mixture of *Pseudomonas* sp., *Bacillus* sp. and *Serratia*sp. The pre-germinated capsicum seedlings were prepared as in section 7.2.1 and then dipped in microbial solution for 45 min. The seedlings were then moved to petri dish supplied with 2 mL of different concentrations of can residue leachate, as prepared in the section 7.2.1. A control with deionised water and microbial inoculation was included in the experiment. Three replicated petri dishes were maintained for each treatment and control. Each replicated petri dish contained 4 pre-germinated seedlings. The experiment was laid out in a completely random design. The seedling incubation and root system assessments were performed as described above in section 7.2.1. For data analysis, 3 replications (n=3) were considered as described in section 7.2.1.

7.2.4 Effect of varying inoculation and leachate exposure time on root damage

Pre-germinated capsicum seedlings were prepared as in section 7.2.1 and inoculated with microbial solution for different times (0, 0.5, 1, 12 and 24 hours). The pre-germinated, inoculated capsicum seedlings were then treated with cane residue leachate for different time periods (1, 2, 3, 4 and 5 days), with the experiment incorporating all possible combinations of inoculation time and leachate exposure time. The treatments were replicated thrice and each replication contained 4 pre-germinated seedlings. The petri dishes were sealed and incubated as described in section 7.2.1. The experiment was laid out in a completely random design. The root systems from each seedling was separated and scanned for the root morphological characters after 5 days. For data analysis, 3 replications (n=3) were considered as described in section 7.2.1

7.2.5 Effect of temperature and inoculation time on root damage in cane residue leachate exposed seedlings

Pre-germinated capsicum seedlings were inoculated with the commercially available microbial solution, which was prepared as described in section 7.2.3, for 0, 0.5, 1, 12 and 24 hours. Pre-germinated capsicum seedlings and cane residue leachate were prepared as in the section 7.2.1. The microbial solution treated capsicum seedlings were kept on a filter paper moistened with 2 mL 100% cane residue leachate. Each microbial solution treatment time was combined in all possible combination with 3 different temperatures (15, 25 and 35 ^{o}C). For each treatment 4 pre-germinated capsicum seedlings were kept in a petri dish. Each treatment was replicated thrice and incubated at corresponding temperatures for four days. The experiment was laid out in a completely random design. When root damage was prominent in treatments, roots were separated from the seedlings and scanned to measure the root morphological parameters. For data analysis, 3 replications (n=3) were considered as described in section 7.2.1

7.2.5.1 Root separation and scanning

Capsicum root systems were separated from each seedling using a scalpel and a brush. Root systems were placed in a tray with 1 cm water and scanned using a colour scanner (Epson perfection V700, Digital ICE Tech). The total root length, surface area, volume for the total root, damaged and healthy root were measured for each capsicum seedling using WinRHIZO software as mentioned in chapter 2. At the time of root morphological measurement, damaged roots were defined as any deviation from the white colour of healthy roots, with browning on the root surface and black necrotic root tips the most common damage symptoms.

7.2.5.2 Data analysis

To determine the effect of cane residue leachate solution exposure time, concentration effect or microbial inoculation on proportion of damaged root length or damage root surface area, one-way analysis of variance (ANOVA) was performed. Interaction between different time of microbial inoculation and extract exposure time on capsicum seedling root length and surface area damage was analysed by using the

Generalised Linear Model (GLM). The effect of different temperatures and microbial inoculation times on root damage in leachate-applied seedlings was also analysed by using GLM. Anderson–Darling and Bartlett's test were performed to test the normality and homogeneity of variance respectively. Non-normal data were transformed and results were presented for the original data. Data analysis for the normalized data was conducted with MINITAB-16 (Minitab, 2009). Treatments mean were separated by using Tukey's test. All the data analysis was conducted with MINITAB-16 (Minitab, 2009), and data were presented at significance level of $p < 0.05$.

7.3 Results

7.3.1 Effect of cane residue leachate exposure time on root damage

The results showed that capsicum seedling total root length decreased with the leachate exposure time. The root damage increased with the seedling exposure time to the cane residue leachate. The total root length for the 2, 3, 4 and 5 days seedling exposure to cane trash leachate reduced the root length by 15.18, 35.07, 50.26and 43.45% compared to the one-day exposure (Table 7.1). The proportion of damaged root length in the lowest exposure time (1 day) was significantly lower than that recorded after 4 or 5 days exposure. The damaged root length in 2, 3, 4, and 5 days exposure were 2, 3, 4 and 8 time more than for one-day exposure time (Table 7.1). At the same time the proportion of healthy roots increased in the similar fashion, showing a statistically higher proportion of healthy roots in the lowest exposure time compared to the longer exposure times (Table 7.1).

Table 7.1 Effects of cane residue leachate exposure time on capsicum seedling root length damage presented as per plant. Values are means of n=3 and df=4. Means are separated by Tukey's test. Different letters in a column are significantly different at $p < 0.05$.

Leachate Exposure time (Day)	Total root length (cm)	Healthy root length (cm)	Damage root length (cm)	Proportion of damage root length	Proportion of healthy root length
1	1.91	1.80	0.09	0.06^{c}	0.92^{a}
2	1.62	1.42	0.18	0.12^{bc}	0.86^{ab}
3	1.24	1.00	0.22	0.18^{bc}	0.80^{ab}
4	0.96	0.68	0.26	0.27^{b}	0.71^{b}
5	1.08	0.52	0.54	0.51^{a}	0.46^{c}

Similar to the effect of leachate exposure time on capsicum total root length, the root surface area also decreased with the leachate exposure time. Exposing pre-germinated capsicum seedling for 5 days showed that total root surface area decreased by 31, 50, 58 and 63% in 2, 3, 4 and 5 days exposure compared to the one-day exposure (Table 7.2). The proportion of damaged root surface area, after exposing capsicum seedlings to cane trash leachate, increased with exposure time (Table 7.2). The least damaged surface area was in the one-day exposure while the proportion of damaged root surface area increased with increasing exposure time. Exposing seedlings to cane trash leachate for 5 days produced significantly higher damaged root surface area compared to the 1 and 2 days exposure (Table 7.2). At the same time, the proportion of healthy root surface area decreased from exposing seedling for 1 to 5 days (Table 7.2).

Table 7.2 Effects of cane residue leachate exposure time on capsicum seedling root surface area damage presented as per plant. Values are means of n=3 and df=4. Means are separated by Tukey's test. Different letters in a column are significantly different at p < 0.05.

Leachate Exposure time (Day)	Total surface area (cm^2)	Healthy Surface area (cm^2)	Damage Surface area (cm^2)	Proportion of Damage surface area	Proportion of healthy surface area
1	0.58	0.56	0.02	0.06[d]	0.93[a]
2	0.40	0.34	0.05	0.15[cd]	0.83[ab]
3	0.29	0.23	0.06	0.21[bc]	0.77[bc]
4	0.24	0.13	0.07	0.33[b]	0.64[c]
5	0.21	0.09	0.13	0.58[a]	0.40[d]

7.3.2 Effects of cane residue leachate concentration on root damage

The proportion of root length damaged increased with increasing leachate concentration. The proportion of damaged root length at 10% and higher leachate concentration was significantly higher than in the control (Table 7.3). The lowest concentration extract produced the least damaged root length and the 100% extract concentration produced the highest damaged root length. The proportion of seedling root damage at 20% and above extract concentrations were statistically similar ($p > 0.05$).

The proportion of healthy root length was higher at lower leachate concentrations (Table 7.3). Among treatments, 10% extract concentration produced significantly longer proportion of healthy root length compared to treatments above 40% leachate concentration other treatments (Table 7.3).

Table 7.3 Effects of cane residue leachate concentrations on capsicum seedling root length damage presented as per plant. Values are means of n=3 and df=10. Means are separated by Tukey's test. Different letters in a column are significantly different at $p < 0.05$.

Leachate concentrations (%)	Healthy root length (cm)	Damage root length (cm)	Proportion of damage root length	Proportion of healthy root length
0	2.33	0.00	0.00^{c}	0.99^{a}
10	1.49	0.63	0.34^{bc}	0.65^{b}
20	0.84	1.04	0.54^{ab}	0.44^{bc}
30	1.21	1.46	0.54^{ab}	0.45^{bc}
40	0.85	1.50	0.63^{ab}	0.36^{c}
50	0.92	1.47	0.61^{ab}	0.37^{c}
60	0.68	1.07	0.70^{ab}	0.28^{c}
70	0.67	1.80	0.72^{ab}	0.27^{c}
80	0.70	1.54	0.68^{ab}	0.30^{c}
90	0.50	1.27	0.70^{ab}	0.28^{c}
100	0.43	1.84	0.78^{a}	0.20^{c}

Similar to root length, the proportion of damaged root surface area increased with increasing leachate concentration (Table 7.4). Cane residue leachate at 0% and 100% produced the lowest and highest proportion of damaged root surface area, respectively. Leachate concentrations at 40% and above produced statistically similar root damage. The leachates at 10, 20 and 30% concentration also produced statistically similar proportion of damaged root surface area, but were significantly higher than control.

Table 7.4 Effects of cane residue leachate concentration on capsicum seedling root surface area damage presented as per plant. Values are means of n=3 and df=10. Means are separated by Tukey's test. Different letters in a column are significantly different at $p < 0.05$.

Leachate concentrations (%)	Healthy surface area (cm^2)	Damage surface area (cm^2)	Proportion of damage surface area	Proportion of healthy surface area
0	0.65	0.00	0.01^{d}	0.98^{a}
10	0.51	0.19	0.29^{c}	0.70^{b}
20	0.27	0.28	0.50^{bc}	0.48^{bc}
30	0.51	0.5	0.49^{bc}	0.50^{bc}
40	0.30	0.42	0.57^{ab}	0.41^{cd}
50	0.33	0.44	0.56^{ab}	0.42^{cd}
60	0.21	0.46	0.67^{ab}	0.31^{cd}
70	0.21	0.51	0.70^{ab}	0.29^{cd}
80	0.23	0.45	0.66^{ab}	0.33^{cd}
90	0.14	0.35	0.70^{ab}	0.29^{cd}
100	0.15	0.57	0.76^{a}	0.23^{d}

7.3.3 Effects of microbial inoculation on root damage by cane residue leachate

In this experiment the seedling with deionised water and microbial inoculation produced similar proportion of healthy root length (Table 7.5). The effect of microbial inoculation on root damage by cane residue leachate was assessed by comparing data from table 7.3 vs 7.5, and 7.4 vs 7.6. The proportion of root damage when microbes were inoculated reduced the extent of damage when compared with the damage just with leachate application (as presented in Table 7.3). However, the proportion of damaged root length increased with leachate concentration (Table 7.5). The proportion of root length damage at 100% was statistically similar with 90% leachate concentration but significantly higher than 80% leachate concentration, and other lower leachate concentrations and control. Leachate concentration at 90% produced similar proportion of damaged root length with 50, 60, 70 and 80% leachate concentration but significantly higher than 10, 20, 30 and 40% leachate concentrations (Table 7.5).

The proportion of healthy root length was relatively higher at lower leachate concentration compared to high leachate concentrations (Table 7.5). Statistically, the control with microbes added to seedlings produced a similar proportion of healthy root length compared up to 40% leachate concentration. The 100% leachate concentration produced least healthy proportion of root length (Table 7.5).

Table 7.5 Effects of microbial inoculation on capsicum seedling root length damage presented as per plant. Values are means of n=3 and df=11. Means are separated by Tukey's test. Different letters in a column are significantly different at p < 0.05.

Leachate concentrations (%)	Microbial inoculation	Healthy root length (cm)	Damage root length (cm)	Proportion of damage root length	Proportion of healthy root length
0	-	2.33	0.00	0.00^{f}	0.99^{a}
0	+	1.85	0.00	0.00^{f}	0.99^{a}
10	+	2.01	0.11	0.05^{ef}	0.93^{a}
20	+	1.75	0.17	0.08^{def}	0.90^{ab}
30	+	1.48	0.21	0.12^{cdef}	0.86^{abc}
40	+	1.42	0.23	0.14^{cdef}	0.84^{abc}
50	+	1.08	0.37	0.26^{bc}	0.73^{cd}
60	+	1.21	0.36	0.23^{bcd}	0.75^{bcd}
70	+	1.26	0.32	0.20^{bcde}	0.78^{bcd}
80	+	1.09	0.33	0.23^{bcd}	0.76^{bcd}
90	+	0.95	0.47	0.33^{ab}	0.65^{de}
100	+	0.89	0.72	0.44^{a}	0.54^{e}

Note: - and + indicates the pre-germinated seedlings were either not inoculated with microbes or inoculated before exposing to the cane residue leachate.

As like the root length, the proportion of healthy root surface area in control with deionised water and with microbial inoculation produced statistically similar results (Table 7.6). The proportion of damage root surface area in microbial inoculated treatment was much lower compared to the without microbes inoculation in cane trash leachate applied treatments (e.g. Table 7.4 vs Table 7.6). The proportion of damaged root surface area after microbial inoculation was similar among treatments

(Table 7.6). However, the proportion of healthy root surface area was significantly higher in the control and lower leachate concentrations compared to the higher leachate concentrations (Table 7.6). Leachate concentrations at 10, 20, 30 and 40% produced similar proportion of healthy root surface area, but were significantly higher than the 90 and 100% leachate concentration.

Table 7.6 Effects of microbial inoculation on capsicum seedling root surface area damage presented as per plant. Values are means of n=3 and df=11. Means are separated by Tukey's test. Different letters in a column are significantly different at $p < 0.05$.

Leachate concentrations (%)	Microbial Inoculation	Healthy surface area (cm^2)	Damage surface area (cm^2)	Proportion of damage surface area	Proportion of healthy surface area
0	-	0.65	0.00	0.01^{a}	0.98^{a}
0	+	0.44	0.01	0.03^{a}	0.95^{a}
10	+	0.52	0.03	0.05^{a}	0.93^{ab}
20	+	0.51	0.05	0.08^{a}	0.90^{abc}
30	+	0.44	0.07	0.13^{a}	0.85^{abcd}
40	+	0.42	0.20	0.48^{a}	0.86^{abcd}
50	+	0.34	0.12	0.26^{a}	0.73^{def}
60	+	0.38	0.11	0.22^{a}	0.76^{cde}
70	+	0.41	0.10	0.19^{a}	0.79^{bcde}
80	+	0.37	0.10	0.21^{a}	0.75^{cde}
90	+	0.27	0.12	0.31^{a}	0.68^{ef}
100	+	0.25	0.17	0.41^{a}	0.58^{f}

Note: - and + indicates the pre-germinated seedlings were either not inoculated with microbes or inoculated before exposing to the cane residue leachate.

7.3.4 Effects of microbe inoculation time and leachate exposure time on capsicum seedling root damage

In this experiment, both the individual effect of microbial inoculation time, leachate exposure time and their interaction on the proportion of damaged root length were significant. The level of damage increased with the exposure time at all inoculation times (Fig. 7.1). Irrespective of the microbial inoculation time, the damaged root length following 1 or 2 days leachate exposure time was similar to the controls. The inoculation of seedlings with microbial solution produced significantly lower damage compared to the control following 4 days cane trash leachate exposure. Although not significant, the shorter microbial inoculation time and longer leachate exposure produced a higher percentage of damaged root length compared to the longer microbial inoculation time and shorter leachate exposure time (Fig. 7.1).

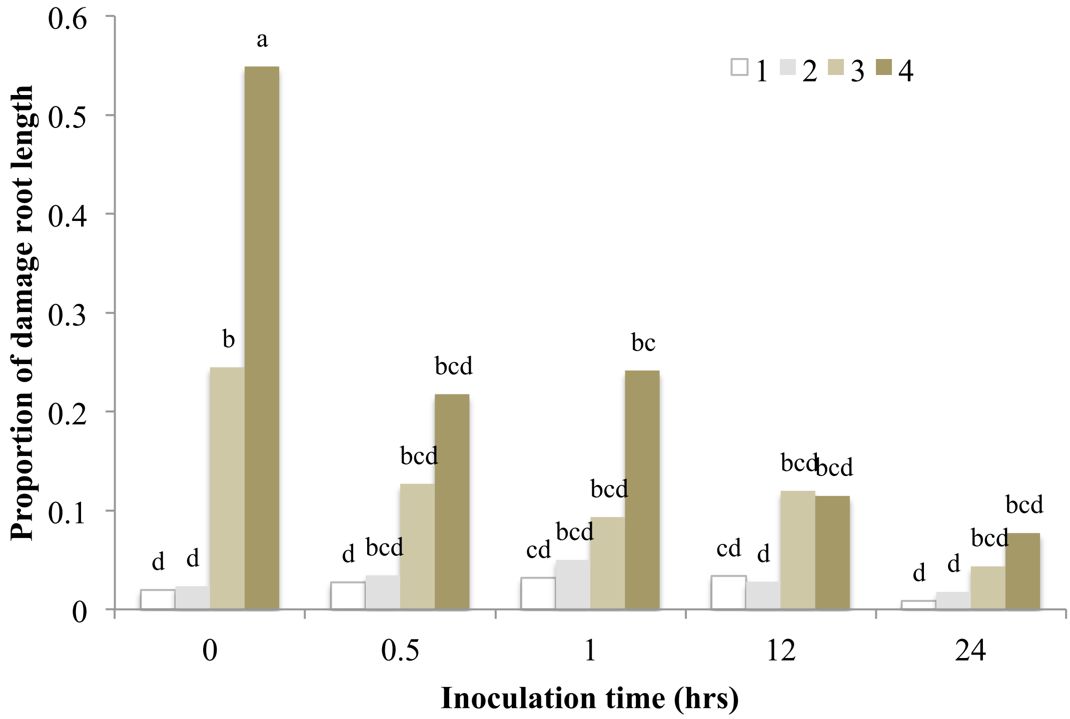

Figure 7.1 Interaction effects of microbial inoculation time and cane residue leachate exposure time on proportion of damaged root length presented as per plant. Values are means of n=3 and df=12. Means are separated by Tukey's test. The different letters on each bar are significantly different at $p < 0.05$. The numbers (1, 2, 3 and 4) in legend represents days of exposure.

The proportion of healthy root length did not vary between control and microbial inoculated treatments following exposure of roots for 1 or 2 days. The healthy root length at 0.5 and 1 hours microbial and 4 days of extract exposure produced lower healthy root length compared to the 24 hours inoculation and same leachate exposure. Control with 4 days leachate exposure produced significantly lower healthy root length compared to any combination of microbial inoculation and leachate exposure time (data not presented).

The effects of the main factors and their interactions were significant for the proportion of damaged root surface area. Similar to the root length, the proportion of damaged surface area decreased with microbial inoculation (Fig. 7.2). Control with seedlings exposed to cane trash leachate for 4 days produced the highest proportion of damaged surface area. Exposing seedlings to cane residue leachate for 1 or 2 days both for control and microbial inoculated treatments produced the lowest damaged area. However, the damaged surface area increased with leachate exposure time in all treatments. As for root length, the longer microbial inoculation time and shorter leachate exposure produced the lowest damaged surface area compared to the shorter inoculation time and longer extract exposure time (Fig. 7.2).

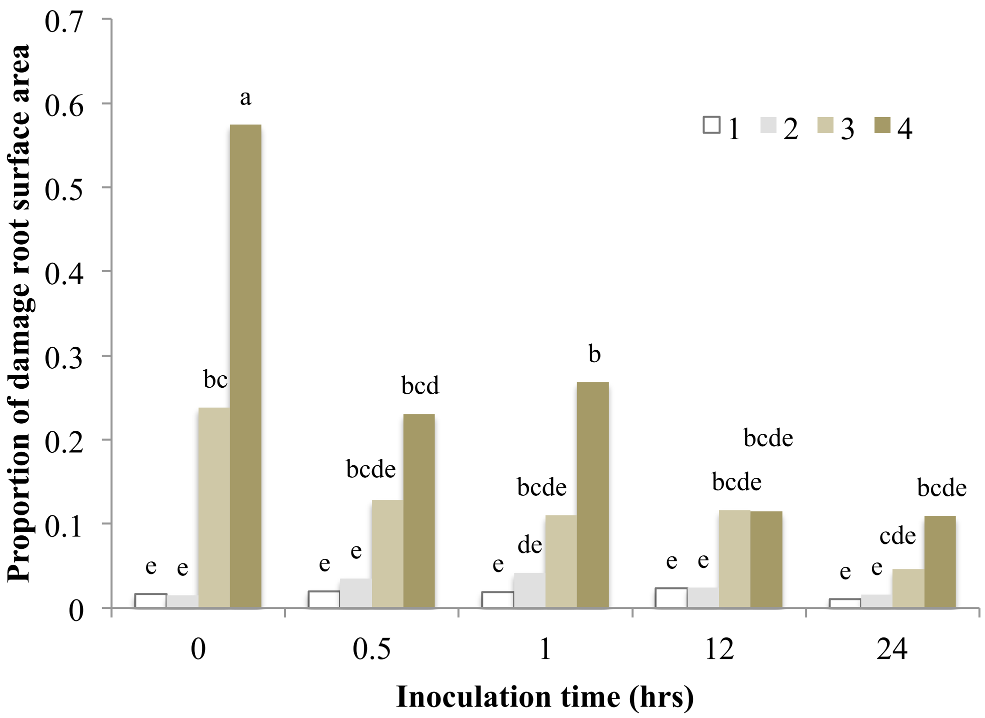

Figure 7.2 Interaction effects of microbial inoculation time and cane trash leachate exposure time on proportion of damaged root surface area presented as per plant. Values are means of n=3 and df=12. Means are separated by Tukey's test. The different letters on each bar are significantly different at $p < 0.05$. The numbers (1, 2, 3 and 4) in legend represents days of exposure.

The healthy root surface area followed a similar pattern as the proportion of healthy root length, showing increased healthy root surface area with longer microbial inoculation time (data not presented).

7.3.5 Effects of temperature on root damage by cane residue leachate in microbial inoculated capsicum seedling

The analysis of variance revealed that temperature and microbial exposure time and their interactions had a significant effect on level of root damage. Proportion of root damage decreased with increasing microbial inoculation time (Fig. 7.3). However in the control, uninoculated treatment root damage at 35^{o}C was similar with 15^{o}C but significantly higher than at 25^{o}C. Root damage was significantly higher at 15^{o}C compared to 25 and 35^{o}C at shorter microbial inoculation times. Root damage was statistically similar at 25 and 35^{o}C for all microbial inoculation times, but at 35^{o}C the proportion of root damage was much lower than at any other temperature combinations. At 24 hours inoculation time the proportion of root length damage at 25 and 35^{o}C was statistically lower than at 0.5 and 1 hour inoculation at 15^{o}C (Fig. 7.3).

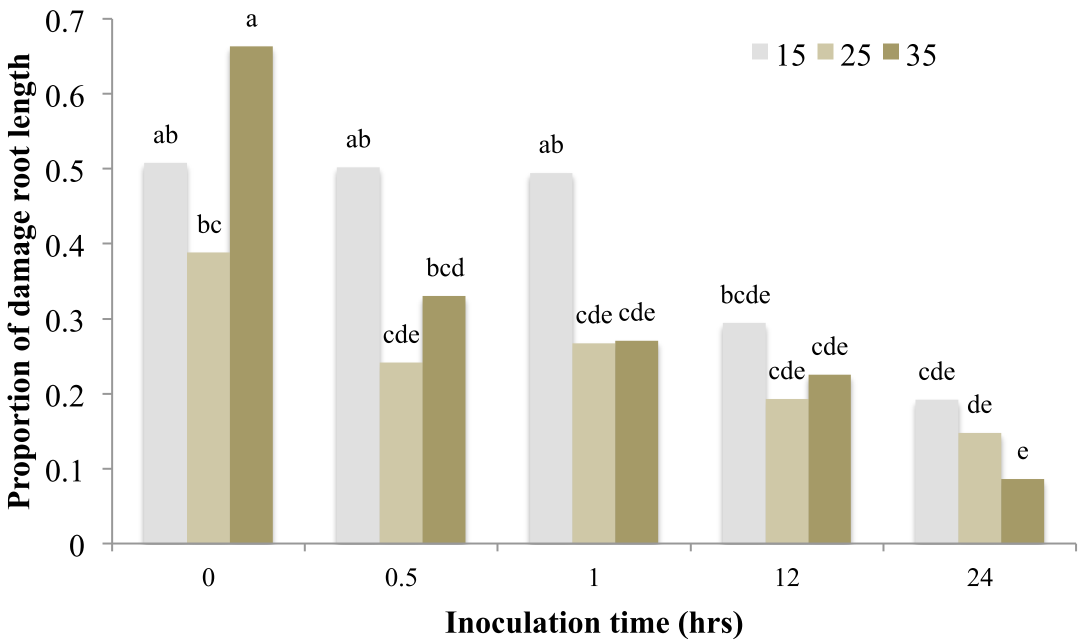

Figure 7.3 Interaction effects of microbial inoculation time and temperature on proportion of damaged root length presented as per plant. Values are means of n=3 and df=8. Means are separated by Tukey's test. The different letters on each bar are significantly different at $p < 0.05$. The numbers (15, 25 and 35) in legend represents incubation temperature (^{o}C).

Both temperature and microbial inoculation time and their interaction produced a significant effect ($p < 0.05$) on the proportion of damaged root surface area. Similar to the root length, the proportion of damaged root surface area increased with decreasing temperature (Fig. 7.4). Inoculating seedlings for longer times and incubating them at 25 and 35^{o}C produced lower root damage compared to inoculating seedlings for shorter times and lower temperature. Inoculating seedlings for a longer time (12 or 24 hours) produced significantly smaller damaged root surface area compared to the control at 15^{o}C or 0.5 hr inoculation at 15^{o}C. However at all inoculation times, the damaged root surface area did not vary statistically between 25 and 35^{o}C.

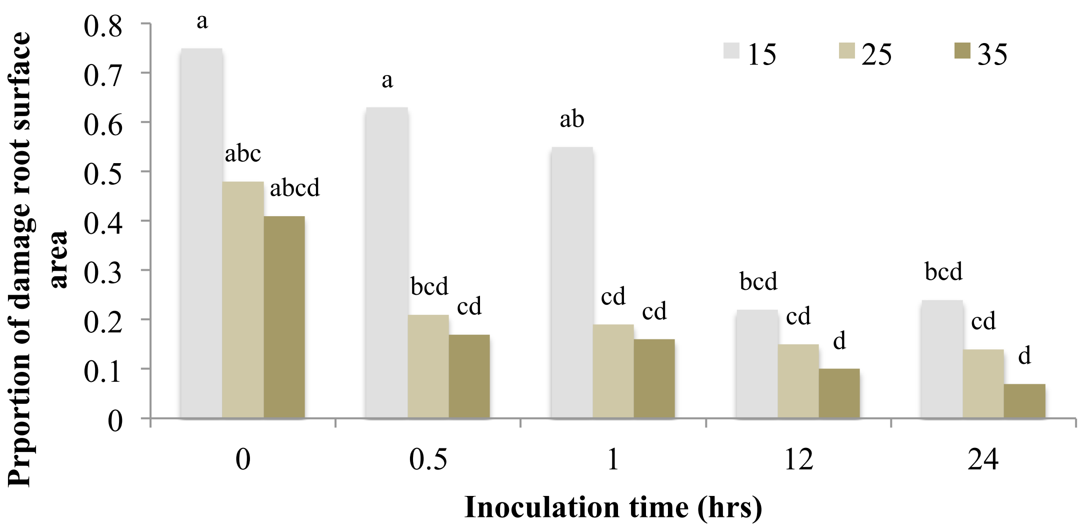

Figure 7.4 Interaction effects of microbial inoculation time and temperature on proportion of damaged root surface area presented as per plant. Values are means of n=3 and df=8. Means are separated by Tukey's test. The different letters on each bar are significantly different at $p < 0.05$. The numbers (15, 25 and 35) in legend represents incubation temperature (^{o}C).

Broadly, the proportion of healthy root surface area increased with increasing inoculation time and increasing temperature. The lowest healthy surface area was with the control without microbial inoculation. The healthy root surface area was also found to be lower at short inoculation times (0.5 and 1 hour) at 15^{o}C compared to the higher temperatures at the same inoculation time (data not presented).

7.4 Discussion

The laboratory experiment demonstrated that cane residue leachate damages the capsicum root system, and that the damage was significantly increased by increasing exposure time. The root damage caused by cane residue leachate in capsicum seedling was minimized by inoculating microbial solution. It is known that crop residues can contain allelochemicals that are inhibiting the root growth (Singh et al., 2003; Turk & Twaha, 2002). Sampietro et al. (2006) isolated phenolic compounds including ferulic, vanillic and syringic acids from cane residue leachates. While the leachate was not examined for its allelopathic effects of transplanting vegetable crops, the compounds identified in the leachate have been associated with allelopathic effects previously (Sampietro et al., 2006; Singh et al., 2003) so it is not unexpected that the cane residue would be demonstrated for the first time in this research to have allelopathic effects.

The increased root damage when seedlings were exposed to higher concentration or for longer exposure time to cane residue leachate may be due to the higher concentration of the allelochemicals present in leachate, and longer exposure of plant root to those chemicals present in the leachate. Cane residues contain organic acids and are known to affect the plant root growth negatively in both crops and weeds (Sampietro et al., 2006; Singh et al., 2003). Although this research did not investigate the role of organic acids on root damage, the root damage in cane residue leachate applied treatment is supported by the evidence that the benzoic, vanillic and ferulic acids present in the cane residue significantly increased the superoxide dismutase (SOD) and Peroxidase (POD) activities in plant root system (Yu et al., 2003), responses associated with plant cell damage. Benzoic acid treated roots also displays increased catalase (CAT) activity (Yang et al., 2006) while the activity of SOD, POD and CAT increased when tomato seedling roots was treated with benzoic acids (Zhang et al., 2010). Increased SOD and CAT activities increase the generation of active oxygen (AOS), and AOS causes the peroxidation of membrane lipids, causing membrane damage (Foyer et al., 1994).

The use of microbial inoculation of pre-germinated capsicum seedlings subsequently exposed to different concentrations of leachate showed microbes were effective in reducing the root damage caused by the leachate at low concentration. The level of root damage at higher concentration indicated microorganisms were not fully effective in protecting the root against the allelochemicals. The reduced root damage with the inoculation of microbes in cane residue leachate applied treatment could be related with the capacity of the plant tolerance to the toxic chemicals present in the leachates. Plants with beneficial microbes are known to increasing tolerance to abiotic stresses such as drought, temperature, salinity and metal toxicity as well (Dimka et al., 2009). Microbes such as *Bacillus* spp. used in this experiment is known to produce the siderophores, and the siderophores producing bacteria are capable of reducing lipid peroxidation (POD) and superoxide dismutase (SOD) activities

(Dimkpa et al., 2009a) caused by the allelochemicals present in the cane residue leachates that causes the root membrane damage.

The interaction between inoculation time and seedling exposure time to cane residue leachate shows that longer microbial inoculation time with lesser cane residue leachate exposure time produced a reduced proportion of damaged root length and root surface area compared to the low inoculation time and high exposure time. The other intermediate combination of inoculation time and leachate exposure time produced a medium proportion of damaged root length and surface area. The reduced proportion of damaged root length and root surface area may be associated with the increased microbial growth when exposed to inoculum for a longer time before exposure to the allelochemicals, allowing a thicker biofilm to form This research did not measure the biofilm thickness around the root, however, the lesser root damage with longer microbial inoculation time supports that there could have produced a thicker microbial film around the root surface so that better protected the root against the allelopathic effect of cane residue leachate. A research shows a visual demonstration of thicker biofilm cross-section of *Rhizobium leguminosarum* bv. *trifolii* strain 242 cells in a densely populated clover root cross-section compared to the low populated roots (Janczarek et al., 2015). But, little quantitative evidence has been presented in the literature on how the biofilm formation varies in the plant root with microbial inoculation over a course of time, and their efficacy in protecting the roots from toxic chemicals or pathogens. However, laboratory experiments shows that the bacterial exopolysaccharide (EPS) synthesis and biofilm formation in the inert material is correlated with the bacterial culture density, cell culture density, and culture time (Janczarek et al., 2015; Rinaudi et al, 2006).

The results from this research show that root damage by allelochemical reduced at higher temperature with microbe inoculation. Although there are little evidence in the plant root system, previous laboratory studies have shown that biofilm formation by biofilm forming bacteria in solid/inert materials in the laboratory are affected in a temperature dependant manner (Janczarek et al., 2015; Rinaudi et al., 2006). A research by Berenjian et al. (2013) shows the cell density and biofilm formation by *Bacillus subtilis* was optimum at 40^{o}C in an artificial media, and temperature above this reduces the rate of biofilm formation. The reduced biofilm formation at high temperature is associated with the reduced ability of microbes to produce extracellular polymeric substances (EPS) (Else et al., 2003), which enhance to adhare the microbes on the root surface.

On the other hand, at the lower temperature (15^{o}C), the short time microbial inoculation may have produced thinner microbial biofilm that could not sufficiently protect the root from damage. At lower temperature, the motility of the beneficial microbes present around the seeds could have reduced and could not cover the elongating radicle, thus the allelochemicals present in leachate in the seedling growing media damage larger proportion of root length and surface area. Lower temperature (15^{o}C) also reduces the bacterial-cell propogation compared to the higher temperature (37^{o}C) (Budde et al., 2006), which could have affected biofilm formation. At the same time, at higher temperature (35^{o}C), the seed inoculated with microbes quickly increase its population, covers root surface and reach maximum diameter, and therefore protect the root against allelochemicals present in the leachate. The reduced swimming and swarming motility of microbial biocontrol agent, *B. amyloliquefaciens* strain S499, at lower temperature (15^{o}C) compared to higher temperature (35^{o}C) (Puopolo et al., 2013) supports the role of inoculated microbes in protecting capsicum seedling root at variable temperatures.

The reduced root damage in microbes inoculated seedlings is also supported by the evidence that the extracellular polymeric matrix-exopolysaccharide produced by the bacterial cells in biofilm provides a diffusive barrier to toxic compounds and antibiotics (Qureshi et al., 2005; Walker et al., 2004). Also, microbes such as *Bacillus subtilis* biofilm colonies are non-wetting and repellent to commercial biocides, thus limiting the penetration of antimicrobial compounds (Epstein et al., 2011). This function of biofilm may have provided the physical barrier to the allelochemicals contacting the plant root system. Hence, microbe inoculated plants produced a lesser proportion of damaged root length and surface area compared to the uninoculated treatments when exposed to the cane trash residue.

7.5 Conclusions

This research shows that applying uncomposted organic material leachate damages the plant root system via an allelopathic effect. Application of higher amounts of organic matter in order to build soil organic carbon may therefore also increase the level of damage to the root system. This study demonstrates that inoculating plant roots with a microbial solution is beneficial in reducing the allelopathic effect of organic material residue on plant roots. This result highlights the opportunity for the farmers to use beneficial microbes to reduce the risk of root damage when planting in crops where uncomposted organic materail has been incorporated.

8 General Discussion

Vegetable production in mechanised farming systems using intensive cropping practices is often associated with several soil healths related problems. The soil health problems related to intensive cropping systems in Australia and elsewhere include increase in pathogen population and increase damage to the plant root systems (Stirling & Eden, 2008). Damaged plants root reduce the plant's capacity to support shoot development, and therefore affect the plants growth and crop yield. The plant root health problem that damages the plant root system includes root rot diseases caused by pathogenic fungi, bacteria and nematode, for example, root rot diseases caused by *Pythium* sp. *Phytophthora* sp., *Rhizoctonia* sp. and root knot disease caused by root knot nematode (*Meloidogyne* sp.), and they are the major soil borne diseases in vegetable growing regions in Australia, causing severe yield loss (DAF, 2012). The root damage by the soil borne diseases is more prevalent with the present of their wider host range among vegetable crops. These root rot problems are usually severe in soil managed with intensive cropping systems with little or no organic materials incorporated in the soil in which crop production is mainly based on inorganic fertilizer (Stirling & Eden, 2008). Thus, low or reduced soil organic carbon content, which affect a number of soil properties, is considered one of the indicators of poor soil health.

To improve soil organic carbon, soil management practices such as use of organic materials, are encouraged. Organic material applications are known to improve soil microbial activity, plant root shoot growth and crop yield (Bulluck et al., 2002; Hou et al., 2012; Marinari et al., 2006). Farmers in Australia use both composted and uncomposted organic amendments in the cereals and vegetable field with the aim to have positive impact on soil organic carbon, reducing soil compaction and improving soil aggregate. The research presented in this thesis investigated the effect of use of both composted and uncomposted organic material on soil biology (respiration), root growth and root health. To answer this question, specific research questions related to the effect of different types and rates of compost application on soil respiration, root health and root growth, effect of variable C:N ratio on root growth and soil microbial diversity, effect of fresh crop residue leachate on root growth and finally the effect of use of beneficial microbes were tested to reduce the allelopathic effect of fresh cane residue on root system development in capsicum.

Despite the wide recognition of the positive crop growth response to organic material, the results from the field experiments conducted in this research demonstrated that the addition of fresh or composted organic material to improve the soil health is not always beneficial for the vegetable root system development, shoot growth and crop yield. Incorporation of both composted and uncomposted organic materials increased soil respiration rate, but the total root length, surface are and root volume, shoot growth and fruit yield of capsicum was reduced in uncomposted cane residue applied treatment. In this research, increased microbial growth was indicated by the higher soil respiration with uncomposted cane residue providing higher organic carbon for microbial growth. Cane residue applied as an organic material has a very high carbon to nitrogen ratio (> 90). Organic residue with a C:N ratio greater than 30 can restrict the nitrogen availability to plant during early decomposition of the organic material (Hodge et al., 2000; Yu et al., 2015). Nitrogen is essential for the plant root growth, but microbes are better competitors for the available soil nitrogen compared to the plants (Hodge et al., 2000) and therefore the increased soil microbial activity in uncomposted cane residue was originally thought to have locked up available soil nitrogen, thus restricting plant growth. That means, there is need to have sufficient time given between the organic materials incorporated into the soil and before transplanting seedlings so that organic materials can decompose and stabilize the C:N ratio.

The benefits of crop residue incorporated into soil depend on the fate of how quickly it is decomposed or when the crop residue was incorporated. Plant nutrients like nitrate nitrogen from the incorporated crop residue is only available when crop residues are decomposed. This means timing of organic residue incorporation is important, allowing sufficient decomposition time for incorporated residue. But, the decomposition of crop residue and mineralization of nitrogen varied with the crop residue types (Pare et al., 2000). For example, the nitrogen mineralization from maize residue was smaller than from the alfalfa residue. In the same experiment, after 140 days of incubation period the total nitrogen derived was much smaller for maize residue compared to the lower C:N ratio alalfa residue (5.6 vs 30.6 $mgkg^{-1}$ soil). The difference in derived nitrogen was due to the difference in decomposition rate which is determined by the composition of crop residue e.g. crop residue with higher C:N ratio, high in cellulose, hemicellulose and lesser soluble fibre releases less nitrogen compared to the alalfa residue (Pare et al., 2000). Net nitrogen mineralization from the crop residue negatively correlates with C:N ratio and lignin content, thus *Zea mays* incorporated soil is always in negative nitrogen balance and *Glycine max* incorporated soil is in positive nitrogen balance (Abbasi et al., 2015).

The cane residue used in the field and pot experiment in this research also had very high C:N ratio and very high proportion of lignin and cellulose component (Fortes et al., 2013; Oliveira et al., 2001) that caused slow in mineralization process, but continuously provide the carbon source to the soil microbes to increase in their growth and activity, measured in term of soil respiration. While the compost used in field had lower in C:N ratio (< 30) and easily decomposed so that plant easily access the available mineralized nitrogen. For the crop residue, the time that crop residue incorporated to soil in the field experiment and time taken for the decomposition become crucial. The crop residue having very high C:N ratio and lignin content, the decomposition rate is very slow, for example the winter wheat stubbles has 113 and 231 initial C:N ratio and lignin content (g kg^{-1} dry matter), respectively, takes 14.5 months to get down to 20 C:N ratio during the decomposition (Kariauciuniene et al., 2012). Cane residue also has longer (about 12 months) decomposition time in Queensland weather condition, during which nitrogen loss from the residue is very negligible (Robertson & Thorburn, 2007a), thus crops may not benefit from its use. This shows, cane residue incorporated farmers need to keep their land fallow for considerable time to get benefit out of cane residue decomposition.

Farmers are always interested to keep minimum gap between the crops in order to maximize the benefit. Soil amended with crop residue with high C:N ratio such as straw increase nutrient mineralization with the increase in nitrogen application rate (Henriksen & Breland, 1999). In the field experiment, nitrogen fertilizer was applied at field recommendation rate, but the cane residue was applied at very high rate. Thus, it is advised to add nitrogen externally to access the mineralized nitrogen from high C:N ratio materials. The shorter root length and smaller surface area and volume in cane residue incorporated treatment might be associated with smaller amount of external nitrogen. Adding high rate nitrogen application may provide sufficient nitrogen for crop growth, but that may create an issue related to soil and water pollution. Beside high C:N ratio of the cane residue, some other mechanism such as allelopathic effect of toxic chemicals released from the cane residue during the long time decomposition process could be the problem for crop growth causing necrotic root tip and reduced root growth as presented in chapter 6 and 7.

Common agriculture practices in the cane-growing region in Australia is that after couple of years of growing sugarcane, vegetable or small fruit crops such as watermelon are grown as complementary crops or in rotation. Also in the recent years, the sugarcane cultivation area is decreasing and sugarcane-growing area is turning in to fruit and vegetable farms (ABS, 2016). After several years of sugarcane cultivation, large quantity of cane residue is built in the sugarcane grown field. While the use of uncomposted organic material such as cane residue in a sugarcane growing region such as Bundaberg provides a good source of organic materials to improve soil organic carbon levels in the long-term, but in the short-term cane residue provides the food and energy to the soil microbes for its growth and immobilize the available soil nitrogen.

The effect of using uncomposted cane residue was clearly seen in this research in the field experiment when large amount of cane residue was incorporated in the soil just few months before capsicum transplanting, producing shorter root system and lower fruit yield compared to the use of composted cane residue or no use of organic materials. Thus, the practice of using the uncomposted fresh crop residue incorporation must be managed carefully to avoid a negative impact on vegetable and fruit crops grown in rotation or as complementary crop production with sugarcane. Incorporating the uncomposed cane residue in soil and allowing it to decompose completely in the field takes more than a year in ropical Queensland (Robertson & Thorburn, 2007a). The incorporation of late harvested cane residue also remains C:N ratio more than 40 for significant time (> 9 months) of decomposition period (Robertson & Thorburn, 2007a), which is not beneficial for crop growth. Thus, to get benefit out of composted cane residue land should keep idle for long time period if farmers want to use cane field to vegetable production or to use cane residue as organic materials, but represents an opportunity cost for the farmers who may desire to utilise the field for crop production for most of the year. To solve this problem adding external nitrogen in high does could be a solution, but higher rate of nitrogen application may cause soil and water pollution.

Use of composted organic material at a high rate was also shown to not be beneficial for plant root system development and crop growth, opposed result that conflicts with other finding that suggest increasing compost application rate improves crop growth and yield (Barzegar et al., 2002). The highest rate of compost application used in this research increased the soil respiration rate showing the highest biological activity in soil, which has been used as an indicator of healthy soil (Araújo et al., 2009; Pankhurst et al., 1995), but root development and crop growth was reduced.

Organic materials addition are described as beneficial in reducing the soil borne diseases in crops like snap bean and cucumber (Sabet et al., 2013; Stone et al., 2003), in contrast this research shows the higher percentage of wilted plant and higher proportion of root damage in the high rate of compost applied treatment. Those results indicate that only soil respiration cannot be used as sole indicator of soil health. The higher root damage in high rate of compost application indicates that detrimental microbial effects on the root system, potentially linked to a higher rate of nitrogen availability, might have occurred. In the field, soil with very high rate of compost applied treatment shows increase in nitrogen availability. Examples in cereal and vegetable crops shows that increase in nitrogen availability in soil are detrimental with the increased severity of root rot diseases e.g. fusarium root rot in maize (Abiodun et al., 2015), corky root rot disease in tomato (Workneh et al., 1993). The disease severity index of *Fusarium* is linearly associated with the nitrogen application rate (Abiodun et al., 2015). Although the field research in chilli with different rate of compost did not identify which pathogen was causing the root damage, the increased nitrogen availability in the soil system with the increase root damage in the plant root shows that the root damage is nitrogen-induced response. This might be one reason that highest rate of compost applied field in Austchilli produce more wilted plants with higher proportion of root damage compared to medium rate of compost application.

Pathogens were considered unlikely to be responsible for all of the detrimental effects associated with composted organic matter additions to the soil. At high compost application rates, with no evidence of disease in a field research in 2014 chilli field the number of first order lateral roots in plants was also reduced along with the total root length. In 2014, soil nitrogen was not analysed, but 2013 field experiment shows that very high rate of compost releases higher nitrogen in soil. It is concluded that the reduced root system development at high rates of compost application was due to an increase in soil nitrate availability. As the compost applied in the field was thoroughly mixed with the rotary hoe tractor before transplanting chilli seedlings, the mineralized nitrogen might have homogenously available in the root zone soil profile. The negative effect of high rate of nitrogen application on root development are suggested in cereal crops, for example, total root length in maize (*Zea mays*) was reduced both at early growth stage and the rapid growth stages at very high rate of nitrogen application (175 kg ha^{-1}) compared to the lower application rate (60 kg ha^{-1}), which in turn affected the crop yield (Shen et al., 2012). Very high dose of nitrogen application (200 and 400 kg ha^{-1}) also reduced the root elongation and number of root in maize (Wang et al., 2008). Available soil nitrogen can produce a localized or systemic effect on lateral root initiation. Localized zones of high nitrogen availability in soil promotes proliferation of lateral root initiation in plants while homogenously mixed nitrogen in soil media retards lateral root initiation (Zhang et al., 2007). The reduced lateral number in homogenously mixed high compost application rate in 2014 field experiment results shows that an optimum compost application rate is required in order to improve the benefits of increasing soil organic carbon without decreasing lateral root growth and crop yield. Thus, identifying the optimum rate of compost application is required to improve root health and to increase the root growth.

From the above experiments and the evidences, it is clear that two different mechanisms controls the root development in the crops; i) at nitrogen non-limiting condition such as use of very high rate of compost in capsicum and chilli crops, the excess nitrogen available through mineralization reduces the elongation and proliferation of root in vegetable crops ii) at the nitrogen limiting condition e.g. high rate of uncomposted cane residue applied in the field or in the pot experiment, the increased microbial activity caused the nitrogen lock-up and nitrogen become unavailable to the plants, which in turn reduced the root growth and plant performances.

Beside the nutrient lock-up by microbes, the allelopathic effect of uncomposted cane residue also works in the same condition when uncomposted cane residue was applied at higher rate. Uncomposted organic material was found to not only affect crop growth in the field but also reduced crop seed germination. Higher application rates of powdered, unleached cane residue in pot experiments significantly decreased capsicum seed germination. This experiment identifies that the cane residue used as organic matter at very high rate inhibit the crop seed germination. The allelopathic effect of cane residue on capsicum seed germination and growth of transplanted capsicum in the field condition is the first experiment of its kind.

The allelopathic effect of crop residue on seed germination and crop growth has influential role in the areas where organic matter such as cane residue is incorporated into the soil. This research used capsicum as model crop, but the effect could be similar in other vegetable crops. In the sugarcane growing region cane residue are incorporated into soil before vegetable or fruit seedling transplanting. Visual evidences in field in Bundaberg have been seen that crop such as watermelon (*Citrullus lanatus*) and pumpkin (*Cucurbita pepo*) growth are negatively affected when grown after cane residue incorporation, with no evidences of disease or nutrient deficiency (personal communication with extension worker), which might be the effect of toxic chemicals present in the cane residue that affected the root growth. Laboratory results from this research with similar seed germination and root growth in capsicum and cabbage at 100% leachate concentration and 100% sterile solution supports that the detrimental effect of leachate is chemical not biological. Evidences collected elsewhere are there that crop residue leachates inhibit crop seed germination, root and shoot growth (Singh et al., 2003; Viator et al., 2006). Researches have demonstrated that organic matter leachate contains water-soluble allelochemicals (Sampietro et al., 2006; Singh et al., 2003). Although allelopathy is a useful mechanism in controlling weeds in crops, it has the potential to also decrease crop seed germination and that affects the crop stand population, and thus ultimately affects the crop yield. Allelopathic effects are either autotoxic, affecting the growth of same plant species, or heterotoxic as is the case for the response noted in affecting the cane residue on capsicum seed germination and root growth of different species (Viator et al., 2006).

Organic material leachates reduced seed germination rate, root and shoot growth. Organic material leachates are known to have different water-soluble toxic chemicals including phenolic and benzoic acids and their derivatives (Sampietro et al., 2006; Singh et al., 2003). Phenolic compounds and their derivatives have been shown to negatively affect plant physiology for instance photosynthesis and transpiration (Yu et al., 2003). However the effect of organic material leachates on seed germination and crop growth varies with the plant parts and leachate concentration (Turk & Tawaha 2002, Turk et al., 2005; Viator et al., 2006). The experimental evidence in this research clearly shows that the detrimental effects of uncomposted organic material on crop growth occurred by other mechanisms other than a high C:N ratio, with allelochemicals likely to be having the greatest effect in the field.

The use of leachates from uncomposted cane residue containing water-soluble allelochemicals into the soil has previously been shown to have an inhibitory effect on lettuce, radish, sorghum, wheat and lentil (Sampietro et al., 2006; Singh et al., 2003). In the laboratory research, cane residue, sorghum and lucerne residue also had an inhibiting effect on capsicum and cabbage seed germination and root growth. Under field conditions in this research, transplanted capsicum plants grown in cane residue incorporated soil showed significantly reduced the root and shoot growth. The negative effect of uncomposted crop residue could have significant implication in field condition in Bundaberg, Queensland, as well as other tropical and subtropical environment where sorghum and lucerne are often used as cover crops in the summer season to build soil organic carbon content as well as protect soil from erosion. Allowing cover crop residues to decompose in the soil after incorporation or choosing proper cover crops with lower allelopathic effect on crops can be utilized as current management strategy to reduce the allelopathic effect of cover crops on crop seed germination and seedling transplant growth. The effect of toxic compounds present in the uncomposted organic material reduces during decomposition (Rajbanshi & Inubushi, 1998; Tiquia & Tam, 1998), but using fresh organic material like cane residue in the cropping system requires the farmer to keep the land idle may be up to 12 months for sufficient decomposition (> 80%) (Robertson & Thorburn, 2007a), and therefore an alternative solution to reduce the negative effect of toxic allelochemicals present in the organic material is required.

This research demonstrates a novel approach to reduce the allelopathic effect of organic material on crop seedling emergence in pot experiments, and crop growth and root system development of transplanted vegetable under field conditions by inoculation of seeds or seedling root systems with beneficial microbes. This procedure increased the seedling emergence percentage and rate, and plant growth and root development in the pot and in the field, respectively. Inocluation also reduces the time needed between incorporation of uncomposted organic material. Researchers have shown the benefits of using beneficial microbes in crop growth through nutrient supply, by hormonal influence, reducing the soil pathogen population or reducing the plant disease incidence or increasing tolerance environmental factors and other effect, but there has not been seen any evidence of using microbes to reduce the allelopathic effect of crop residue and improving the crop growth in transplanted vegetable crops. This research is the first of this kind that demonstrates the benefit of beneficial microbes in reducing the allelopathic effect of cane residue in the fresh cane residue incorporated vegetable field. This claim is supported with the result of increased crop capsicum seed emergence, increased root growth in transplanted capsicum seedling and reduced root damage by using microbes in cane residue leachate or cane residue incorporated treatments in this research.

The increased root development in the microbial inoculation treatment was the result of beneficial microbes protecting the seedling root system from the possible damage caused by the allelochemicals. Microbes when inoculated to the seeds or seedlings form bacterial communities encased in an extracellular polymeric matrix known as biofilm around the root surface (O'Toole et al., 2000). Biofilm formation around the root surface by the colonization of *Bacillus subtilis* an isolate from the natural environments, protect the tomato plant against wilting problem caused by the pathogen *Rolstonia solanacearum* (Chen et al., 2013). Several strain of *Bacillus subtilis* are capable of forming biofilm around the wheat root surface that protected the root against the root rot problem caused by *Fusarium culmorum* in a greenhouse experiment (Khezri et al., 2011). Khezri et al. (2011) suggested that the biofilm formation and antagonistic ability of *B. subtilis* are significantly and positively correlated. Biofilm, besides protecting crop against diseases, are also effective to reduce the negative effect of different types of environmental stress (Bais et al., 2004; Qurashi & Sabri, 2012).

The severity of root damage caused by the cane residue leachate on the capsicum seedling root system increased with decreasing microbial exposure time, as presented in the laboratory experiment in chapter 7. This experiment demonstrates the effectiveness of microbes in reducing the vegetable root damage caused by the leachate released from the organic material. Although in this research, biofilm formation and its thickness were not measured, one possible explanation would be that the biofilm formation provides physical barrier against leachate. Microbes when inoculated proliferate and form biofilm on the root surface. Evidence shows that microbes proliferate with time, and biofilm is formed (Janczarek et al., 2015), but they are mainly demonstrated in the inert materials or glass rod in the lab. This PhD research experiment showed that microbes protect the root from the allelochemicals potentially by forming biofilm of differential thickness on the root surface, which depends on the microbial exposure time in leachate applied treatments. Seedlings inoculated for longer time (24 hrs) provides less damaged root in terms of their length and surface area compared to the short time exposure (0.5 hr). The short time exposure to microbes produced similar root damage to the no use of microbes in cane residue leachate applied treatment, which indicates there is need of minimum exposure time for the microbes to be used as a plant protection measure. The longer the inoculation times provided, the more microbes proliferate and potentially provided a thicker biofilm, as suggested in a laboratory experiment in the glass surface (Janczarek et al., 2015).

In this research, capsicum seeds or seedlings dipping in microbial solution or adding microbial solution in the planting hole at the time of seedling transplanting might have provided opportunity to cover the seeds or root by microbes, which form biofilm around the root system. Root protection by the inoculation of microbes in leachate or freshly applied cane residue would be stronger with thicker biofilm formation. Thus a thicker biofilm around the capsicum root system would be expected to protect the root against the toxic chemicals present in the leachate solution better than a thinner biofilm. However, the root protection by microbes in cane residue leachate applied treatments shows temperature dependant response. Less damage root length and surface area were found in microbes inoculated capsicum root at higher temperature (25 or 35^{o}C) than at lower temperature (15^{o}C) in cane leachate applied treatments. These results suggest that the use of microbes as inoculants is not effective at the colder soil temperature area. As the root damage was lesser at 25^{o}C temperature, which is similar to the soil temperature in vegetable growing region in the arid and semi-arid region of the world. Further, in the high soil temperature zone microbes also increase tolerance against other abiotic factors such as heat and drought stresses (Dimkpa et al., 2009a). The result from this research shows that farmers in arid and semi-arid area can make use of microbes at the time of seeding or transplanting more effectively to protect plant root from the allelopathic effect of uncomposted crop residue as well as stresses from other abiotic factors. This benefit of microbes is associated with the increase in microbial proliferation with the increase in temperature and inoculation time. The longer inoculation time at relatively higher temperature reduced the root damage occurring after subsequent exposure to organic residue leachate. This result is supported with biofilm development around the root being faster at higher temperature than at lower temperature (Puopolo et al., 2013).

The better germination and root growth in compost leachate applied treatment in laboratory, and better root growth in microbes applied treatments in cane residue applied treatment in field suggest that the biofilm formation as one of the mechanisms, but this research did not explore the biofilm formation around the capsicum root. As equal to the physical barrier mechanism of the biofilm, the detoxification of allelochemicals present in cane residue would be another mechanism. The microbes used in this experiment also support the decomposition of crop residue and organic waste, such as fruit waste, vegetable waste, leaves, hay, wheat straw and rice husk were decomposed by using *Bacillus subtilis*, *Pseudomonas* spp. and their consortium (Pan et al., 2012). Bacteria like *Bacillus*, *Pseudomonas* and *Serratia* app are also known having cellulolytic activity in the presence of crop residue (Hameda et al., 2006; Singh et al., 2010), which support the degredation of crop residue that are rich in cellulose content. *Pseudomonas* and *Serratia* spp. are also effective in lignolytic activities, which disintegrate the lignin rich compounds (Kaplan et al., 1980; Perestelo et al., 1994). For example, the use of *Bacillus* spp. decompose corn residue (*Zea mays*) much quicker and produce better growth of *Brassica rapa* plant compared to the plants grown just with corn residue without *Bacillus* sp. (Li et al., 2013). When crop residue are decomposed, the effect of toxic chemicals present in the uncomposted crop residue are reduced (Bonanomi et al., 2011), thus not affecting the crop seed germination and root growth and justify the benefits of similar root development in compost leachate as in control in the laboratory experiment for capsicum, barley and cabbage.

Further, the beneficial microbes used in this research might benefit the capsicum seedling in cane residue leachate applied treatments and crop growth in field with cane residue incorporated soil by detoxifying the toxic chemicals released from the residue. Several evidences show that microbes are beneficial in detoxifying the toxic chemicals and complex hydrocarbons present in soil (Bento et al., 2003; Ghazali et al., 2004; Saranraj & Stella, 2012), thus, the inoculation of vegetable crop seedlings and use of microbial solution in the transplanting hole would benefit the crop growth. Makoi & Ndakidemi (2012) review presents that crop residue such as cane, sorghum, alalfa and other cereal crops contains the allelopathic chemicals, e.g.vanillic, ferulic, cinnamic, coumaric, propionic, gallic, benzoic and hydroxybenzoic acids, hydroxamic acid etc. These chemicals are known to inhibit plant root growth, and plant physiology such as photosynthesis and stomata conductance (Yu et al., 2003). *Bacillus* spp. are effective in degradation of cinnamic, coumaric, propionic, ferulic, benzoic and hydroxybenzoic acids (Peng et al., 2003) and *Pseudomonas fluorescens*, also shows prompt degradation of ferulic and coumaric acids (Ruzzi et al., 1997), thus plants get benefit by using microbial solution while transplanting seedlings.

In summary, this research provided insights into how the different types and rates of organic matter incorporated into soils to improve vegetable root system development, using capsicum and chilli as model crops. This research also demonstrated that the detrimental effect of uncomposted organic material application could be biologically reduced. Increased organic material application rates, which is considered beneficial for the crop growth, in contrast to wider belief may not produce better root development and higher crop performance.

The detrimental effects of higher application rates of organic material could be due to:

i) uncomposted organic material increases soil microbial activity that competes with the root system for soil nitrogen and may create nutrient starvation for the crop,
ii) composted organic material increases the microbial activity, and increases nitrate availability to the soil system, generating an environment supportive of pathogen induced root damage,
iii) very high rate of composted organic material application supress the lateral root branching and thus smaller surface area and root volume and, shorter total root length.
iv) seed germination and root growth are inhibited by allelochemicals released from the freshly applied organic material.

The allelopathic effect of organic material on crop seed germination and emergence can be reduced by using beneficial microbes. Dipping vegetable seedlings in microbial solution and pouring the microbial solution in the hole before transplanting seedling reduce the negative effect of freshly incorporated crop residue and improves crop growth and yield. The efficiency of microbes in reducing allelopathic effect of crop residue leachate on seedling root damage is affected by the environmental factors such as temperature as well as the exposure time.

This research presents several possible future research directions including more detailed assessment of the effect of C:N ratio on root development focussing on microbial composition relationships, the effects of different commercial microbial inoculations on root damage as a crop management practice, quantifying the biofilm formation process under different soil environments and further research in effectiveness of different microbes in degrading the allelochemicals released from the fresh uncomposted crop residue.

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