AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

TITLE: Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop

AUTHORS: Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., Brunton, N., Grant, J. and Griffiths, B. S.

NOTICE: This is the pre-peer reviewed version of the following article: Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., Brunton, N., Grant, J. and Griffiths, B. S. (2013), Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop, J. Sci. Food Agric., 93: 3700–3709. doi: 10.1002/jsfa.6206 which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6206/full.

Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop

Running title:

Kinsealy_soils_JSFA

Reilly, Kim [1,3*] , Cullen, Eileen [2,4] , Lola-Luz, Theodora [1,3] , Stone, Dorothy, [2] Valverde, Juan [3] , Gaffney, Michael [3], Brunton, Nigel, [3, 5] Grant, James [3]and Griffiths, Bryan [S2., 6]

[1] Teagasc Kinsealy Research Centre, Dublin 17, Ireland; [2]Teagasc , Johnstown Castle, Wexford, Ireland; [3]Horticulture Development Department, TFRCA, Ashtown, Dublin 15, Ireland; [4]University of Wisconsin, Madison WI 53706, USA; [5] School of Agriculture and Food Science, University College Dublin, Ireland; [6]SRUC Crop and Soil Systems Research Group, Edinburgh EH9 3JG, UK.

***Correspondence:** kim.reilly@teagasc.ie

Abstract

BACKGROUND

Responses of the soil microbial and nematode community to organic and conventional agricultural practices were studied using the Teagasc Kinsealy Systems Comparison trial as the experimental system. The trial is a long term field experiment which divides conventional and organic agriculture into component pest-control and soil treatment practices. We hypothesised that management practices would affect soil ecology and used community level physiological profiles (CLPP), microbial and nematode counts, and denaturing gradient gel electrophoresis (DGGE) to characterise soil microbial communities in plots used for onion (*Allium cepa* L.) cultivation.

RESULTS

Microbial activity and culturable bacterial counts were significantly higher under fully organic management. Culturable fungi, actinomycete and nematode counts showed a consistent trend towards higher numbers under fully organic management but these data were not statistically significant. No differences were found in the fungal/bacterial ratio. DGGE banding patterns and sequencing of excised bands showed clear differences between treatments. Putative onion fungal pathogens were predominantly sequenced under conventional soil treatment practices whilst putative soil suppressive bacterial species were predominantly sequenced from the organic pest-control treatment plots.

CONCLUSION

Organic management increased microbial activity and diversity. Sequence data was indicative of differences in functional groups and warrants further investigation.

Keywords

Biolog Eco-plates; microbial diversity; community level physiological profile; denaturing gradient gel electrophoresis; organic agriculture

INTRODUCTION

Good soil husbandry is fundamental to sustainable long term crop production and plant health, and is a focus of many techniques used by organic and conventional growers. However our understanding of how agricultural practices affect soil ecology and contribute to soil functioning is incomplete. Soil health is broadly defined as "the competence with which soil functional processes (e.g. nutrient cycling, energy flow) are able to support viable, self sustaining (micro) faunal and (micro) floral ecosystems" [1]. In contrast soil quality is defined by its "suitability for a specific use". This definition encompasses biological, physical and chemical attributes and is dependant on the soil type and land use context [2.]

A number of studies have shown that biotic measures of soil quality and soil health can be affected by agricultural practices. Some studies have indicated that practices used in organic and sustainable conventional agriculture such as addition of organic matter, use of green manure or ley crops, crop rotations, reduced application of synthetic nutrients, and the absence of synthetic pesticides can increase indices of soil quality, [2-4] soil disease suppressiveness [5], and soil microbiological activity and diversity [6-8] . Conversely certain agrochemicals for example phenyl urea herbicides such as linuron, the benzimidazole fungicide carbendazim, and the biological pesticide azadirachtin have been shown to impact soil microbial community structure [9, 10].A number of techniques have been used to assess soil microbial diversity. Traditional techniques relied on the use of selective or semi selective media, however CLPP (Community Level Physiological Profiling) and DGGE (Denaturing Gradient Gel Electrophoresis) have become widely used. CLPP is carried out using Biolog Eco-plates or Biolog GN plates which contain 31 or 95 different sole carbon sources respectively. The subset of sole C sources used in the Eco-plates are known to occur in plant root exudates and/or to have a high discriminatory power among soil communities [11,12] . Biolog plates enable assessment of soil functional (rather than taxonomic) diversity and of changes in substrate utilization patterns. However it is a culture dependant method with a bias towards culturable, fast growing species. DGGE is a technique based on electrophoresis of PCR amplified DNA samples across a denaturing gradient in an acrylimide gel. To date most soil DGGE community studies have used PCR products generated using primers based on the 16S rRNA gene for bacteria and archaea [9,13-15] and the 18S small subunit region (SSU) of the fungal ribosomal RNA gene cluster [16-18] . Separation of the mixture of PCR products generated on a DGGE gel gives rise to a community fingerprint which can be analysed using image analysis software and/or by sequencing of excised bands.

The aim of the present study was to examine the effect of organic, conventional and mixed agricultural practices on soil microbial and nematode community structure. The experimental system is a long term field trial that was set up so as to divide “organic” and “conventional” agriculture into component parts, namely a) soil management, and b) pest-control. The trial is a factorial split plot design which investigates the effect of, and any interaction between, production system components. Onion plots within this trial were was selected for study since onion crops are highly dependant on rhizosphere associations and susceptible to soil-borne pathogens.

MATERIALS AND METHODS

Field trial

Soil samples were from plots used for onion cultivation in the systems comparison field trial at Teagasc, Kinsealy (53° 25' N Lat 6° 10' W), located in north county Dublin, Ireland. Soil type is loam to clay loam belonging to the grey brown podzolic soil group. (Altitude: 28 metres O.D., Slope: 1^{o}, moderately well drained). The trial is a factorial split plot design with 4 replicates and follows commercial vegetable production practices in Ireland. Carrot (*Daucus carota*), broccoli (*Brassica oheracea* var. *itahica*) and onion (*Ahhium cepa*) are grown each year and are assigned as the main plot. There are 2 levels of soil treatment – an organic soil treatment (OS) and a conventional soil treatment (CS); and 2 levels of pest-control – an organic pest-control treatment (OP) and a conventional pest-control treatment (CP). The trial was established in spring 2009 on land that had previously been under grass set-aside for over 10 years. The organic cultivation practices used were in compliance with EC1990/92, EC 834/2007 [19,20]and with standards for organic certification set out by the Irish organic certification bodies, with the exception that for experimental purposes the separation distance required between adjacent organic and conventional commercial enterprises was not practised between organic and conventional treatment plots.

The organic soil (OS) treatments comprised certified organic fertilizer inputs; a 4 year horticultural crop rotation including a fertility building red clover ley (*Trifohium pratense*); and use of small grain or legume winter cover crops. The conventional soil (CS) treatment comprised use of mineral fertilizers, with no set crop rotation (crops randomly allocated each year) and no winter cover crop. Equivalent rates of nitrogen (N), phosphorus (P) and potassium (K) were applied to both CS and OS treatments for each crop following a spring soil test and the rates applied were according to Teagasc recommendations for the crop [21.] Fertilizer was applied as calcium ammonium nitrate (CAN), single super-phosphate and sulphate of potash for the CS treatment; or Greenvale (3:3:1) and ProKali for the OS treatment. Conventional pest-control (CP) treatments comprised pesticide spray applications for the control of weeds, pests and diseases in accordance with Integrated Pest Management strategies typical of north county Dublin commercial vegetable production and in accordance with [22] . Organic pest-control (OP) treatments comprised mechanical weed and pest-control methods; certified treatments of biological origin if required and appropriate to the crop; and provision of a refuge area to encourage beneficial insects. Applied inputs for onion cultivation in 2011 are shown in Table 1. Mean harvested bulb weight was in the range 178 to 191 g and did not differ between treatments, although total yield per plot was significantly higher under fully conventional (CSCP) management. No differences in disease incidence between treatments were observed. Additional information on the field trial layout, and climatic conditions is available at http://www.ipfn.ie/publications/agronomic/

Onions cv. 'Hyskin' were transplanted to V1 onion main plots as multi-seeded modular transplants at 3 rows per 152cm bed and 25cm in row spacing on 26th April 2011 and were harvested on 19th September 2011. Soil samples used for BIOLOG and DGGE analyses were collected on 4th July 2011. Initial nematode counts were lower than expected, therefore culturable microbial population and total nematode counts were assessed from soil samples taken 7th November 2011 after onion harvest and before winter cover crop initiation in OS split plots. On both sample dates, 6 to 8 soil cores (10cm depth) were taken using a cone auger from each onion plot and hand mixed to form a composite sample.

CLPP analysis

The substrate utilization pattern of soil samples was assessed using Biolog Eco plates (Biolog Inc. Hayward, CA) according to the recommendations of the manufacturer. Freshly obtained composite soil samples from each plot were sequentially reduced and a 5g sub-sample was weighed into a sterile falcon tube with 50ml 1/4 strength Ringers solution (Oxoid). Samples were placed in a shaking incubator at room temperature and 300 rpm for 30 minutes and were then allowed to settle for 10 minutes. An aliquot (10ml) of each solution was transferred to a fresh tube and further diluted an absorbance $_{A590}$ of 0.4 to standardise inoculum densities. Experimental samples were randomly assigned to each Biolog plate replicate, giving a total of 6 Eco-plates. Wells were inoculated with soil suspension (150 jtl per well) and incubated at 25^{o}C for 7 days. The $_{A590}$ was measured on day 0 and on day 2, 3, 4 and 5 after inoculation using an automated plate reader (Dynatech MRX). Prior to further analysis each well absorbance reading was blanked against its own initial reading and the control well. The average well colour development (AWCD), area under the curve (AUC) and Shannon diversity Index (H') were then calculated as described below.

Culturable microbial population

Five grams of each soil sample were added to 45ml sterile distilled water in a 50ml Falcon tube and shaken on a wrist action shaker (flask shaker, Stuart scientific) for 10 minutes. Ten-fold serial dilutions were prepared and 1 00tl aliquots were spread onto trypic soy agar for total bacterial counts; potato dextrose agar with streptomycin (50 tg ml^{-1}) for total fungal counts and water agar with nystatin (50 tg ml^{-1}) for actinomycete counts. Plates for bacterial and fungal counts were incubated at 25^{o}C for 2-4 days. Fungal genera were identified by morphological and microscopic examination. Plates for actinomycete counts were incubated at 28 ^{o}C for 10-14 days.

Nematode abundance

Nematodes were extracted from 100 g composite soil samples by Oostenbrink elutriator and Baermann funnel techniques, collected after 48 h, and preserved in DESS according to [23]. Total nematode numbers were determined. Counts were adjusted for soil moisture content and expressed as nematode abundance g dry soil^{-1}.

Data analysis

Statistical analysis was carried out using SAS 9.1 (Cary, NC). For CLPP analysis AWCD for each experimental treatment was calculated over time. The area under the curve (AUC) for each substrate was calculated according to [24] and the Shannon diversity index (H') was calculated according to the formula:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

The AUC for each sole carbon source in the fully conventional (CSCP) and fully organic (OSOP) treatments were subjected to a paired t-test for significant differences between treatments. AWCD data, H' data, microbial and nematode count data and F/B ratio data were analysed using an ANOVA mixed model containing a contrast code to compare the fully organic (OSOP) and fully conventional (CSCP) treatments as well as the individual treatments and interactions (SAS 9.1).

DGGE analysis

Microbial DNA was extracted from soil samples using the MOBIO Ultraclean Soil DNA Isolation kit according to the specifications of the manufacturer. For the analysis of fungal diversity a segment of the fungal 18S small subunit (SSU) rDNA gene (around 390bp) was amplified using primer pair FR1 and FF390 as described by Vainio and Hantula [17]. Each PCR reaction contained 25jtl MyTaqRed (Bioline), 0.1jtM of each primer and around 10ng of template DNA to a final volume of 50jtl. PCR cycles were as described in [17]. For the analysis of soil bacterial diversity a 433bp fragment of the bacterial 1 6s rDNA gene was amplified using primers F984GC and R1 378 described in [14]. PCR was carried out in a volume of 50jtl as above using the following cycle: initial denaturing step 5 minutes at 94^{o}C, 35 cycles comprising 1 minute denaturing at 94^{o}C, 1 minute annealing at 53^{o}C, 2 minutes extension at 72^{o}C, followed by a final extension step of 10 minutes at 72^{o}C. Aliquots (5jtl) of each reaction were electrophoresed on a 1% (w/v) agarose gel to verify similar concentrations of PCR products had been amplified from each soil sample. The remaining PCR reaction products (45 jtl) were then analysed by DGGE using a 45-60% denaturing gradient (100% denaturant contained 7M urea and 40% fomamide) on a 6.5% (w/v) polyacrylimide gel for fungal samples or a 30 - 60% denaturing gradient for bacterial samples [14,17,25]. DGGE gels were prepared using a Biorad 475 Gradient Delivery System (Biorad) and were allowed to polymerise overnight. Gels were run at 50v and 60^{o}C for 17 hours in 1X TAE re-circulating buffer for separation of fungal DNA or at 150V and 60^{o}C for 5 hours in 0.5X TAE for bacterial gels. Bands were stained by immersing the gel in 1x TAE buffer containing Gelstar GelRed stain (Lonza) according to the specifications of the manufacturer and were visualised under UV light. To compare the separation patterns of all four soil treatments (OSOP, OSCP, CSOP, CSCP) on a single DGGE gel, initial DGGE gels containing all four replicates of each treatment (16 lanes) were run and checked for consistency across the replicates. Subsequently representative samples for each treatment were re-amplified as above and run on a single DGGE gel.

DGGE band sequencing and sequence analysis

Bands of interest were excised from the DGGE gel and eluted into a small volume (20 – 50jtl) ofsterile PCR grade water using a “crush and soak” method. Five microlitres of the solution was used to re-amplify the excised fragment using the same primer pair and PCR conditions as previously described. PCR products were purified from a 1% (w/v) agarose gel using the QiaxII gel extraction kit (Qiagen), sub-cloned into the pGEMT-easy vector (Promega) and transformed into competent *E.coli* JM109 cells (Promega). Since co-migration of multiple sequences within the same DGGE band has been noted [26-28] three to four clones containing an insert of the expected size were sequenced for each expected band. Where identical duplicate sequences were obtained from the same band, one sequence only is presented. Sequencing was carried out using universal pUC13/M13 forward sequencing primer (5'-CGCCAGGGTTTTCCCAGTCACGAC-3'). Bacterial sequences were identified by a blastn search alignment against 16s ribosomal DNA sequences on the NCBI database (http://blast.ncbi.nlm.nih.gov) [29] . Fungal sequences were identified by blastn search alignment using the nucleotide collection (nr/nt) database. Uncultured/environmental sample sequences were excluded from both fungal and bacterial search parameters. For identification based on blast search homology the criteria used were consistent similarity at ≥98% to the same species or genus. Sequences identified were submitted to the Genbank database using the Bankit submission tool.

Nucleotide sequence accession numbers

Nucleotide sequences for bacterial 16s bands B1-B6 and fungal 18s bands K1-K22 have been deposited in the Genbank database. Accession numbers are shown in Tables 4 and 5.

RESULTS AND DISCUSSION

CLPP analysis

Our data indicated significantly higher levels of microbial activity and functional diversity in soil under the fully organic (OSOP) treatment compared to the fully conventional (CSCP) treatment. Three indices – average well colour development (AWCD), Shannon diversity index (H'), and area under the curve (AUC) for individual carbon sources - were calculated using the Biolog substrate utilization data (Figure 1 and Table 3). Where initial inoculum density has been standardised the AWCD is a measure of the rate of microbial activity. Values for AWCD were significantly lower under the CSCP treatment than under the OSOP treatment ($p< 0.01$) indicating higher levels of microbial activity under fully organic management. The Shannon diversity index (H') is a measure of the potential functional diversity of the microbial community and takes into account both the number of substrates and their degree of utilization [30.] Differences shown by AWCD were more pronounced than those observed using H' as has been noted elsewhere [30] . Soil treatment had a significant main effect ($p<0.05$) in the AWCD and Shannon diversity index data. In the experimental design used here the organic soil (OS) treatment is itself composed of crop rotation, use of organic fertilizer inputs and use of cover crops. Thus it is not possible to identify the relative contribution of specific individual practices within the soil treatment.. Several studies [31] [32] [33] report a proliferation of bacterial and fungal populations and activity under organic management. Conversely some studies have shown crop rotation, and in particular the specific crop species grown in the previous 3 years, to be the dominant management factor [34.]

The area under the curve (AUC) is useful as a summary statistic in analysing BIOLOG data since it incorporates both the maximum colour development and the rate of colour development [24] . Mean AUC values for utilisation of the 31 individual sole carbon sources found on Biolog Eco-plates for the OSOP and CSCP treatment soils were calculated (Table 3). The microbial community under the fully organic treatment (OSOP) showed significantly higher utilisation of sugar derivatives, carboxylic acids and polymers ($p<0.05$) than under the fully conventional CSCP treatment. In terms of individual sole carbon sources the sugars D-cellobiose and D-xylose, and the carboxylic acids pyruvic acid methyl ester and 4-hydroxybenzioc acid were utilised at higher levels in the organic (OSOP) plots than in the conventional (CSCP) plots. Conversely the sugar i-erythritol showed significantly lower utilization under the OSOP treatment.

Culturable microbial population and total nematode counts

Culturable bacteria were in the range 0.87×10^8 cfu g^{-1} under the fully conventional CSCP treatment to 7.87×10^8 cfu g^{-1} under the fully organic OSOP treatment and were significantly higher under the fully organic (OSOP)) treatment (Figure 2). Culturable fungal and actinomycete counts were in the range 1.93×10^7 cfu g^{-1} to 8.87×10^7 cfu g^{-1}; and 4.71×10^8 cfu g^{-1} to 7.71×10^8 cfu g^{-1} respectively. Nematode abundance ranged from 1.33 ± 0.22 nematodes g^{-1} dry soil under the fully conventional (CSCP) treatment to 2.14 ± 0.16 nematodes g^{-1} dry soil under the fully organic (OSOP) treatment. Although there was a clear trend of higher fungal and nematode counts under organic management the differences were not statistically significant. Increased bacterial counts or bacterial biomass, and microbial biomass and activity have been reported elsewhere in organically managed systems [3, 35] and in response to crop rotation practices [36] . Similarly total nematode abundance has been reported to increase in response to organic matter fertiliser [37].Animal manure or composts are a very important component of organic agriculture, and several studies report on pathogenic nematode and disease suppression after soils are amended with animal manures [38]. A linear decrease in the population of the plant pathogenic nematode *Meloidogine incognita* with increasing numbers of bacteria after the application of chicken litter in the soil, and a lower infection by nematodes on tomato plants when un-sterilised as compared to sterilised chicken manure was used has been demonstrated [39,40] . Other studies report suppression of soil borne fungal pathogens such as *Pythium*, *Fusarium*, *Phytophthora* and *Rhizoctonia* [38,41].

It has often been suggested that the soil fungal/bacterial ratio is indicative of ecological succession, with bacteria dominant (low F/B ratio) in disrupted soils such as arable and horticultural soils, whilst fungi are increasingly dominant (high F/B ratios) under established grassland and forest soils. For horticultural production F/B ratios in the region of 0.3 to 1 are generally considered optimal. Increased F/B ratios have been reported in response to a number of agricultural practices including continuous cropping of a single crop, adoption of no tillage systems, application of organic matter with a high C:N ratio and transition to grass [2 36, 42]; whilst decreases in the F/B ratio have been reported following addition of compost, slurry, FYM or mineral N, and in response to tillage [2,42] . The fungal/bacterial ratio based on count data was in the range 0.08 to 0.29 (Figure 3) however no significant differences in F/B ratio under different treatments were found.

DGGE and sequence analysis

Molecular techniques provide powerful new tools to investigate soil microbial populations. Fungal and bacterial DGGE and sequence data in this study indicated that soil fungi predominantly belonged to the Ascomycetes whilst isolated bacterial sequences belonged primarily to the Actinobacteria and Proteobacteria (Tables 4 and 5). A number of previous studies found ascomycete fungi to be predominant in agricultural and grassland soils [43.]

Fungal and bacterial community fingerprints obtained by DGGE were generally consistent across the 4 field replicates for each treatment (data not shown). Representative samples for each treatment were then compared on a single gel (Figure 4). For the fungal DGGE profile some variability in banding patterns between replicates was observed in the OSCP treatment, and two lanes encompassing this variability were run. Comparison of sequence data suggests that both organic soil and organic pest-control treatments may have beneficial effects in terms of disease and soil disease suppresiveness. Putative fungal onion pathogens were sequenced primarily under the conventional soil (CSCP and CSOP) treatments, whilst putative soil suppressive bacterial species (*Lysobacter* sp. and the Actinomycetes) were sequenced predominantly under the organic pest-control treatment.

The majority of fungal species obtained belonged to the Ascomycetes, with smaller groups of Basidiomycetes, Chitridiomycetes and Zygomycetes. Two sequences showing relatively low homology to yeasts and to the unclassified soil flagellate *Proleptomonas faecicola* formed a small out-group. Two common soil saprophyte fungi *Chaetomium globosum* and *Mortierella* were widely distributed and were found in soil samples from all four treatments. Sequences corresponding to the causative organisms of several important onion diseases including Fusarium basal rot (*Fusarium* spp.); leaf blotch (*Cladosporium* spp.); pink root (*Phoma* spp.); stunting (*Rhizoctonia* spp.) and storage black mould (*Aspergillu*s spp.) were identified and were predominantly found under the conventional soil (CS) treatment. Although the pathogenicity of the individual strains present is not known it is of interest that 10 of the 11 putative onion pathogen sequences were found in the CSCP and CSOP treatment soil samples. *Verticillium* spp. can cause disease in a number of dicot crops and ornamentals, but do not affect monocots such as onion and were sequenced from OSOP and OSCP treatments. Fungal genera which can contribute to soil disease suppressiveness include non-pathogenic *Fusarium* spp., *Penicillium* spp., and *Trichoderma* spp. [43].Potential soil suppressive DGGE bands included one *Trichoderma* sequence found under the OSOP treatment, and a second found in the CSCP treatment. A number of parasitic or entomopathogenic fungi were also identified.

Nine unique bacterial sequences including *Nocaroides, Friedmaniella*, *Cellvibrio,* and *Lysobacter* species were obtained for the fully organic treatment. All nine sequences belonged to the Proteobacteria or Actinobacteria (including Actinomcyete) groups. The occurrence of Actinomycetes and *Lysobacter* species are of particular interest since they have been implicated in soil disease suppresivenesss [44-46] . Seven unique bacterial sequences including *Friedmaniella* and *Mycobacterium* sp. were obtained from the fully conventional treatment. These sequences were phylogenetically more diverse and belonged to Verucomicrobia, Parachlamidiae, Actinobacteria (including Actinomycete and Mycobacteriaceae) groups. No onion pathogenic bacterial species were identified under any treatment.

CONCLUSIONS

Data presented here indicate that organic management practices can have a beneficial effect on biotic aspects of soil health in a cultivated onion crop, including microbial activity and diversity. The sequence data is indicative of the predominance of different functional groups (i.e. soil suppressive vs. potential pathogens) under different management practices and warrants further investigation. Application of large scale pyrosequencing [43] and/or use of primers for specific species or for specific functional groups in combination with DGGE, qPCR and conventional PCR [34,47-49] would be of interest over a range of time-points to characterize functional microbial groups under the agricultural management treatments used in this study.

ACKNOWLEGEMENTS

The authors thank Prof. Carlo Leifert and Dr. Charles Merfield for helpful discussion in designing the Systems Comparison trial; and L. Finn and C. Roberts for maintaining the trial. The Irish Department of Agriculture, Fisheries and Food (FIRM 06/NITARFC6) is gratefully acknowledged for financial support. TLL thanks IRCSET and OGT for financial support.

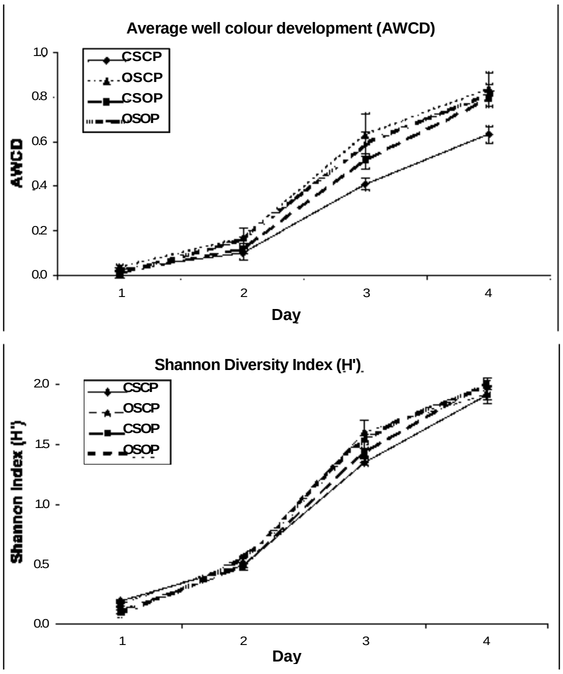

Figure 1. Average Well Colour Development (AWCD) and Shannon Diversity Index (H') over time on BIOLOG Eco-plates for different soil treatments. Values shown are the mean ± SE (n=4). Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control.

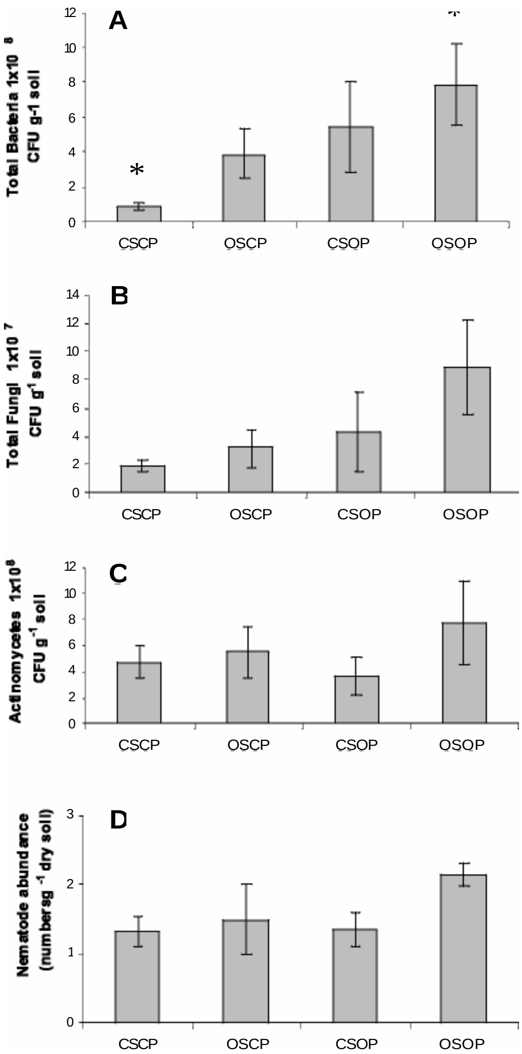

Figure 2. Culturable (A) bacteria, (B) fungi, (C) actinomycetes, and (D)total nematode counts under different treatments. Each value is the mean ± SE of 4 field replicates. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Asterisks show significant difference ($p<0 .05$).

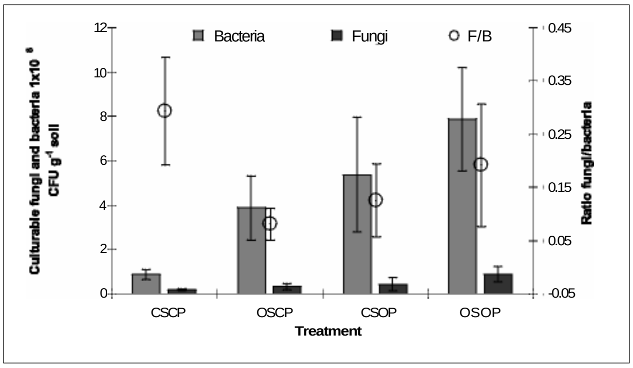

Figure 3. Culturable bacterial and fungal counts and F/B ratio under different cultivation treatments. Each value is the mean ± SE of 4 field replicates. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control.

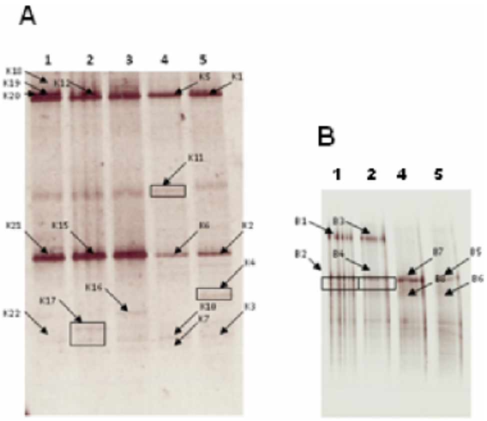

Figure 4. Denaturing gradient gel electrophoresis profile of (A) fungal and (B) bacterial rDNA sequences amplified from soil under different management treatments. Lane 1= OSOP, 2 =OSCP, 3= OSCP, 4 =CSCP, 5= CSOP. Treatment codes as before. Bands indicated by arrows were excised and sequenced. Where regions of the gel clearly contained >1 band the excised region is shown boxed.

Table 1. Onion pest-control and soil treatments used in the Teagasc Kinsealy Systems Comparison trial in 2011

PEST-CONTROL	
Organic pest-control (OP)	Mechanical weeding (hand hoeing).
Conventional pest-control (CP)	Proplant[2] (10ml m^{2-1} modular drench) , Roundup[1] (4L ha^{-1}) , Stomp[1] (3.3L ha^{-1}), CICP[1] (4.2L ha^{-1}), Defy[1] (3.3L ha^{-1}), Folio Gold [2] (2L ha^{-1}), Penncozeb[2] (4.4 kg ha^{-1}).
SOIL TREATMENT	
Organic soil treatment (OS)	Previous crop – broccoli cv. Belstar N 70 kg ha^{-1} P20kgha^{-1} K215 kg ha^{-1} Applied as Greenvale plant food (4.5:3:3) (pelleted chicken manure + calcified seaweed) and ProKali (3:0:14). A top dress equivalent to 35kg ha^{-1} N, and contributing 25kg ha^{-1} P and 24kg ha^{-1} K was applied on 22nd June 2011.
Conventional soil treatment (CS)	Previous crop – broccoli / carrot / lettuce N 70 kg ha^{-1} P20kgha^{-1} K215 kg ha^{-1} Applied as CAN (27% N), single superphosphate (7.8%P) and sulphate of potash (42% K). A top dress equivalent to 35kg ha^{-1} N, 25kg ha^{-1} P and 24kg ha^{-1} K was applied on 22nd June 2011.

[1] Herbicide, [2] Fungicide. No insecticide treatments were required.

Table 2. Primers used for DGGE PCR.

Primer	Target	Sequence 5' - 3'	Product size (bp)	Ref.
FR1	Fungal 18S rDNA	* AICCATTCAATCGGTAIT		
FF390	Fungal 18S rDNA	CGATAACGAACGAGACCT	390	1
R1378	Bacterial 16S rDNA	CGGTGTGTACAAGGCCCGGGAACG		
F984GC	Bacterial 16S rDNA	*AAC GCG AAG AAC CTT AC	433	2

* GC clamp 5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG -3'

Table 3. Sole carbon source utilisation profiles on BIOLOG Eco-plates for soils under fully organic (OSOP) and fully conventional (CSCP) treatments. Values shown are the mean area under the curve (AUC) values for each C source ± SE (n=4). Values with the same letter were significantly different between treatments.

		OSOP	CSCP
SUGAR DERIVATIVES	D-Cellobiose	2.82 ± 0.06 [a]	0.36 ± 0.21 [a]
	a-D-Lactose	0.74 ± 0.05	0.24 ± 0.24
	p-Methyl- D-Glucoside	1.91 ± 0.35	0.71 ± 0.51
	D-Xylose	0.54 ± 0.10 [b]	0.03 ± 0.10 [b]
	i-Erythritol	-0.07 ± 0.02 [c]	0.03 ± 0.02 [c]
	D-Mannitol	2.20 ± 0.27	1.81 ± 0.16
	N-Acetyl-D-Glucosamine	2.62 ± 0.24	1.79 ± 0.50
	TOTAL SUGAR DERIVATIVES	**10.75 ± 0.79** [f]	**4.97 ± 1.47** [f]
SUGAR PHOSPHATES	D,L-a-Glycerol Phosphate	0.35 ± 0.03	0.39 ± 0.06
	Glucose-1-Phosphate	0.09 ± 0.15	-0.07 ± 0.07
	TOTAL SUGAR PHOSPHATES	**0.44 ± 0.17**	**0.31 ± 0.08**
CARBOXYLIC ACIDS	Pyruvic Acid Methyl Ester	1.96 ± 0.13 [d]	1.36 ± 0.08 [d]
	D-Glucosaminic Acid	0.29 ± 0.04	0.23 ± 0.04
	D-Galactonic Acid y-Lactone	1.80 ± 0.07	1.79 ± 0.12
	D-Galacturonic Acid	1.90 ± 0.15	1.55 ± 0.36
	2-Hydroxy Benzoic Acid	0.60 ± 0.42	-0.01 ± 0.06
	4-Hydroxy Benzoic Acid	2.36 ±0.32 [e]	1.43 ±0.10 [e]
	y-HydroxybutyricAcid	0.15 ± 0.11	0.20 ± 0.13
	Itaconic acid	1.19 ± 0.02	1.22 ± 0.61
	aKetobutyric Acid	-0.18 ± 0.03	-0.17 ± 0.03
	D-Malic Acid	1.37 ± 0.16	0.57 ± 0.24
	TOTAL CARBOXYLIC ACIDS	**11.45 ± 0.46** [g]	**8.16 ± 1.15** [g]
AMINO ACIDS	L-Arginine	2.03 ± 0.23	1.48 ± 0.21
	L-Asparagine	3.75 ± 0.32	3.25 ± 0.22
	L-Phenylalanine	0.49 ± 0.07	0.16 ± 0.11
	L-Serine	2.37 ± 0.18	2.13 ± 0.15
	L-Threonine	0.16 ± 0.19	-0.09 ± 0.04
	Glycyl-L-Glutamic Acid	0.25 ± 0.12	0.09 ± 0.12
	Phenylethylamine	1.46 ± 0.38	1.02 ± 0.14
	Putrescine	1.90 ± 0.27	1.94 ± 0.07
	TOTAL AMINO ACIDS	**12.40 ± 1.09**	**9.98 ± 0.42**
POLYMERS	Tween40	1.05 ±0.06	0.64±0.21
	Tween80	1.72 ± 0.13	1.86 ±0.15
	a-Cyclodextrin	0.29 ± 0.12	0.03 ± 0.07
	Glycogen	2.67 ± 0.58	0.92 ± 0.69
	TOTAL POLYMERS	**5.73 ± 0.64** [h]	**3.44 ± 0.87** [h]

Table 4. Identification, classification and assigned Genbank accession numbers of cloned fungal 1 8s SSU ribosomal DGGE bands from soil under different treatments.

Potential onion pathogens are indicated in bold. Potential soil disease suppressive fungal genera are highlighted in grey text.

Soil treatment	Pest control treatment	DGGE Band	Identity	Closest hit (blast n)	Maximum identity	Genbank Accession number
CS	CP	K5	*Mortierella* sp.	*Mortierella* sp (EU71 0842.1)	99%	JX560271
CS	CP	K5b	*Mortierella* sp.	*Mortierella* sp. (JF895929.1)	99%	JX560272
CS	CP	K5c	*Mortierella* sp.	*Mortierella* sp. (JF895929.1)	99%	JX560273
CS	CP	K5d	*Mortierella* sp.	*Mortierella* sp. (JF895929.1)	99%	JX560274
CS	CP	K6	*Tricholoma* sp.	*Tricholoma ponderosum* (D84673. 1)	98%	JX560275
CS	CP	K6b	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560276
CS	CP	K6c	***Fusarium* sp.**	*Fusarium oxysporum* (JF807401 .1)	100%	JX560277
CS	CP	K1 1b	Similar to *Proleptomonas faecicola*	*Proleptomonas faecicola* (GQ377682. 1)	86%	JX560284
CS	CP	K1 1 d	Similar to *Stilbella fimetaria*	*Stilbella fimetaria* (FJ939395. 1)	99%	JX560285
CS	CP	K1 1 e	Similar to *Nectria lugdunensis*	*Nectria lugdunensis* (AY357278.1)	99%	JX560286
CS	CP	K1 1f	Similar to *Spathaspora* sp.	*Spathaspora passalidarum* (DQ232894.1)	89%	JX560287
CS	CP	K1 1g	Similar to *Resupinatus alboniger*	*Resupinatus alboniger* (DQ851 586.1)	100%	JX560288
CS	CP	K1 1 h	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560289
CS	CP	K7c	*Thelebolus* sp.	*Thelebolus* sp. (GU004234.1)	99%	JX560278
CS	CP	K7b	*Tricholoma* sp.	*Tricholoma ponderosum* (D84673. 1)	98%	JX560279
CS	CP	K7d	*Anguillospora* sp.	*Anguillospora mediocres* (AY357264.1)	99%	JX560280
CS	CP	K10a	***Phoma* sp.**	*Phoma* sp. (JQ83801 1.1)	99%	JX560281
CS	CP	K10b	***Trichoderma* sp.**	*Trichoderma harzianum* (JQ806366.1)	99%	JX560282
CS	CP	K1 0c	Similar to *Tricholoma* sp.	*Tricholoma ponderosum* (D84673. 1)	97%	JX560283
CS	OP	K1	*Mortierella* sp.	Mortierella sp. (EU71 0842.1)	98%	JX560256
CS	OP	K1 b	Similar to *Acremonium*	*Acremonium minutisporum* (HQ2321 99.1)	98%	JX560257
CS	OP	K1 c	***Fusarium merismoides***	*Fusarium merismoides* (AF1 41950.1)	100%	JX560258
CS	OP	K1d	*Chaetomium globosum*	*Chaetomium globosum* strain (JN639021 .1)	100%	JX560259
CS	OP	K1e	***Phoma* sp.**	*Phoma exigua* var. *Exigua* (EU342941 .1)	99%	JX560260
CS	OP	K2a	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560261
CS	OP	K2b	***Fusarium oxysporum***	*Fusarium oxysporum* (JQ926985.1)	100%	JX560262
CS	OP	K3	***Phoma* sp.**	*Phoma* sp. (JQ83801 1.1)	99%	JX560263

CS	OP	K3b	***Aspergilus* sp.**	*Aspergillus niger* (JX1 12703.1)	99%	JX560264
CS	OP	K3c	***Phoma* sp.**	*Phoma* sp. (JQ83801 1.1)	99%	JX560264
CS	OP	K3d	***Rhizoctonia solani***	*Rhizoctonia solani* (D85643.1)	99%	JX560266
CS	OP	K4a	*Thelebolus* sp.	*Thelebolus* sp. (GU004234.1)	100%	JX560267
CS	OP	K4b	***Cladosporium* sp.**	*Cladosporium bruhnei* (JN397376.1)	99%	JX560268
CS	OP	K4e	*Thelebolus* sp.	*Thelebolus* sp. (GU004234.1)	98%	JX560269
CS	OP	K4f	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560270
OS	CP	K1 2a	Similar to *Elaphocordyceps sp.*	*Elaphocordyceps ophioglossoides* (JN941 734.1)	99%	JX560290
OS	CP	K12b	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560291
OS	CP	K12d	Similar to *Verticillium* sp.	*Verticillium* sp. (AJ557787.1)	99%	JX560292
OS	CP	K1 5a	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560293
OS	CP	K1 5b	Similar to *Phyllachora graminis*	*Phyllachora graminis* (AF064051 .1)	98%	JX560294
OS	CP	K1 5c	***Fusarium* sp.**	*Fusarium oxysporum* (JQ926985.1)	100%	JX560295
OS	CP	K16a	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	100%	JX560296
OS	CP	K16b	Similar to *Humicola* sp.	*Humicola* sp. (EU71 0839.1)	99%	JX560297
OS	CP	K1 7b	*Trichosporon* sp.	*Trichosporon gracile* (JN939432. 1)	100%	JX560298
OS	CP	K1 7c	Similar to *Chytridium polysiphoniae*	*Chytridium polysiphoniae* (AY032608. 1)	96%	JX560299
OS	OP	K1 8a	*Cordyceps gunnii*	*Cordyceps gunnii* (HM1 35160.1)	99%	JX560300
OS	OP	K18b	Similar to *Miladina lecithina*	*Miladina lecithina* (DQ646538.1)	99%	JX560301
OS	OP	K18c	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	99%	JX560302
OS	OP	K18d	*Verticillium* sp.	*Verticillium* sp. (AJ557787.1)	99%	JX560303
OS	OP	K1 9a	*Mucor* sp.	*Mucor hiemalis* (JN397378. 1)	99%	JX560304
OS	OP	K19b	*Mortierella* sp.	*Mortierella* sp. (JF895929.1)	99%	JX560305
OS	OP	K20a	*Mortierella* sp.	*Mortierella indohii* (EU688965. 1)	99%	JX560306
OS	OP	K20c	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	100%	JX560307
OS	OP	K21 c	*Powellomycetaceae* sp.	*Powellomycetaceae* sp. (HQ901 755.1)	99%	JX560308
OS	OP	K21d	*Chaetomium globosum*	*Chaetomium globosum* (JN639021 .1)	100%	JX560309
OS	OP	K21b	Similar to *Clavicepspurpurea*	*Clavicepspurpurea* (AB490177.1)	99%	JX560310
OS	OP	K22b	**Similar to *Trichoderma* sp.**	*Trichoderma* sp. (JF895925.1)	99%	JX56031 1

REFERENCES

1. Sturz AV and Christie BR, Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil & Tillage Research* **72**:107-123 (2003).

2. Griffiths BS, Ball BC, Daniell TJ, Hallett PD, Neilson R, Wheatley RE, Osler G and Bohanec M, Integrating soil quality changes to arable agricultural systems following organic matter addition, or adoption of a ley-arable rotation. *Applied Soil Ecology* **46**:43-53 (2010).

3. Wu SM, Hu DX and Ingham ER, Comparison of soil biota between organic and conventional agroecosystems in Oregon, USA. *Pedosphere* **15**:395-403 (2005).

4. Ghorbani R, Wilcockson S, Koocheki A and Leifert C, Soil management for sustainable crop disease control: a review. *Environmental Chemistry Letters* **6**:149-162 (2008).

5. Tamm L, Thurig B, Bruns C, Fuchs JG, Kopke U, Laustela M, Leifert C, Mahlberg N, Nietlispach B, Schmidt C, Weber F and Fliessbach A, Soil type, management history, and soil amendments influence the development of soil-borne (Rhizoctonia solani, Pythium ultimum) and air-borne (Phytophthora infestans, Hyaloperonospora parasitica) diseases. *European Journal of Plant Pathology* **127**:465-481(2010).

6. Shannon D, Sen AM and Johnson DB, A comparative study of the microbiology of soils managed under organic and conventional regimes. *Soil Use Manage* **18**:274-283 (2002).

7. Wu T, Chellemi DO, Graham JH, Martin KJ and Rosskopf EN, Comparison of soil bacterial communities under diverse agricultural land management and crop production practices. *Microbial Ecology* **55**:293-310 (2008).

8. Birkhofer K, Bezemer TM, Bloem J, Bonkowski M, Christensen S, Dubois D, Ekelund F, Fliessbach A, Gunst L, Hedlund K, Mader P, Mikola J, Robin C, Setala H, Tatin-Froux F, Van der Putten WH and Scheu S, Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. *Soil Biology & Biochemistry* **40**:2297-2308 (2008).

9. El Fantroussi S, Verschuere L, Verstraete W and Top EM, Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Applied and Environmental Microbiology* **65**:982-988 (1999).

10. Zhang QC, Shamsi IH, Xu DT, Wang GH, Lin XY, Jilani G, Hussain N and ChaudhryAN, Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Applied Soil Ecology* **57**:1-8 (2012).

11. Hitzl W, Rangger A, Sharma S and Insam H, Separation power of the 95 substrates of the BIOLOG system determined in various soils. *Fems Microbiology Ecology* **22**:167-174 (1997).

12. Insam H, and Goberna, M., Use of Biolog for the Community Level Physiological Profiling (CLPP) of Environmental samples, in *Molecular Microbial Ecology Manual*

ed. by Kowalchuk GA, Bruijn, F.J., Head, I.M., Akkermans, A.D., van Elsas, J.D. (Eds.). Springer, pp 853 -860 (2004).

13. Cocolin L, Manzano M, Cantoni C and Comi G, Denaturing gradient gel elec trophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. *Applied and Environmental Microbiology* **67**:5113-5121(2001).

14. Heuer H, Krsek M, Baker P, Smalla K and Wellingto n EMH, Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology* **63**:3233-3241 (1997).

15. Wallis PD, Haynes RJ, Hunter CH and Morris CD, Effect of land use and management on soil bacterial biodiversity as measured by PCR-DGGE. *Applied Soil Ecology* **46**:147-150 (2010).

16. Duong LM, Jeewon R, Lumyong S and Hyde KD, DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. *Fungal Diversity* **23**:121-138 (2006).

17. Vainio EJ and Hantula J, Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research* **104**:927-936 (2000).

18. Zhong WH, Gu T, Wang W, Zhang B, Lin XG, Huang QR and Shen WS, The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant Soil* **326**:511-522 (2010).

19. EU, Council Regulation No. 2092/91 of 24th June on organic production of agricultural pro ducrs and indications referring thereto on agricultural products and foodstuffs. *OJL 198 227p1* (1991).

20. EU, Council Regulation (EC) No 834/2007 of 28th June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/ 91 *Official Journal of the European Uniion* **20.7.2007** (2007).

21. Lalor S, and Coulter, B., *Major and minor micronutrient advice for productive agricultural crops*. Teagasc, Oakpark, Carlow (2008).

22. Alexander S, *Approved Pesticides for use on vegetable crops 2011*. Teagasc, Kinsealy, Dublin 17.

23. Yoder M, De Ley IT, King IW, Mundo-Ocampo M, Mann J, Blaxter M, Poiras L and De Ley P, DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* **8**:367-376 (2006).

24. Hackett CA and Griffiths BS, Statistical analysis of the time-course of Biolog substrate utilization. *Journal of Microbiological Methods* **30**:63-69 (1997).

25. Macek M, Denaturing gradient gel electrophoresis (DGGE) protocol, in *MD for Hum Mutation 9:136*, Ed (1997).

26. Green SJ, Leigh, M.B. and Neufeld, J.D, Denaturing gradient gel electrophoresis (DGGE) for microbial community analysis, in *Handbook of Hydrocarbon and Lipid Microbiology*, ed. by Timmis K, N., Heidelberg, pp 41 37-4158 (2009).

27. Kusar D and Avgustin G, Optimization of the DGGE band identification method. *Folia Microbiologica* **57**:301-306 (2012).

28. Jackson CR, Roden, E.E., and Churchill P.F., Denaturing Gradient Gel Electrophoresis Can Fail to Separate 16S rDNA Fragments with Multiple Base Differences. *Molecular Biology Today* **1**:49-51(2000).

29. Altschul SF, Madden TL, SchafferAA, Zhang JH, Zhang Z, MillerW and Lipman DJ, Gapped BLAST and PSI - BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**:3389-3402 (1997).

30. Marongiu R, Garau G, Caredda M and Dei ana P, Impact of soil management on the functional activity of microbial communities associated to cork oak rhizosphere
in *First International Symposium on Environment Identities and Mediterranean Area* Ed, pp 46-50 (2006).

31. Tejada M, Benitez C, Gomez I and Parrado J, Use of biostimulants on soil restoration: Effects on soil biochemical properties and microbial community. *Applied Soil Ecology* **49**:11-17 (2011).

32. Bastida F, Kandeler E, Moreno JL, Ros M, Garcia C and Hernandez T, Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. *Applied Soil Ecology* **40**:318-329 (2008).

33. Liu B, Gumpertz ML, Hu S and Ristaino JB, Long -term effects of organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight. *Soil Biology & Biochemistry* **39**:2302-2316 (2007).

34. Orr CH, James A, Leifert C, Cooper JM and Cummings SP, Diversity and Activity of Free -Living Nitrogen-Fixing Bacteria and Total Bacteria in Organic and Conventionally Managed Soils. *Applied and Environmental Microbiology* **77**:911-919 (2011).

35. Reganold JP, Andrews PK, Reeve JR, Carpenter -Boggs L, Schadt CW, Alldredge JR, Ross CF, Davies NM and Zhou JZ, Fruit and Soil Quality of Organic and Con ventional Strawberry Agroecosystems. *Plos One* **5** (2010).

36. Liu XB and Herbert SJ, Fifteen years of research examining cultivation of continuous soybean in northeast China: A review. *Field Crops Research* **79**:1-7 (2002).

37. Verhoeven R, Response of soil microfauna to organic fertilisation in sandy virgin soils of coastal dunes. *Biol Fertil Soils* **34**:390-396 (2001).

38. Oka Y, Mechanisms of nematode suppression by organic soil amendments -A review. *Applied Soil Ecology* **44**:101-115 (2010).

39. Kaplan M, Noe JP and Hartel PG, The role of microbes associated with chicken litter in the supression of *Meloidogyne arenaria Journal of Nematology* **24**:522-527 (1992).

40. Kaplan M and Noe JP, Effects of chicken excrement amendments on *Meloidogyne arenaria Journal of Nematology* **25**:71-77 (1993).

41. Hoitink HAJ, Zhang WZ, Han DY and Dick WA, Making compost to suppress plant disease. *Biocycle* **38**:40-42 (1997).

42. de Vries FT, Hoffland E, van Eekeren N, Brussaard L and Bloem J, Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology & Biochemistry* **38**:2092-2103 (2006).

43. Xu LH, Ravnskov S, Larsen J, Nilsson RH and Nicolaisen M, Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. *Soil Biology & Biochemistry* **46**:26-32 (2012).

44. Folman LB, Postma J and van Veen JA, Characterisation of Lysobacter enzymogenes (Christensen and Cook 1978) strain 3.1T8, a powerful antagonist of fungal diseases of cucumber. *Microbiological Research* **158**:107-115 (2003).

45. Postma J, Schilder MT, Bloem J and van Leeumen-Haagsma WK, Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. *Soil Biology & Biochemistry* **40**:2394-2406 (2008).

46. Govaerts B, Mezzalama M, Sayre KD, Crossa J, Lichter K, Troch V, Vanherck K, De Corte P and Deckers J, Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. *Applied Soil Ecology* **38**:197-210 (2008).

47. Wakelin SA, Nelson PN, Armour JD, Rasiah V and Colloff MJ, Bacterial community structure and denitrifier (nir-gene) abundance in soil water and groundwater beneath agricultural land in tropical North Queensland, Australia. *Soil Res* **49**:65-76 (2011).

48. Barrett MB, Bhreathnach, N.B., O'Flaherty, V., Johnston, P., Khalil, M. I. and Richards, K. , *Subsurface denitrification and microbial diversity within soils and groundwater* . Teagasc (2009).

49. Ipsilantis I, Samourelis C and Karpouzas DG, The impact of biological pesticides on arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* **45**:147-155 (2012).

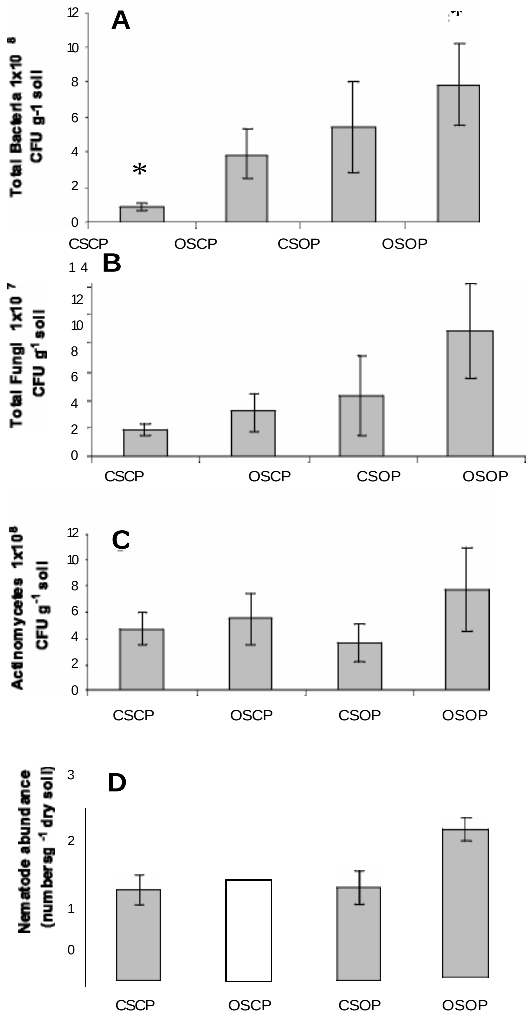

Figure 2. Culturable (A) bacteria, (B) fungi, (C) actinomycetes, and (D)total nematode counts under different treatments. Each value is the mean ± SE of 4 field replicates. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Asterisks show significant difference ($p<0.05$).