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Project leader:	Dr Mark Pawlett
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This report comprises of a student MSc thesis investigation conducted over a five month period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations. Names of the products tested have been removed to protect commercial sensitivity.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

There were no positive short term impacts on soil biological and chemical characteristics of adding organic amendments to an organically-grown sweet pepper crop.

Background

This project was undertaken by an MSc thesis student at Cranfield University in collaboration with a UK commercial sweet pepper grower. The student was supervised by Dr. Mark Pawlett and co-supervised by Professor Karl Ritz. The project evaluated the ability of two proprietary products and a nursery-brewed aerated compost tea to alter soil microbial characteristics with a view to optimising soil health for sustainable soil management practices. An MSc student was selected to perform the supervisor-led research for a dissertation which included a literature review, an experimental trial, and formulating conclusions as to the ability of the treatments to influence soil chemical and biological characteristics.

The project objectives were:

- Perform a search of the scientific literature, focusing on the manipulation of microbial communities to improve both soil and hydroponic crops including salads.
- Establish an experimental trial to investigate the abilities of the selected products (stated below) to alter i) microbial biomass ii) phenotypic and iii) functional characteristics of the soil microbial community
- Investigate the effects of the products on soil chemical characteristics such as pH, organic matter, and nutrients
- Compare results obtained by the Cranfield student to those of the Soil Foodweb laboratories
- Evaluate and compare the results to determine the effectiveness of the individual products for manipulation soil microbial communities.

Both organic and conventional growers are becoming increasingly aware that soil and microbial processes and health are critical for nutrient cycling, and that a biologically diverse rooting medium may reduce disease occurrence. Management practices that maintain microbial health may thus improve crop yields. As such growers are increasingly attempting to manipulate the soil or substrate microbial community to optimise production. Along-side commercial formulations sold to alter soil microbial communities, some growers have opted

to produce their own products, often known as “compost teas”. There is, however, very little empirical evidence regarding the true benefit of these products. This is unsurprising as there is currently very little mechanistic information in peer reviewed journals. Thereby our intention was to evaluate a selection of products and their abilities of manipulating soil microbiology under a selection of cultivation techniques. The current research focused on soil with sweet peppers as the crop, however it is envisaged that results may open up opportunities to investigate the abilities of the products to maximise yields of many other soil- and substrate-grown crops.

It is intended that the information will benefit the industry by providing the grower with scientifically rigorous information regarding the usefulness of various products that are used to influence soil microbial populations to increase crop productivity. It is envisaged that the information will assist the grower in deciding which products are likely to be the most beneficial to improve yields.

Summary

The global increase of organically-managed horticultural soil has increased the use of organic amendments and proprietary products in such systems. The intention is to effect nutrient cycling and hence increase crop yields while improving soil health. In this research, it was hypothesised that such organic amendments impact on soil microbiological properties, such that they are able to, at least in part, replace the need for inorganic fertilisers. Experimental trials were established in glasshouses (Taunton, UK) with monoculture sweet-peppers (*Capsicum annuum*), grown in either a bark- or compost-amended soil. The intention was to determine the effects of adding either aerated compost tea, or two commercial products, viz. a rhizosphere inoculant referred to as ‘Rhizosphere inoculant’[*], or a nutrient supplement referred to as ‘Nutrient supplement’[*], compared to an unamended control. Effects on chemical (nutrient content and pH) and microbiological phenotypic structure (via the measurement of the associated soil microbial community phospholipid fatty acid (PLFA) structure), and basal and substrate (glucose) induced respiration were monitored over time.

None of the products tested resulted in statistically significant impacts on either chemical or microbiological parameters, in either the compost or bark media. However, significant variation in the properties occurred over time, whereby total C significantly decreased (irrespective of application of the test products), in both soil matrices. Total N and pH significantly decreased over time (irrespective of application of the test products) in the

[*] Trade names of the products are anonymous to protect commercial sensitivity

compost matrix. Microbiological parameters, including phenotypic (PLFA) profiles and respiration rates, also varied significantly over time. Thereby evidence that the products impacted upon soil chemical or biological properties in any functional sense is minimal. Impacts upon crop performance or disease suppression were not directly assessed in this component of the study. In conclusion, there was no evidence that the products stimulated microbial activity or nutrient cycling within the current research context, notably where soils had received high organic matter inputs.

Financial Benefits

Approximate costs of the products used in this work for a soil grown organic pepper crop:

Rhizosphere inoculant: £2100/ha per season*

Nutrient supplement: £1400/ha per season (materials) + £1500/ha per season (labour)

Compost tea: £200 one-off construction materials + approx. 2 hours labour per 'brew' (ingredient costs are negligible per brew)

*For this research labour costs of applying product were negligible as it was included in the fertigation programme.

No impact was recorded on the parameters measured, so no financial benefit can be established. Further investigations would be required to investigate effects on disease occurrence and yields, and potential long-term impact.

Action Points

- Growers should be aware that application of proprietary products to manipulate the soil microbial community for the benefit of the crop may not show an impact on the soil chemical and biological characteristics in the short term.
- For growers who already incorporate large amounts of organic matter into their soil, inoculation with proprietary mixes of microorganisms may be of limited value.
- With application costs of up to approximately £3000/ha, growers should attempt to quantify the benefits of any application, ideally by taking measurements from a well designed commercial trial over several seasons.

SCIENCE SECTION

Introduction

This research represented herein was written and submitted by Marketa Hermova in accordance with the MSc thesis requirements of Cranfield University. An extended literature review is presented, following which there is a shorter introduction written to be ready for a scientific journal. The target journal for this thesis was Biology and Fertility of Soils (BFS), and as such the student formatted in accordance with BFS requirements.

Extended Literature Review

The importance of soil and soil management as a fundamental basis for human subsistence is well established. Simultaneously, to reach the best soil quality for the range of crops grown across the world remains an issue. To affect such a heterogeneous system such as soil requires considerable focus on the physical, chemical and biological level. Factors which contribute to effective soil fertility, high crop yields and soil health are as diverse as the soil itself (Mader et al., 2002). Soil biota belongs to these factors and despite the biomass comprising a small proportion of the total mass of soil (DEFRA, 2010), organisms have direct impact on physical and chemical soil properties including soil structure, C and nutrient cycling and food web interactions (Barrios, 2007). Through manipulation of the biological processes, soil biota is able to improve soil conditions and shift horticulture practices towards more sustainable modes (Swift et al., 2004). Recently, when more than 24 million hectares of arable land on the world was placed under organic management (Cong Tu et al., 2006), the role of organic amendments received great attention. In comparison with conventional systems, well-established organic systems have shown low incidence and severity of plant disease caused by soilborne pathogens (van Bruggena and Termorshuizen, 2003; Tu et al., 2006).

Soil chemical properties: In many studies soil type is considered as the primary factor which affects the development of soil microbial communities (Buyer et al., 1999; Girvan et al., 2003). Variable soil matrixes have different ability to hold nutrients and make them available for microbes. Generally, soil condition could be improved by addition of many types of fertilizers or plant based products. These additional products may manipulate soil microbial structure which requires C, O, H, N, S and P as the key components of carbohydrates, lipids, proteins and nucleic acid in their cells. Well-balanced and adequate concentration of these compounds support microbial growth and development. Organic amendments can partially cover energy demand of microbes and are due to become the key drivers of their activity

(Larkin, 2008). Organic matter provides additional microbiology to the soil, and thereby improved nutrient cycling and nutrient availability which may thus act as a substitute for chemical fertilisers. Different type of amendments provides different potential to stimulate the growth of microbes measurable through their respiration and biomass. Predominantly, variable content of nutrients, vitamins and acids are factors influencing the development of a functional microbial community. Chemical properties including pH, O_2 and CO_2 concentration may be also considered as the factors affecting abundance and activity of microorganisms.

Nutrient content: Nutrients are not uniformly dispersed in a soil, but tend to cumulate in the hotspots such as plant rhizosphere, spermosphere or surroundings of plant debris. Microorganisms of various ecological groups with different requirements for nutrient supply are cumulated in these hotspots. Microbial communities need the nutrients for their metabolic processes and their requirements are the key drivers of nutrient cycling involving the capture, storage and release of energy in a system (Defra, 2010). Biological N-fixation is the process by which the microorganisms increase the nitrogen content in the soil. Simultaneously, the microbial population regulates P availability through transformation of P to the PO_4 form available for plant uptake. Furthermore, microbial populations represent a large pool of nutrients in an organic form and their contribution would be important in natural systems. However, the amount of nutrient stored by soil microorganisms is minor in comparison to the massive inputs of nutrients including nitrogen and phosphorus required by cultivated soils. Both of these key macronutrients are applied to crops in both an organic and an inorganic form. In the case of nitrogen, nominally appropriate amounts are applied in an inorganic form as ammonium or nitrate. Organic forms include urea, manures, slurries and industrial waste include sewage sludge and compost. All products derive N for crop to promote the growth. Phosphorus is added to the cultivated soil mainly as the inorganic apatite. Both nutrients are crucial to control soil fertility and to balance them in appropriate rates, fertiliser and organic amendments are added to the cultivated soils and soil matrices.

pH: Different physico-chemical characteristics of individual soil types include soil structure, moisture content, concentration of O_2 and CO_2 affect the final pH. pH value is the primary chemical factor influencing the nutrient form present in a soil as well as distribution of the nutrients in the system. The distribution of microorganisms also varies in a soil according to their pH optima preference (Kilham, 1985). Prokaryotes prefer the optimal pH in a range of 5.0-5.5, however majority of fungi prefer surroundings with lower pH in the range about 4.0-6.0 in contrary with bacteria which predominantly grow in soils with pH about 5.0-6.5 (Prescott et al., 2001). For example, Lim and Kim, 2010 reported in their research that the

optimal pH for *Bacillus spp*. is 6.8. Cultivated soil tends to have lower pH which naturally supports fungi as the community which prefers these slightly acidic soils.

Variable functional group of microorganisms: Microbial communities include main groups of bacteria, protozoa, actinomycetes and fungi acting as pathogens, disease antagonists, microsymbionts, N-fixers and soil decomposers and transformers in a soil. The function of microorganisms would be described through their abundance, diversity and activity. Suitable characteristics, such as microbial biomass or respiration in the soil would be used to indicate the presence/absence of particular communities across a soil profile. Simultaneously, these characteristics would be used to assess the soil condition and the changes in the soil over time. However, majority of these organisms are in a dormant state waiting for suitable conditions (Stenstrom et al., 2001) and in that case the biomass or total number of microorganisms represented the abundance are not necessarily appropriate characteristics for evaluation of soil condition. Therefore, the role of soil biota in soil processes could be expressed rather by their function than species composition.

Pathogens: Stability of each system would be defined in relationship to resistance or resilience (Brussaard et al., 2007), where resistance is the ability to recover from stress whilst resilience could be defined as the rate with which populations renew from disturbance (Griffiths et al., 2000, Tabor-Kaplon et al., 2005). Stability of the system relates directly to the ability to supress disease occurrence and unsuitable conditions in general. The resistance and resilience is all the more important in case of cultivated soils with plant monoculture where the natural distribution of various plant species in different ages is limited, striking power of such ecosystem is broken and potential inherent regulatory mechanism of pests and diseases is reduced (Brussaard et al, 2007).

Recent frequently occurring pathogens include *Aspergillus* spp., *Pythium* spp., *Phoma* spp. or *Fusarium* spp. The appropriate conditions required by these organisms include warmer and wetter environment. Unfortunately, these conditions are often prevailing in horticulture, where the crop has the same demands and soil and soil matrices are cultivated according to these needs. Temperature was considered as the crucial factor affecting the presence of pathogens in a soil by Manici and Cerato (1994) who showed the influence of temperature on *Fusarium oxysporum*. The control of root- inhabiting pathogens that survive saprophytically in soil organic matter and are present in soil in the absence of a host plant (Lampkin, 1990) keep the attention of growers who lose considerable proportion of their yield due to pathogens presence.

Disease antagonists: Soil is an important reservoir for long-term survival of pathogens (Fuxa 1995). Soil-borne plant health problems caused by soil microorganisms such as plant-parasitic nematodes, root-rot fungi and insect larval forms can result in huge crop losses in horticulture. In contrast, other soil communities control and suppress soil diseases through the processes such as competition, predation and parasitism (Susilo et al., 2004). These processes are driven by variability of food web and therefore the competition for energy source is considered as key instrument for antagonist activity and their ability to suppress the soil pathogens. Microbial antagonists provide the possibility to naturally control pest and such characteristics would be beneficial for organically managed soil where other control options are restricted. Therefore, the assessment of microbial community structure performs an important tool to predict the ability of microbial communities to suppress soil disease.

Potential disease suppressive microbial antagonists in soils include fungi, such as *Trichoderma spp., Glomus spp.,* or bacteria such as *Pseudomonas spp*. (Hoitink, 2001; Stone et al., 2004), *Bacillus spp*. (Lim and Kim, 2009) and *Streptomyces (*Lenc et al., 2011). As antifungal antagonists these microorganisms induce plant resistance (Kloepper et al., 2004), produce antibiotics (Lenc et al., 2011) and volatile organic compounds (Leelasuphakul et al., 2007) which reduce plant pathogens*. Bacillus* and *Pseudomonas* belong to well-known rhizobacteria which could stimulate plant growth (Lim and Kim, 2010) by hormone synthesis (Gravel et al., 2007) and enhancement of nutrient availability. The *Streptomyces* spp. bacterium is one of the prolific producers of a broad range of antibiotics which could be possibly used to control disease occurrence.

One of the key factors for the pathogen suppression is an independency on surrounding conditions. Berg and Smalla, 2009 showed that microbial communities have a certain degree of plant specificity regarding the plant species and cultivars. However, *Bacillus ssp*. is strongly tolerant to external environmental changes (Chung et al., 2008) as well as *Pseudomonas spp. (*Fürnkranz et al., 2011) and *Trichoderma* (Cabello and Arambarri, 2002). Positive suppressive effect of all mentioned antagonists has been documented for diseases of wheat, sugar-cane, tobacco, citrus fruit (Andersen et al., 2003; Leelasuphakul et al., 2007) and tomato (Workneh et al, 1993).

Another important factor influencing the ability of beneficial microorganism to suppress soil pathogens is time. Renault et al., 2011 reported that disease antagonists achieve the top of their efficiency within 6 months and thereafter the efficiency start decreasing. Research showed that after 9 months, the efficiency was on the same level as in those soils without inoculation.

Microsymbionts: Plant surface and interiors are important habitats for microorganisms and some of them are able to grow only in association with plants (Dandurand and Knudsen, 1997). Such microsymbionts prefer surroundings of plant roots (rhizosphere and phylosphere) where there is a higher content of sugars and amino or organic acids which are easily degradable by their metabolism. The organic exudates produced by plants provide another source of energy for microbial population. Simultaneously, microbial communities increase the rate of nutrient cycling and provide the nutrients in a form available for plants. Endomycorrhiza or arbuscular mycorrhizal fungi (AMF) belong to the fundamental symbionts, good plant colonisers and growth stimulators. Such characteristics make AMF a potentially interesting agent for use in horticulture. The crop production and final yield would be supported by improvement of soil condition through the inoculation of microorganisms. The enhancement of mineral uptake by plants, weed control and reduction of soil-borne pathogens would be the beneficial function of symbionts provided to crop. The impact of AMF on nutrient availability, especially on P is well documented in many reports (Karagiannidis et al., 2002; Ozgonen and Erkilic, 2007; Ozgonen et al., 2010; Barrios, 2007). Van der Heijden et al. (1998) showed that with increasing arbuscular mycorrhizal diversity, plant P at the vegetation level increased, while soil P decreased. It was suggested that AMF could increase phosphorus in the available forms for plant predominantly through the exploration of a large soil volume by hyphal networks (Jacobsen et al., 1992) where the activity of AMF directly affect the amount of P taken up by fungi. AMF is also able to enhance N supply where ammonium is the predominant ionic form of nitrogen or where soil moisture content is so low that the transport of nitrate is limited (Javaid, 2009). Another positive effect of mycorrhizae could be in weed management, where the weed:mycorrhizal interactions may reduce crop losses by limiting weed species (Jordan et al., 2000). Furthermore, better survival rate of AMF – supported plants and their greater growth has made the AMF inoculation sufficient option for improving land productivity. Efficiency of mycorrhizae is based on appropriate association of crop with AMF and as was suggested by Piotrowski and Rilling (2008), management of a mycorrhizal system on a community scale may minister to better understanding the relationship between both parties concerned.

N-fixers: The process of N_2 fixation includes the reduction of N_2 to NH_3 and simultaneously transformation of NH_3 to amino acids requires large amounts of energy. The bacteria could fix N_2 (i) in their free-living state (non-symbiotic N_2 fixation), (ii) in the association with plant roots (associative N_2 fixation) and (iii) as mutualistic symbionts (symbiotic fixation) (Myrold, 1997). The amount of total N and increase of N in the soil would be the indicator of presence the N-fixers in a soil. However, the method is not sensitive and would be used only for rough estimation. A wide range of free-living N-fixing bacteria may provide N to the associated

plants. Biological nitrogen fixation (BNF) provided by soil bacteria such as *Arthrobacter, Azospirillum* or *Rhizobium* is essential especially in case of organic growing where the inputs of key nutrients are limited. These organisms are able to compensate the nutrient losses caused by plant uptake and thereby participate in sustainable crop production. The main contribution of BNF would be in that case of cropping system where the fixed N goes directly to the harvested product (Barrios, 2007). Okon and Labanderagonzalez 1994 concluded that application of *Azospirillum* supported crop growth and yield by 5 to 30%. However, in many studies the amount of fixed N was not significant. Therefore, it was suggested that N-fixers cannot fully cover demand of plants for N (Dobbelaere et al., 2003). The amount of N fixed by microorganisms relates to the suitable condition for their life, and the efficiency of fixation decreases with the degradation of the habitats. Efficiency of N-fixers is probably driven by soil type or climate condition when appropriate living condition for bacteria directly influences their metabolism. To enhance crop production through N-fixation it is crucial to clearly understand relationships between the symbiont and harvested plant.

Decomposers and elemental transformers: The organo-mineral complex in a soil is transformed, stabilized and preserved by extracellular enzymes (Morra, 1997). The variety of extracellular enzymes originating from bacteria, fungi or plants relate directly to diversity of these communities and many enzymes are responsible for more than one process in soil and soil extracts. Enzymatic activity measurements would be used to assess the microbial activity and thereby their participation on soil fertility and fertilizers use efficiency (Shaffer, 1993). Microorganisms use these enzymes to release energy for metabolism. Brussaard et al., 1997 suggested that in soil, 90% of C is mineralized by bacteria and fungi requiring carbon for their growth. Such transformation is the key process to provide soluble organic and inorganic compounds from detritus and dead organic matter to plants. On the other hand, microorganisms are dependent on plants and their photosynthesis efficiency relating to amount of fixed C. The rate of C flux is the most important factor to sufficiently supply both microorganisms and plants requirements for C. Therefore the transformation of C by microorganisms represented by their enzymatic activity has the impact on crop productivity in horticulture. This part of the feedback cycle would be crucial for the manipulation of soil microbial communities whose metabolic activity supported by provision of energy would be manipulated to improve soil condition in terms of nutrient supply or disease protection.

Organic amendments

Proprietary products

The global increase of organically-managed horticultural soil has increased the use of organic amendments in such systems which aim is to manipulate various aspects of the system to improve crop yield, control disease, and 'condition' soil to function more effectively. Generally, by-products provide the organic inputs of carbon and easily degradable energy to the soil which cause the stimulation of microbial activity (Vance and Chapin, 2001). The availability of the energy for microbes is therefore crucial for transformation of nutrients within the system to make them available for crop uptake. The increase of microbial content by use of organic amendments was demonstrated by Fleissbach and Mäder et al., 2000 who suggested that microbial biomass increased by 45-64% in organic farming systems. The same observation was made by Birkhofer et al., 2008 who suggested that application of amendment promotes abundance of soil microbes and therefore supports plant growth.

Proprietary products are another possibility for farmers to improve nutrient cycling, stimulate plant growth or suppress disease occurrence. Various products (Actinovate AG®, Bio Inoculant®, Bio-S.I.®, Mpact®, 'PMSLA and EO-12'®, Soil Activator®, Super Bio®) products were tested by Russo and Fish, 2012 who concluded that Bio-S.I., PMSLA, EO-12, and Soil Activator had positive influence on plant height and fresh biomass, but simultaneously none of the products provided particular benefits for pepper, cucumber or maize production.

Proprietary products with specific inoculum of soil pathogen antagonists include Contans® WG, Ballad® Plus Biofungicide, Actinovate® SP or Agriphage™. These products are all marketed in the US. Assessment of these products were made by Raudales, et al., 2010 who suggested that some products are not able to suppress disease caused soil borne pathogens, however some of them especially with inoculums *Baccilus sp, Trichoderma, Streptomyces lydicus or Pseudomonas syringae* are able to improve soil health.

Compost tea

Compost tea is a liquid extract of compost obtained by mixing compost and water for a defined period of time (Ingham, 2002). The application of compost tea may participate in nutrient and organic matter supply for microorganisms (Carballo et al., 2008). Characteristics of compost tea are directly affected by production methods e.g. aerated and none aerated compost tea. The choice of input material, chemical and physical properties (pH, temperature) set during the production were suggested as the key factors influencing microbial content of final product (Scheuerell and Mahaffee, 2002). Furthermore, the product may be supported by additional nutrients in a form of molasses, soluble kelp or rock dust (Carballo et al., 2008). Lastly, compost age at time of application and dilution of compost tea

(Table Apx 1) influence the effectiveness compost tea as well (Hoitink and Boehm, 1999; Stone et al., 2004). Lim and Kim, 2010 suggested that glucose, maltose and sucrose are the most effective carbohydrates in promoting microbial growth. The content of such carbohydrates in compost tea may therefore be an important factor able for predicting the microbial response to compost tea addition. Through the manipulation of microbial community, the compost extracts could improve soil condition in terms of preventing, suppressing, or controlling pests and diseases, increasing N-biological fixation.

Literature Review-short version

With some 24 million hectares of arable soil being cultivated organically, the demand from farmers for eco-friendly approaches to crop production is rising (Tu et al., 2006). Organically cultivated land used in horticulture represents 6% of this area which means approximately 1.44 million hectares (FiBL and IFOAM, 2010). In such production systems, addition of organic amendments such as plant residues, composts, manures, and liquid preparations are essential not only as a source of crop nutrients and organic matter (Carballo et al., 2008), but also as an effective tool to enhance the development of beneficial microorganisms such as N-fixers, disease antagonists and microsymbionts. The addition of organic materials can alter the environment experienced by microorganisms (Annabi et al., 2009) and therefore influence microbial community structure (Brussaard et al., 2007). Simultaneously, the shift of microbial composition could affect interactions between individual members, change the dynamics within the community (Garbeva et al., 2004) and thus influence their function in a soil. An active manipulation of these organisms by the application of a variety of organic amendments can apparently have a beneficial effect on soil health and enhance plant/food web interactions (Paterson et al., 2011). Furthermore, studies have shown that soil with high microbial biomass, activity and diversity in organically cultivated soils correlate with low disease incidence (Tu et al., 2006; Hu et al., 1997; Workneh and van Bruggen, 1994). Hence the manipulation of microbial communities by organic amendments is a promising approach to stimulate plant growth, suppress soil disease (Berg and Smalla, 2009; Birkhofer et al., 2008) and therefore increase crop yield. The impact of organic amendments on general soil properties is well documented (e.g. Brady and Weil, 2000; Paterson et al., 2011), as well as specific impacts on microbial community structure (e.g. Joshi et al., 2009; Yin et al., 2011). However, specifications which would make the use of organic amendments efficient in horticulture practice are still missing and the variability in physical, chemical and biological soil properties frequently makes such amendments ineffective. More case studies are needed to understand the linkage between organic amendment use and changes in soil properties.

Our intention was to determine the effects of adding organic fertilisers, including compost tea defined as a liquid extract of compost obtained by mixing compost and water for a defined period of time (Ingham, 2002) and two commercial proprietary products; a rhizosphere inoculant and a nutrient supplement and compare them to an unamended control. The rhizosphere inoculant is a dried concentrated inoculum of numerous microbial species considered to consist predominantly spores of beneficial bacteria such as actinomycetes or fungi whilst the nutrient supplement is a mix of plant derived nutrients in the form of amino acids, carbohydrates, phosphorus, potassium and calcium that serve as a source of nutrition for soil microbes. The impact of such products was assessed by measuring the chemical (nutrient content and pH) and microbiological (phenotypic structure, and basal and substrate-induced respiration) properties over time following application. An experimental trial was established in a glasshouse with a monoculture of sweet peppers (*Capsicum annum*) using two substrate matrices - bark and compost. It was hypothesised that such organic amendments would both generally and specifically impact on soil microbiological properties, such that they may be able to reduce the need for inorganic fertilisers.

Materials and methods

Soils and experimental treatments

Soil samples were collected from a glasshouse-based trial located at Cantelo Nurseries Ltd. in Taunton, West England (50° 58.1' N/ 2° 54.5'W). Soil was a well cultivated clay loam with over 10 years of annual compost or bark additions. Compost (West Country Compost: http://www.ecosci.co.uk/westcountry.php) or bark (Melcourt: http://www.melcourt.co.uk/pdf/Melcourt%20Hortibark-Mixed%20Conifer.pdf) was laid on top of the soil in rows at 2.5 m^3 per 50m^2. Prior to the experiment, both the Bark and Compost were analysed by Laverstoke Park Laboratories (Appendix 3 and 4). Both soil matrices contained small stones and macrofauna consisting predominantly of earthworms. Woody clusters of various shape and size occurred in the bark matrix. Pepper (*Capsicum annum* L.) plants were grown in the respective media at a spacing of 0.5m, in 1 x 56 m parallel rows across the glasshouse bays. Four treatments were applied to the two soil based mixes: (i) control, no additions; (ii) aerated compost tea (CT) containing 13.3 mg ammonia-N/L, 0.1 mg TON-N/L, and 2.1 P mg/L, made by mixing 400 l of water with 7.5 l of compost, 500 ml of seaweed extract and 500g of molasses; start pH of water was set on 6.2 and the start temperature 20.7°C , the CT being applied as a solution, the compost tea brew time was 48 hrs; (iii) rhizosphere inoculant, at the manufacturer's recommended rate of 2kg/ha; (iv) nutrient supplement at the manufacturer's recommended rate of 5-20 litres per Ha as 1% solution. Four independent (randomly prescribed) replicate rows, located in separate

glasshouse bays, each of bark and compost, were treated with each formulation. Due to commercial practicalities, the arrangement of the bark and compost rows were such that they were not oriented randomly and hence matrix effects cannot be compared. The first soil samples were taken to represent all of the experimental variables and replicated, and collated at the beginning of the growing season (16 April; referred to as T0) before application of any organic amendment. Subsequently samples were taken after 21, 52, and 82 days (referred to as T1, T2 and T3), followed continuous application of the products. Samples were removed by excavating the soil close to stem bases, to a depth of 15 cm. The moist soil samples included cut roots of plants and were sieved through a 2 mm sieve and stored in polyvinyl chloride bags in the fridge at 4°C for no more than two weeks. Aliquots of samples from T0 and T3 were dried (105°C for 24h) for certain chemical analyses (as described below).

Soil chemical and physical analyses

Soil pH (1:5 ratio of soil suspension in a solution of 1M KCl), total C and total N content (Perkin-Elmer 2400 CHN elemental analyser), available P (Olsen method) were determined on dried soil samples. The content of soil nitrate-N and nitrite-N summarised as TON and ammonium-N content (1:5 ratio of soil suspension in a solution of 2M KCl), were determined on moist soil samples. The soil sieved through 2 mm mesh was used for soil pH, available P, TON and ammonium-N, however the soil was ground to a fine powder for total analyses. All of the analyses were made for soil samples taken before any amendment addition and samples taken 82 days thereafter. Interim samples were analysed for microbial components only.

The moisture content of the soil was determined by drying 30 g of moist samples kept on a drying tin in an oven set at 105°C for approximately 24 hours, after which the moisture content was calculated from the difference of weight.

Soil biological analyses

Microbial respiration

Soil respiration was assessed using the technique of Ritz *et al*, (2006). Aliquots of soil (20g fresh weight) were pre-incubated at 25°C for one week in the dark and subsequently respiration rates determined using a Rapid Automated Bacterial Impedance Technique (RABIT) instrument (Don Whitley Scientific Ltd.). One-g subsamples of soil were amended with 1.5 ml of water (basal) or 1M glucose solution (substrate-induced) to achieve 100% water-holding capacity. Microbial respiratory responses were monitored every 6 minutes

over 16 hours of incubation interval set at 25°C. Respiration rates were determined before any amendment addition and at 21, 52 and 82 days thereafter.

Phospholipid fatty acid analysis

Phospholipid fatty acids (PLFA) profiles were determined using the method of Frogstegard et al. (1993) based on the method described by Bligh and Dyer (1952). Approximately 7 g of fresh unincubated soil was freeze dried for 24 h and stored in sealed glass vitals prior to analysis. PLFAs were extracted using the solution containing chloroform, methanol and citrate buffer at a ratio of 1:2:0.8 (*v/v/v*). Solid phase extraction was used to fractioned phospholipids from neutral and glycol-lipids. The phospholipids were subjected to an alkaline methanolysis to produce fatty acid methyl esters (FAMES), and resultant fatty acids were measured using gas chromatography. FAME nomenclature was used to identify individual microbial communities according the PLFA profile, where the fatty acids are designated as X:YwZ, where X is the carbon chain length, Y is the number of double bonds and Z indicates the position of the double bond from the aliphatic methyl (w) end of the molecule. Some of the fatty acids presented in soil samples were unknown in terms of nomenclature and they will need to be investigated in an additional study. PLFA profiles were determined for samples taken before amendment and at 21 and 82 days thereafter.

Statistical analysis

Treatment effects on nutrient content (total N, total C, TON, ammonium-N, available P) were tested using a one-way analysis of variance (ANOVA). Respiration rates and PLFA-derived data (analysed by principal component analysis, (PCA) were assessed by repeated measures and post hoc tests using one-way analysis of variance (ANOVA). For all tests, $P < 0.05$ was considered to indicate a statistically significant difference. The software used for analysis includes STATISTICA and R 2.13.

Results

Soil chemical properties

The addition of a rhizosphere inoculant, a nutrient supplement or a mix of Compost tea did not significantly affect the soil chemical properties in either the bark or compost matrix (Table 1). However, there was a significant decrease ($P < 0.05$) over time for total C in both types of soil matrices (Figure 1 and Figure 2), and significant decrease ($P<0.05$) in both total N (Figure 3) and pH (Figure 4), but only in the compost matrix.

Table 1. Soil chemical properties include analyses before amendment addition (start) and after 82 days of amendment addition for control (CONT), ´Compost tea‘ (CT), ´rhizosphere inoculant‘ (A) or ´nutrient supplement‘ (B).

	Start	CONT	CT	A	B	Start	CONT	CT	A	B
pH	7.1 (0.1)	7.2 (0.0)	7.7 (0.1)	7.1 (0.1)	7.8 (0.0)	8.1 (0.0)	7.6 (0.0)	7.6 (0.0)	7.7 (0.0)	7.6 (0.0)
Tot C %	35.5 (0.3)	20.3 (1.2)	25.6 (1.3)	24.0 (0.9)	22.6 (1.5)	23.7 (0.2)	19.4 (0.7)	15.0 (0.6)	15.7 (0.4)	14.5 (0.8)
Tot N %	1.2 (0.0)	0.9 (0.0)	1.0 (0.0)	0.9 (0.0)	1.0 (0.1)	1.8 (0.0)	1.3 (0.0)	1.0 (0.0)	1.1 (0.0)	1.0 (0.1)
NH_{4+} mg/kg	0.5 (0.0)	8.7 (3.1)	2.8 (0.9)	2.8 (0.7)	4.8 (1.9)	0.5 (0.0)	0.6 (0.0)	1.7 (0.3)	0.9 (0.2)	0.9 (0.1)
TON mg/kg	28.5 (5.8)	51.5 (1.7)	59.8 (4.5)	57.6 (3.8)	50.6 (3.5)	69.9 (6.5)	57.4 (3.3)	50.2 (3.9)	72.3 (7.3)	91.3 (11.0)
P mg/kg	177.0 (3.1)	174.6 (3.7)	161.7 (7.8)	176.8 (6.3)	189.0 (10.8)	202.2 (4.6)	210.1 (4.0)	230.2 (26.2)	179.2 (5.2)	196.0 (2.8)

Data represent means with standard deviation in parenthesis (n=5)

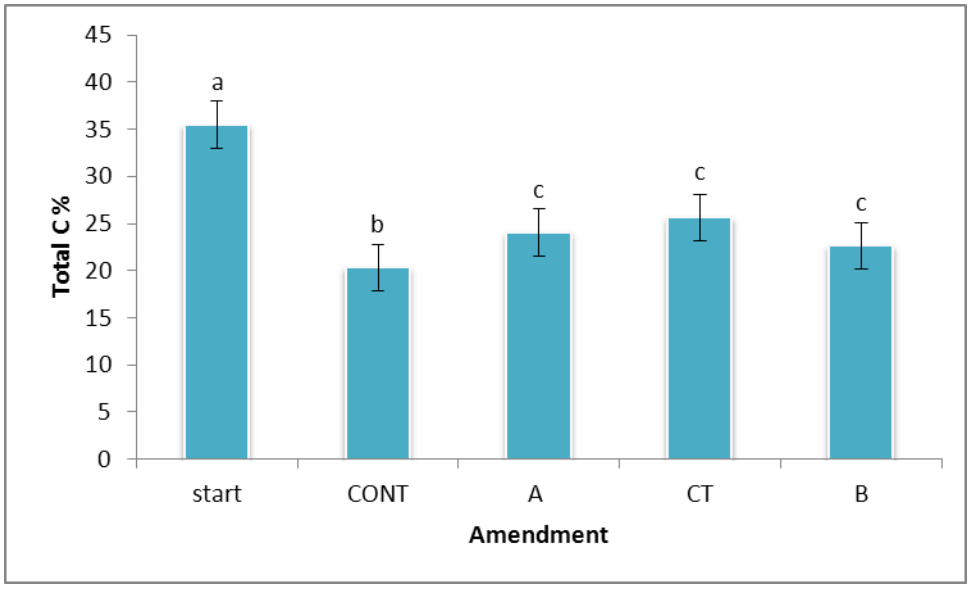

Figure 1. Temporal changes of C across all treatments in the bark matrix

Data show means with standard error (n=5)

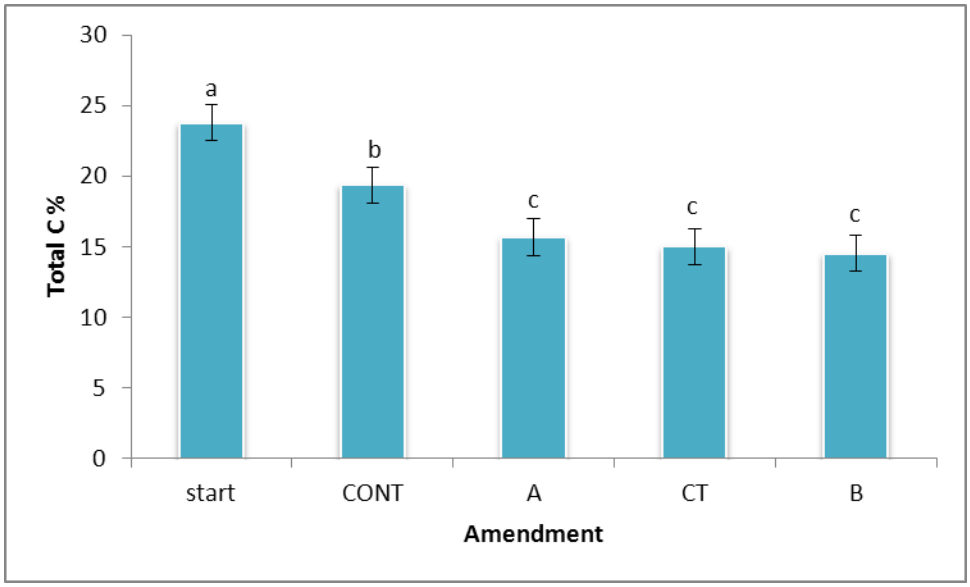

Figure 2. Temporal changes of C across all treatments in the compost matrix

Data show means with standard error (n=5)

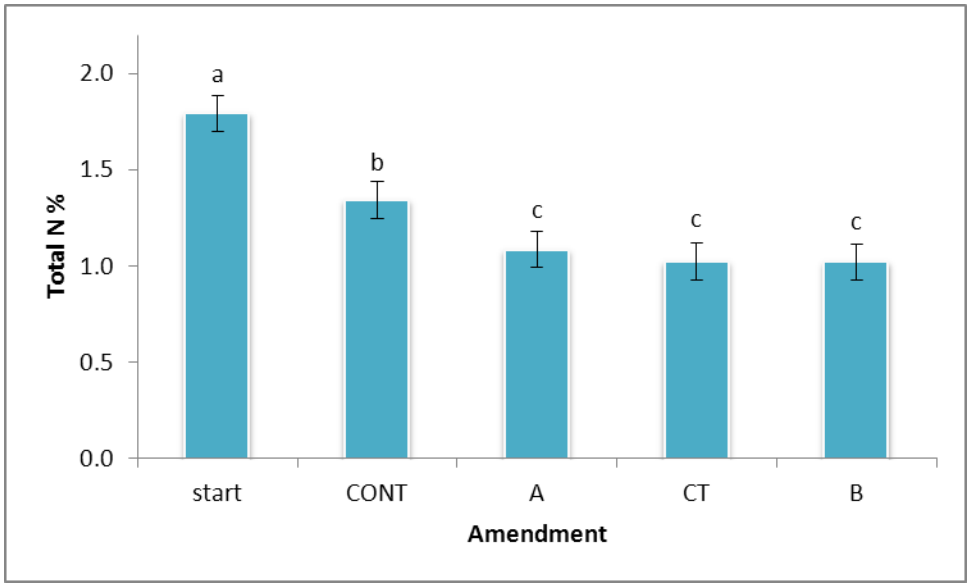

Figure 3. Temporal changes of N across all treatments in the compost matrix

Data show means with standard error (n=5)

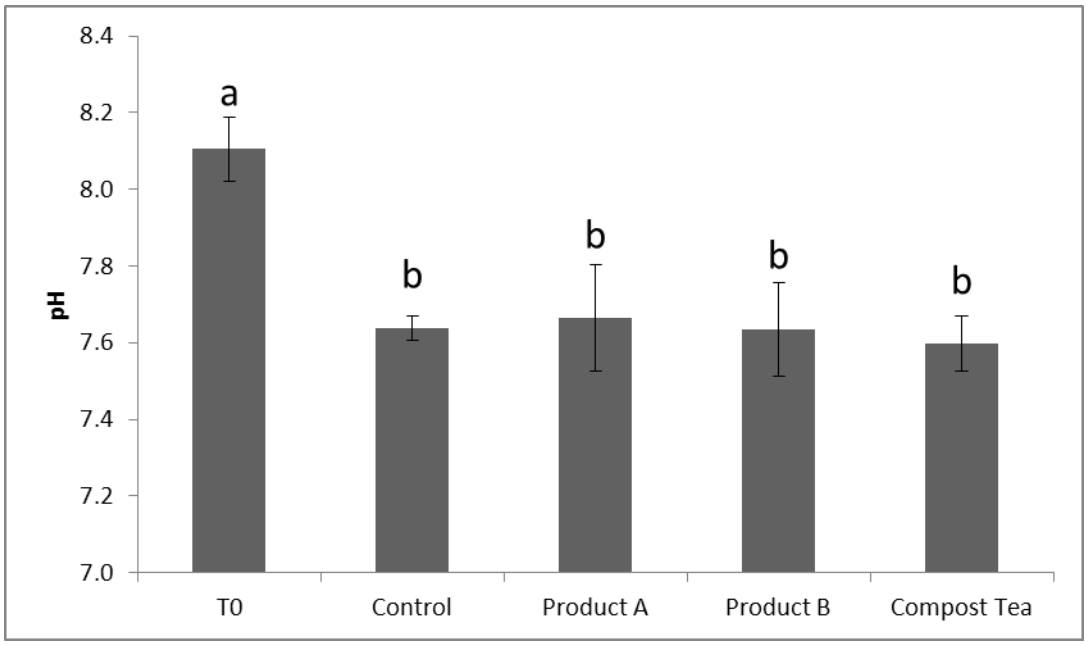

Figure 4. Temporal changes of pH across all treatments in the compost matrix

Data show means with standard error (n=5)

Soil microbial respiration

The basal metabolic rate in the bark matrix did not differ significantly between individual treatments nor was there a significant time effect (P=0.14; Table 2). Overall means of CONT and CT treatments fluctuated around 3.6 µg CO_2 g^{-1} dw soil, 3.9 µg CO_2 g^{-1} dw soil, respectively. The means of A and B treatments fluctuated over time around 4.6 µg CO_2 g^{-1} dw soil, 3.9 µg CO_2 g^{-1} dw soil respectively.

Similarly, substrate-induced respiration rate in glucose in the bark matrix did not change significantly either between the treatments or over time (P=0.62; Table 2). The overall means fluctuated in the case of CONT and A treatment around 11.7 µg CO_2 g^{-1} dw soil and 13.3 µg CO_2 g^{-1} dw soil, respectively. The means of CT and B varied around 11.5 µg CO_2 g^{-1} dw soil and 15.6 µg CO_2 g^{-1} dw soil, respectively.

Table 1. Respiration rate (µg CO_2 g^{-1} dry soil) on three occasions following the addition of amendments to the bark matrix, for control (CONT), ´Compost tea' (CT), 'Rhizosphere incoluant' (A) or ´nutrient supplement' (B).

	Basal metabolic rate				Respiration rate in glucose			
	CONT	A	CT	B	CONT	A	CT	B
21 days	5.2 (0.6)	4.3 (0.6)	4.2 (0.3)	2.9 (0.3)	12.7 (1.7)	13.2 (1.8)	9.8 (1.0)	9.6 (0.9)
52 days	2.5 (0.2)	6.1 (1.0)	3.3 (0.2)	4.0 (0.5)	9.7 (0.54)	14.9 (1.8)	12.0 (1.0)	16.3 (3.2)
82 days	3.1 (0.3)	4.1 (0.9)	4.2 (0.3)	3.2 (0.4)	12.6 (1.1)	11.7 (1.4)	12.5 (0.8)	17.2 (2.1)

Data represent means, with standard deviation in parenthesis (n=5)

The basal metabolic rate in the compost matrix was not significantly different between treatments ($P>0.05$). However, there was a significant decrease between 21 and 52 days ($P<0.001$; Figure 5). However, none of the organic amendment treatments alone showed a significant change.

Respiration rate of glucose amended samples did not record any significant change neither between the treatments nor over time in compost matrix ($P=0.41$; Table 2). In case of CONT and A treatments the overall means fluctuated around 5.8 µg CO_2 g^{-1} dw soil, 6.2 µg CO_2 g^{-1} dw soil, respectively. The means of CT and B treatments averaging around 6.6 µg CO_2 g^{-1} dw soil and 6.8, respectively.

Table 2 Respiration rate (µg CO_2 g^{-1} dw soil) at particular time intervals following the addition of amendments to the compost matrix, for control (CONT), ´Compost tea'(CT), ´Rhizosphere inoculant'(A) or ´Nutrient supplement'(B).

	Basal metabolic rate				Respiration rate in glucose			
	CONT	A	CT	B	CONT	A	CT	B
21 days	1.6 (0.0)	2.2 (0.7)	2.6 (0.7)	1.6 (0.1)	4.5 (0.3)	4.7 (0.3)	5.6 (0.4)	6.6 (0.7)
52 days	1.5 (0.1)	1.3 (0.0)	1.2 (0.0)	1.3 (0.1)	8.7 (2.1)	7.3 (1.5)	4.9 (0.3)	9.7 (1.1)
82 days	1.4 (0.1)	1.2 (0.1)	1.2 (0.1)	1.3 (0.1)	4.2 (0.3)	6.8 (0.8)	9.5 (1.7)	4.1 (0.4)

Data represent means, with standard deviation in parenthesis (n=5)

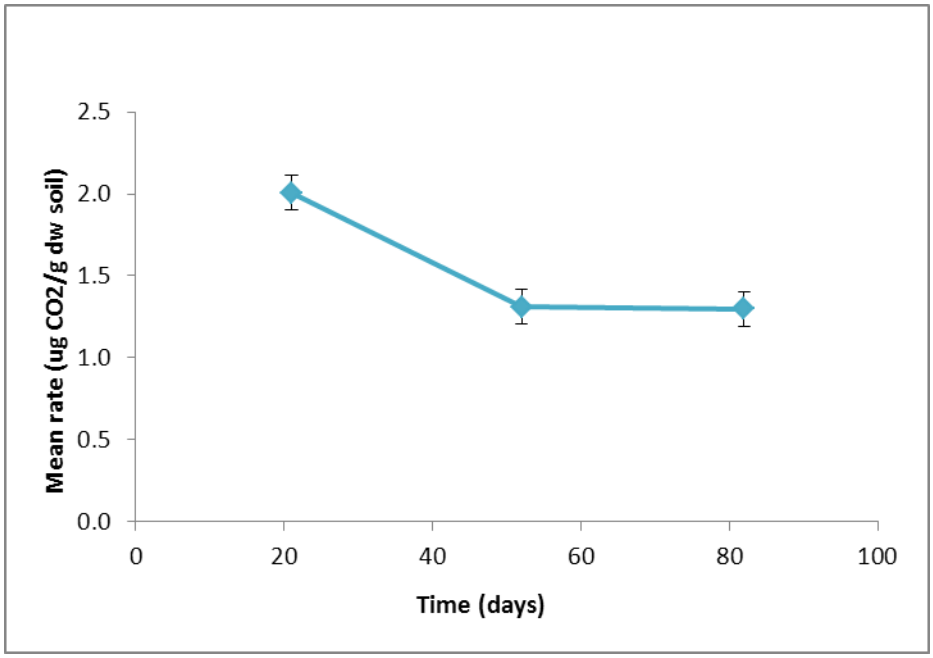

Figure 5 Temporal changes in basal metabolic rate across all treatments in the compost matrix. Points show means (n=20, bars show se)

Microbial community structure

The PCA plot of PLFA data from bark matrix detected clear separation of the microbial communities over time (i.e. between start point, 21 days, 82 days respectively; Figure 6). The first two components accounted for 45% of the total variance. There was the significant difference ($P<0.05$) between particular time intervals. There was marginal evidence for short-time effect of the rhizosphere inoculant on phenotypic structure ($P<0.05$). This effect was recorded after 21 days of application and diminished after 82 days when was no significant separation ($P>0.05$) according to organic amendment addition at all. In comparison, the nutrient supplement had a treatment effect which continued after 82 days.

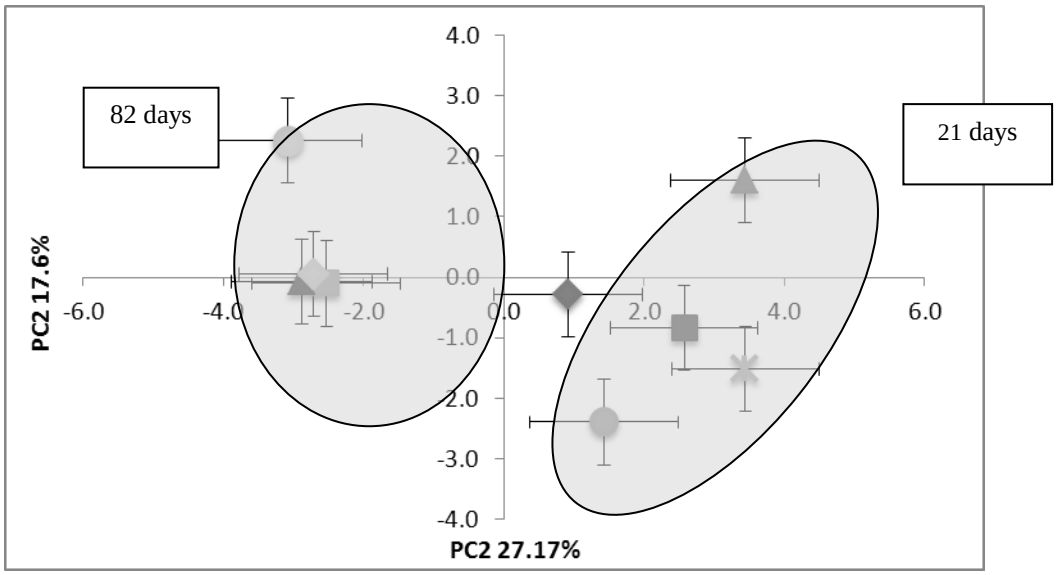

Figure 6. PCA score plot of ordination (n=5 ±standard error) showing the effect of organic amendments addition on microbial community structure by PLFA data obtained from bark matrix. *star* start point; *square* control, *diamond* compost tea, triangle 'Rhizosphere inoculant', *circle* 'Nutrient supplement'.

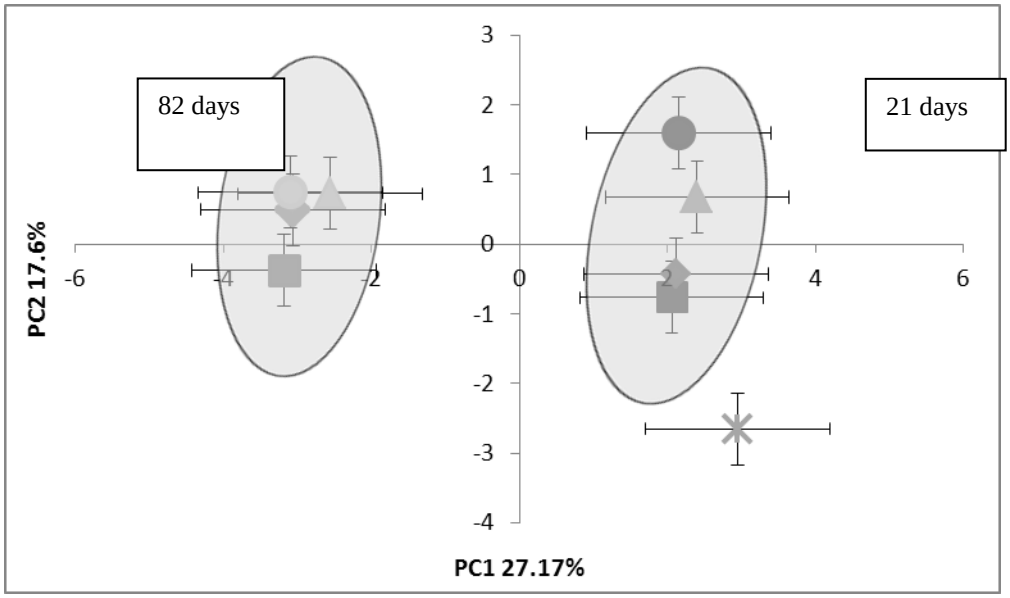

Figure 7 PCA score plot of ordination (n=5 ±standard error) showing the effect of organic amendments addition on microbial community structure by PLFA data obtained from compost matrix. *square* control, *diamond* 'Compost tea', *triangle* 'Rhizosphere inoculant', *circle* 'Nutrient supplement'

Loadings related to individual PLFAs which contributed notably to the Principle Components in the bark matrix 18:1*w*9*c*, 17:1*w*7 and 1 unidentified fatty acid were the main positive

contributors for the first component which explain 27.2% of total variance of variables and 15:0*i*, 15:0*ai* fatty acids were the main negative contributors. The FAMES *ai*17:0, 17:0(12 Me), Me 17:0 isomer 2 were loaded as the positive contributors for the second PC.

Table 4. Microbial PLFA receiving a positive (>0.8) or negative (<0.8) weighting on the first and second principal component for bark

	PC1	**PC2**
Positive weighting	18:1*w*9*c*, 17:1*w*7, 1 unidentified fatty acid,	*ai*17:0, 17:0(12 Me), Me 17:0 isomer 2
Negative weighting	15:0*i*, 15:0*ai*	-

In the compost matrix, fungi represented by 18:2*w*6,9 and actinomycetes represented by 20:0 were present as the positive contributors. In contrast, the bacterial fatty acids 15:0*i*, 15:0*ai*, 16:0, and 1 unidentified fatty acid were the main negative contributors (Table 5).

Table 5 Microbial PLFA receiving a positive (>0.8) or negative (<0.8) weighting on the first, second and third principal component for compost

	PC1	**PC2**
Positive weighting	18:2*w*6,9	20:0
Negative weighting	15:0*i*, 15:0*ai*, 16:0, 1 unidentified fatty acid	-

Fatty acid methyl esters (FAMES) detected, but not noted as contributing to the loadings included: 14:0, 15:0, 16:0i, 16:1w11t, 16:1w7c, 16:1w5, Me17:0 isomer, 17:1w8c, 17:0c, 17:1w8t, 18:1w7t, 18:1w13, 18:0, 19:1w6, 19:0c, 20:4, 20:5w3 and 5 other unidentified FAMES.

Discussion

Soil chemistry

The amendments did not affect either NH_4^+ or available P in either the compost or the bark matrix. Total C in both types of soil matrix decreased significantly after 82 days of amendment addition in comparison to the start point. This indicates high availability of C in both soil matrixes at the beginning of the growing season following by depletion over time. This is likely due to easily degradable forms of C being utilized by microbes as the main

source of nutrients and also taken up by the plants. However, there was no significant (managed) input of C to the system, so the total C decreased. The same consideration would be made for total N in compost matrix, when also this factor significantly declined over time. Therefore, it would be suggested that this depletion is caused by inefficient catabolism of organic substrates whose application should supplement the microbial and plant supply for these key macronutrients to the soil system during the growing season. This is partly contrary to Lenc et al., (2011) who reported that organic amendments such as composts and compost extracts could provide considerable carbon input. The possible reasons could be a very low nutrient content of compost tea (referred in Materials and Methods), method of compost tea production or compost tea age at the time of application. In case of the proprietary products, dilution is the factor affecting their efficiency. There are 4 options of possible dilution in manufacture's recommendation and accordingly a change of dilution could be a means to increase the impact of amendment on nutrient cycling. Lack of information about efficiency of proprietary products in the peer-reviewed literature precludes comparison with the results here.

Similarly to total N, soil pH did not show any significant change as the consequence of any amendment addition. However, there was significant change in the compost matrix over time, when pH in compost declined from alkaline condition in the control treatment to the more neutral in the case of all the treatments. Whilst pH in bark matrix did not show any trend over time and remained neutral for all the time. Therefore, different physicochemical properties of each type of soil matrix would be considered as the key factors which have an impact on nutrient cycling and thereby on amendment efficiency.

Change of pH in compost would likely shift the composition of the microbial community, and possibly improve the potential of soil to suppress disease (Noble, 2011). Particular species of pathogen antagonists may be developed and therefore soil resilience would be improved. This suggestion is similar to Griffiths et al., 2008 who reported that the soil functional resilience is governed by the physicochemical structure of the soil through its effect on microbial community composition and microbial growth. Simultaneously, neutral conditions would suppress the population of soil pathogens which prefer alkaline soil. This was documented by Martinez et al., 2011 who suggested that *Fusarium spp.* survive more in alkaline soils. An incidence of soil-borne diseases would be influenced by soil pH change also indirectly via affecting nutrient availabilities (Yin et al., 2011). Also communities enriching soil for nutrients, such as mycorrhizal fungi prefer more neutral soils instead of alkaline (Wang et al., 1993; Krsek and Wellington, 2006). Overall, effect of pH on soil properties and microbial composition is well documented, but strongly case specific (Ownley et al., 2003; Yin et al., (2011).

Microbial respiration

The substrate-induced respiration rate did not show any significant difference in either of the soil matrices. However, the respiration rate in glucose treated bark was approximately two times higher in comparison with compost. This indicates that the bark matrix contained a greater proportion of active microorganisms in comparison with compost and therefore could be considered as the more suitable matrix for manipulation of soil microbial communities. Nevertheless, basal metabolic rates recorded completely opposite results, when basal metabolic rate in bark did not show any significant change over time, but basal metabolic rate in compost showed significant difference between the first and second time intervals (after 21 and 52 days of amendment addition). In this case, soil respiration after 52 days of application was two times lower in comparison with the soil respiration after 21 days of amendment addition.

The decreased ability of the microbial communities to utilize carbon sources suggest that microbial composition was altered over time and that there was more active microorganisms after 21 days of application than 52 days thereafter. At the same time, it may be considered that compost as the soil matrix is unable to enhance microbial growth over the long term due to lack of energy sources needed for such a quick turnover.

Easily degradable soil organic matter released from soil preparation may have fuelled microbial activity at the start point (Feng, 2009) which would represent the beginning of the growing season in this project. Continuous addition of nutrients is able then to recover this fraction of organic matter in a soil (Ghosh, 2012) and to maintain microbial activity at the same level. Therefore, the decreasing trend of basal metabolic rate would be a consequence of easily available nutrients within the compost being depleted.

Microbial community structure

The phenotypic profiles distinctively separated individual sampling intervals from each other. The impact of time was so dominant in comparison with treatment effect which was recorded only in the bark matrix for ´Rhizosphere inoculant´ and ´Nutrient supplement´. Moreover, both treatment effects tended to diminish over time. The effect of time rather than particular amendments application indicating that time has stronger effect on microbial growth than organic amendment itself. Therefore, PLFA method would be considered as the sensitive method. The soil microbial communities in bark were dominated by fungi, represented mainly by 18:1*w*9*c* and by Gram positive bacteria (15:0*i*, 15:0*ai*). Fungi communities, indicated by the abundance of the fatty acid 18:2*w*6,9 and Gram positive bacteria 15:0*i*,

15:0*ai*, 16:0 were present in compost. As was reported above, the major factor influencing the microbial composition was probably soil C, N content and pH. This suggestion agrees with findings taken by Cookson et al., 2005 who concluded that the shifts in microbial structure are related to aspects of soil C and N pools. Simultaneously, Aciego Pietri and Brookes, 2009 concluded that Gram positive bacteria and fungi are more abundant in soil with neutral and lower pH. Another possible reason for higher abundance of Gram positive bacteria is their ability to quickly recover after challenges posed by acidic environments (Cotter and Hill, 2003). The third community group presented in soil compost matrix were actinomycetes represented only by 20:0. Therefore, it would be considered that fungi and bacteria were main communities presented in soil matrixes. These findings concur with Zhang et al., (2012) who reported that application of organic substrates enhance the bacterial and fungal communities rather than actinomycetes, and that organic matter inputs increased the PLFA biomarkers for bacterial and fungal communities.

Jindo et al., 2012 reported that the microbial community structure depends on the original organic wastes. Furthermore, the properties set during the amendments production such as C/N ratio, temperature, moisture content and bulk density are the key factors able to influence PLFA pattern. Therefore, change of the input materials and different settings of such parameters will play the role in the ability of organic amendments to shift microbial communities. However, dilution used for application would be considered as another factor able to change microbial structure. Such results relate to poor ability of organic amendments suppress diseases caused by soil microbial pathogens.

Conclusions

The manufacture's claims provided for 'Rhizosphere inoculant': (i) promotion of a plant's recovery from stress, (ii) root development and improvement of nutrient uptake, (iv) colonization of the root zone with beneficial microbial populations and lastly (v) getting nutrients available from soil organic material. Simultaneously, the claims provided for 'Nutrient supplement') include: Promotion of healthy fungal growth to stabilise the slow release of nutrients and promote disease resistance. On the basis of the results here, many of these assertions in respect of microbiological effects were not supported. However, effects on plant growth, crop performance and disease control could not be substantiated within the bounds of this study. In the case of compost tea, similar observations were made by Birkhofer et al. (2008) and Scheuerell and Mahaffee, (2004). In comparison, Yin et al., (2011) reported that biochemical properties of soil and plant growth were better in compost-amended plots and also Joshi et al., 2009 suggested that application of compost extracts can alter the population of some pathogen antagonists and therefore may provide effective

control of disease. Such results indicate that the efficiency of the products used in horticulture practice is a complex problem and that cannot be accounted for by any single factors. With this suggestion agree Litterick et al, 2004 whose review reveals the inconsistence between the particular results related to organic amendment use in horticulture.

In a general sense, the analysed parameters suggest that within the parameters of this research, soil quality was not improved by application amendments used. Therefore the manipulation of soil microorganisms for sustainable horticulture require an understanding of microbial function in a soil linked to scale at which each member of the microbial community makes its own contribution for complexity of system. However, soil conditions affected by intensification or crop repetition need to be firstly recovered to provide the support needed for microbial development. This research indicated that single management approach, such as organic amendment addition, alone is not effective in manipulating soil microbial characteristics. Thereby another management practice is necessary to optimize soil properties as the part of active management program. This suggestion is consistent with Hartemink (2006) who also reported that, if the soil does not provide the support needed, biological amendments will not properly establish or be active and will not produce the intended effect.

Knowledge and Technology Transfer

Dissemination:

1. The MSc thesis will be available in the British Library for public viewing
2. There was a poster presentation day at Cranfield University whereby project sponsors were invited to view MSc student's posters.
3. The intention is to publish the results gained in a relevant peer reviewed journal.
4. A short HDC News magazine article or HDC technical note will be published for growers

Glossary

N/A

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Appendices

Tables and Experimental trial

Table Apx 1 Comparison of physical and biological characteristics of compost extracts with different dilution (modified from Shrestha et al., 2011)

Parameter	Non-diluted	1:10	1:100	1:1000
NH_4^+ - N ($\mu g.g^{-1}$ d wt)	0.18±0.07a	1.29±0.10b	0.28±0.03a	0.16±0.04a
PO_4 – P ($\mu g.g^{-1}$ d wt)	37.61±3.20a	33.05±1.52a	39.35±1.15a	40.00±2.27a
Bacterial population (cfu Log10)	9.24±0.04a	11.79±0.01d	10.96±0.04c	9.39±0.03b
Fungal population (cfu Log10)	7.84±0.04a	8.40±0.07b	8.28±0.05b	8.21±0.01b

Table Apx 2 Microbial properties of compost tea applied to the soil

Parameter	Analysis	Results
Microbial Community	Active Bacteria	15.9 µg/ml
	Total Bacteria	512.0 µg/ml
	Active Fungi	2.8 µg/g
	Total Fungi	7.7 µg/ml
Ratio	Total Fungi : Total Bacteria	0.02
	Active : Total Fungi	0.36
	Active : Total Bacteria	0.03
	Active Fungi : Active Bacteria	0.18
Protozoa	Flagellates	152495 No/ml
	Amoebas	3 No/ml
	Ciliates	13 No/ml

Data analysed by Laverstoke Park Laboratories, Southley Farm, Hampshire

Appendix 3:

Soil Microbiology Report of the Bark used in the experiment.

LAVERSTOKE PARK LABORATORIES

Independent Analysis and Advice for Soil Fertility Management

Soil Microbiology Report

02/04/2012

Client: Neal Ward (Cantelo Nurseries Ltd.)

Address: Bradon Farm, Isle Abbotts, Taunton. TA3 6RX.

Date Submitted: 19/04/2012
Plant Species: Peppers
Sample No: 2644
Sample Type: Soil
Sample ID: 1 - 4 Bark

	Analysis	Units	Results	Guideline	Comments
Microbial Biomass	Moisture Content	%	59	15 - 55	Too Wet
	Active Bacteria (AB)	µg/g	125	15 - 25	Good
	Total Bacteria (TB)	µg/g	293	100 - 3000	Good
	Active Fungi (AF)	µg/g	40.0	15 - 25	Good
	Total Fungi (TF)	µg/g	221	100 - 300	Good
	Hyphal Diameter (HD)	µm	2.67	> 2.5	Good

	Analysis	Results	Guideline	Comments
Fungal: Bacterial Ratios	Total Fungi : Total Bacteria (TF:TB)	0.75	0.8 – 1.5	Low (Just)
	Active : Total Fungi (AF:TF)	0.18	0.25 – 0.95	Low
	Active : Total Bacteria (AB:TB)	0.43	0.25 – 0.95	Good
	Active Fungi : Active Bacteria (AF:AB)	0.32	0.75 – 1.5	Low

	Analysis	Units	Results	Guideline	Comments
Protozoa	Flagellates	No/g*	N/A	N/A	Not ordered
	Amoebas	No/g*	N/A	N/A	Not ordered
	Ciliates	No/g*	N/A	N/A	Not ordered

Numbers per gram of soil

Nematodes						
TOTAL NEMATODES per gram of soil	J* (%)	FF* (%)	BF* (%)	PP* (%)	PR* (%)	OTH* (%)
Not ordered	-	-	-	-	-	-
Guideline: N/A						

Juvenile Nematodes (J), Fungal Feeding Nematodes (FF), Bacterial Feeding Nematodes (BF), Plant Parasitic Nematodes (PP), Other Nematodes (OTH).

	Analysis	Units	Results	Guideline	Comments
Mycorrhizal Colonisation	Ectomycorrhizae	%	N/A	40 - 80	Not ordered
	Arbuscular Mycorrhizae	%	N/A	40 - 80	Not ordered

Laverstoke Park Laboratories, Southley Farm, Overton, Hampshire. RG25 3DR
Tel: 01256 772815 Fax: 01256 772809 Email: lab@laverstokepark.co.uk

Appendix 4:

Soil Microbiology Report of the Compost used in the experiment.

LAVERSTOKE PARK LABORATORIES

Independent Analysis and Advice for Soil Fertility Management

Soil Microbiology Report

02/04/2012

Client: Neal Ward (Cantelo Nurseries Ltd.)

Address: Bradon Farm, Isle Abbotts, Taunton. TA3 6RX.

Date Submitted: 19/04/2012
Plant Species: Peppers
Sample No: 2645
Sample Type: Soil
Sample ID: 1 - 4 Compost

	Analysis	Units	Results	Guideline	Comments
Microbial Biomass	Moisture Content	%	57	15 - 55	Too Wet
	Active Bacteria (AB)	µg/g	81.8	15 - 25	Good
	Total Bacteria (TB)	µg/g	323	100 - 300	Good
	Active Fungi (AF)	µg/g	27.9	15 - 25	Good
	Total Fungi (TF)	µg/g	95.2	100 - 300	Low
	Hyphal Diameter (HD)	µm	2.67	> 2.5	Good

	Analysis	Results	Guideline	Comments
Fungal: Bacterial Ratios	Total Fungi : Total Bacteria (TF:TB)	0.29	0.8 – 1.5	Low
	Active : Total Fungi (AF:TF)	0.29	0.25 – 0.95	Low
	Active : Total Bacteria (AB:TB)	0.25	0.25 – 0.95	Good
	Active Fungi : Active Bacteria (AF:AB)	0.34	0.75 – 1.5	Low

	Analysis	Units	Results	Guideline	Comments
Protozoa	Flagellates	No/g*	N/A	N/A	Not ordered
	Amoebas	No/g*	N/A	N/A	Not ordered
	Ciliates	No/g*	N/A	N/A	Not ordered

Numbers per gram of soil

Nematodes						
TOTAL NEMATODES per gram of soil	J* (%)	FF* (%)	BF* (%)	PP* (%)	PR* (%)	OTH* (%)
Not ordered	-	-	-	-	-	-
Guideline: N/A						

Juvenile Nematodes (J), Fungal Feeding Nematodes (FF), Bacterial Feeding Nematodes (BF), Plant Parasitic Nematodes (PP), Other Nematodes (OTH).

	Analysis	Units	Results	Guideline	Comments
Mycorrhizal Colonisation	Ectomycorrhizae	%	N/A	40 - 80	Not ordered
	Arbuscular Mycorrhizae	%	N/A	40 - 80	Not ordered

Laverstoke Park Laboratories, Southley Farm, Overton, Hampshire. RG25 3DR
Tel: 01256 772815 Fax: 01256 772809 Email: lab@laverstokepark.co.uk

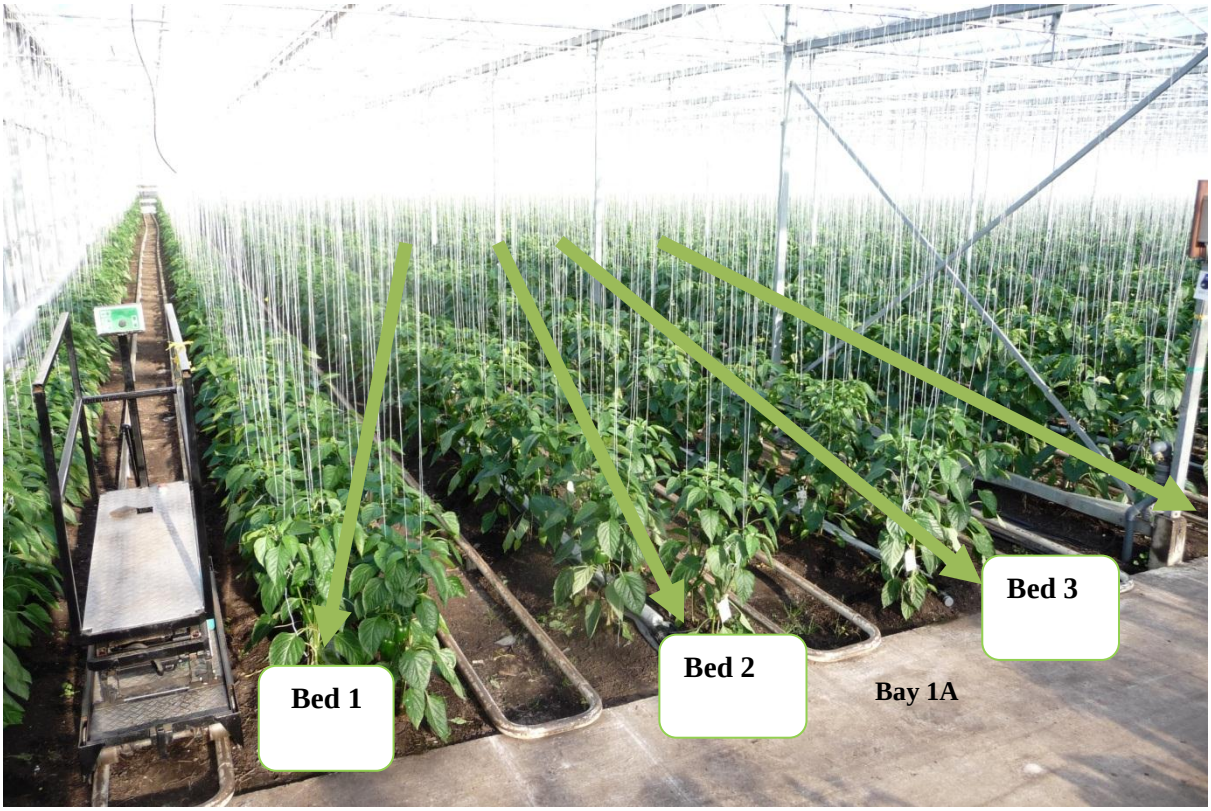

Figure Apx 1 Experimental trial