SOILBORNE DISEASE SUPPRESSIVENESS / CONDUCIVENESS: ANALYSIS OF MICROBIAL COMMUNITY DYNAMICS

by

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Each soil is an individual body of nature, possessing its own character, life history, and powers to support plants and animals

Hans Jenny

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Language and style used in this dissertation are in accordance with the requirements of the journal *Soil Biology and Biochemistry*.

This dissertation represents a compilation of manuscripts, where each chapter is an individual entity and some repetition between the chapters has been unavoidable

SUMMARY

Take-all is the name given to the disease caused by a soilborne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*), an ascomycete of the family Magnaportheaceae (Cook, 2003). This fungus is an aggressive soil-borne pathogen causing root rot of wheat (primary host), barley and rye crops (secondary host). The flowering, seedling, and vegetative growth stages can be affected by the infection of the whole plant, leaves, roots, and stems. Infections of roots result in losses in crop yield and quality primarily due to a lowering in nutrient uptake. Take-all is most common in regions where wheat is cultivated without adequate crop rotation. Crop rotation allows time between the planting dates of susceptible crops, which causes a decrease in the inoculum potential of soilborne plant pathogens to levels below an economic threshold by resident antagonistic soil microbial communities. Soilborne disease suppressiveness is an inherent characteristic of the physical, chemical, and/or biological structure of a particular soil which might be induced by agricultural practices and activities such as the cultivation of crops, or the addition of organisms or nutritional amendments, causing a change in the microfloral environment. Disturbances of soil ecosystems that impact on the normal functioning of microbial communities are potentially detrimental to soil formation, energy transfers, nutrient cycling, and long-term stability. In this regard, an overview of soil properties and processes indicated that the use of microbiological and biochemical soil properties, such as microbial biomass, the analysis of microbial functional diversity and microbial structural diversity by the quantification of community level physiological profiles and signature lipid biomarkers are useful as indicators of soil ecological stress or restoration properties because they are more responsive to small changes than physical and chemical characteristics. In this study, the relationship between physico-chemical characteristics, and different biological indicators of soil quality of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat as caused by the soilborne fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), were investigated using various techniques. The effect of crop rotation on the functional and structural diversity of soils conducive to take-all disease was also investigated. Through the integration of quantitative and qualitative biological data as well as the physico-chemical characteristics of the various soils, the functional and structural diversity of microbial

communities in the soils during different stadia of take-all disease of wheat were characterised. All results were evaluated statistically and the predominant physical and chemical characteristics that influenced the microbiological and biochemical properties of the agricultural soils during different stadia of take-all disease of wheat were identified using multivariate analyses. Although no significant difference ($p > 0.05$) could be observed between the various soils using conventional microbiological enumeration techniques, the incidence of *Gliocladium* spp. in suppressive soils was increased. Significant differences ($p < 0.05$) were observed between agricultural soils during different stadia of take-all disease of wheat. Although no clear distinction could be made between soils suppressive and neutral to take-all disease of wheat, soils suppressive and conducive to take-all disease of wheat differed substantially in their community level physiological profiles (CLPPs). Soils suppressive / neutral to take-all disease were characterised by enhanced utilisation of carboxylic acids, amino acids, and carbohydrates, while conducive soils were characterised by enhanced utilisation of carbohydrates. Shifts in the functional diversity of the associated microbial communities were possibly caused by the presence of *Ggt* and associated antagonistic fungal and bacterial populations in the various soils. It was evident that the relationships amongst the functionality of the microbial communities within the various soils had undergone changes through the different stages of development of take-all disease of wheat, thus implying different substrate utilisation capabilities of present soil microbial communities. Diversity indices were calculated as Shannon's diversity index (H') and substrate equitability (J) and were overall within the higher diversity range of 3.6 and 0.8, respectively, indicating the achievement of very high substrate diversity values in the various soils. A substantial percentage of the carbon sources were utilised, which contributed to the very high Shannon-Weaver substrate utilisation indices. Obtained substrate evenness (equitability) (J) indices indicated an existing high functional diversity. The functional diversity as observed during crop rotation, differed significantly ($p < 0.05$) from each other, implying different substrate utilisation capabilities of present soil microbial communities, which could possibly be ascribed to the excretion of root exudates by sunflowers and soybeans. Using the Sorenson's index, a clear distinction could be made between the degrees of substrate utilisation between microbial populations in soils conducive, suppressive, and neutral to take-all disease of wheat, as well as during crop rotation. Furthermore, the various soils could also be differentiated on the basis of the microbial community structure as determined by phospholipid fatty acid (PLFA) analysis. Soil suppressive to take-all disease of wheat differed significantly

($p < 0.05$) from soils conducive, and neutral to take-all disease of wheat, implying a shift in relationships amongst the structural diversity of microbial communities within the various soils. A positive association was observed between the microbial phospholipid fatty acid profiles, and dominant environmental variables of soils conducive, suppressive, and neutral to take-all disease of wheat. Soils conducive and neutral to take-all disease of wheat were characterised by high concentrations of manganese, as well as elevated concentrations of monounsaturated fatty acids, terminally branched saturated fatty acids, and polyunsaturated fatty acids which were indicative of Gram-negative bacteria, Gram-positive bacteria and microeukaryotes (primarily fungi), respectively. These soils were also characterised by low concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as low soil pH. Soil suppressive to take-all disease of wheat was characterised by the elevated levels of estimated of biomass and elevated concentrations of normal saturated fatty acids, which is ubiquitous to microorganisms. The concentration of normal saturated fatty acids in suppressive soils is indicative of a low structural diversity. This soil was also characterised by high concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as elevated soil pH. The relationship between PLFAs and agricultural soils was investigated using principal component analysis (PCA), redundancy analysis (RDA) and discriminant analysis (DA). Soil suppressive to take-all disease of wheat differed significantly ($p < 0.05$) from soils conducive, and neutral to take-all disease of wheat, implying a shift in relationships amongst the structural diversity of microbial communities within the various soils. A positive association was observed between the microbial phospholipid fatty acid profiles, and dominant environmental variables of soils conducive, suppressive, and neutral to take-all disease of wheat. Hierarchical cluster analysis of the major phospholipid fatty acid groups indicated that the structural diversity differed significantly between soils conducive, suppressive, and neutral to take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*. The results indicate that the microbial community functionality as well as the microbial community structure was significantly influenced by the presence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, and that the characterisation of microbial functional and structural diversity by analysis of community level physiological profiles and phospholipid fatty acid analysis, respectively, could be successfully used as an assessment criteria for the evaluation of agricultural soils conducive, suppressive, and neutral to take-all disease of wheat, as well as in crop rotation systems. This

methodology might be of significant value in assisting in the management and evaluation of agricultural soils subject to the prevalence of other soilborne diseases.

Keywords: Soil microbial communities; Community level physiological profiles (CLPP); *Gaeumannomyces graminis* var. *tritici*; Take-all disease; Crop rotation; Diversity indices; Soil microbial community structure; Phospholipid fatty acids (PLFAs)

"Vrotpootjie" is die naam van die siekte wat veroorsaak word deur die grond-fungus *Gaeumannomyces graminis* (Sacc.) von Arx en Olivier var. *tritici* Walker (*Ggt*), 'n askomyseet van die Magnaportheaceae familie (Cook, 2003). Die fungus is 'n aggressiewe grondgedraagde patogeen wat wortel-verrotting van koring (primêre gasheer), gars en rog (sekondêre gashere) veroorsaak. Die blom-, saailing- en vegetatiewe groei fases kan beïnvloed word deur infeksie van die hele plant, blare, wortels en stamme. Infeksie van die wortels lei tot verliese in gewas-opbrengs en kwaliteit hoofsaaklik weens 'n verlaging in voedingstof-opname. "Vrotpootjie" is veral algemeen in gebiede waar koring verbou word en onvoldoende wisselbou plaasvind. Wisselbou laat tyd toe tussen die aanplantdatums van vatbare gewasse, wat lei tot 'n verlaging in die inokulum-potensiaal van die grondgedraagde patogene tot 'n vlak onder 'n ekonomiese drempel deur reeds teenwoordige antagonistiese grond mikrobiese gemeenskappe. Die onderdrukking van grondgedraagde siektes is 'n inherente eienskap van die fisiese-, chemiese-, en / of biologiese struktuur van 'n gegewe grond wat geïnduseer kan word deur die landboukundige praktyke wat daarop toegepas word, asook aktiwiteite soos die verbouing van gewasse, of die toediening van organismes of voedingsaanvullings, wat lei tot die veranderinge in die grond se mikroflora-omgewing. Versteurings van grond-ekosisteme wat 'n impak het op die normale funksionering van die mikrobiese gemeenskappe, is potensiëel nadelig vir grondvorming, energie-oordragte, voedingstofsirkulering en langtermyn stabiliteit. In hierdie opsig het 'n oorsig van grondeienskappe en -prosesse getoon dat die gebruik van mikrobiologiese en biochemiese grondeienskappe soos mikrobiese biomassa, die analise van mikrobiese funksionele diversiteit en mikrobiese strukturele diversiteit deur die kwantifisering van gemeenskapsvlak fisiologiese profiele en kenmerkende lipied biomerkers as nuttige indikators van grond ekologiese stres of restoreringseienskappe kan dien omdat hulle meer gevoelig is vir klein veranderinge in vergelyking met fisies en chemiese eienskappe. Tydens hierdie projek is die verhouding / verwantskap tussen fisies-chemiese en verskillende biologiese eienskappe, van grond gehalte ten opsigte van die aanleidende, onderdrukkende en neutrale toestand van die grond ten opsigte van *Gaeumannomyces graminis* var. *tritici* (*Ggt*) ondersoek met behulp van verskeie tegnieke. Die effek van wisselbou op die funksionele en strukturele diversiteit van die

grond wat aanleidend is tot "vrotpootjie", is ook ondersoek. Deur die integrasie van kwantitatiewe en kwalitatiewe biologiese data, sowel as die fisies-chemiese eienskappe van die verskeie gronde, is die funksionele en strukturele diversiteit van die mikrobiese gemeenskappe in die gronde tydens verskillende stadia van "vrotpootjie" van koring gekarakteriseer. Alle resultate is statisties geëvalueer en die dominante fisiese en chemiese eienskappe wat die mikrobiese en biochemiese eienskappe tydens die verskeie stadia van "vrotpootjie" beïnvloed het, is geïdentifiseer deur middel van multi-veranderlike statistiese analises. Alhoewel geen statisties betekenisvolle verskille ($p > 0.05$) tussen die verskeie gronde met konvensionele mikrobiese tegnieke waargeneem kon word nie, is 'n toename in die voorkoms van *Gliocladium* spp. waargeneem. Statisties betekenisvolle verskille ($p < 0.05$) is waargeneem tussen die verskeie gronde in verskillende stadia van "vrotpootjie" van koring. Alhoewel geen duidelike onderskeid gemaak kon word tussen grond wat neutraal en onderdrukkend is vir "vrotpootjie" van koring nie, het daar wel duidelike verskille voorgekom tussen grond wat aanleidend en onderdrukkend is vir "vrotpootjie" van koring in terme van gemeenskapsvlak fisiologiese profiele (GVFPe). Grond wat onderdrukkend of neutraal was tot "vrotpootjie", was gekenmerk deur verhoogde verbruik van karboksielsure, aminosure en koolhidrate, terwyl gronde wat aanleidend was tot "vrotpootjie" gekenmerk is deur verhoogde gebruik van koolhidrate. Verskuiwings in die funksionele diversiteit van die geassosieerde mikrobiese gemeenskappe is moontlik veroorsaak deur die teenwoordigheid van *Ggt* en geassosieerde antagonistiese fungus- en bakteriese bevolkings in die verskeie gronde. Dit is opvallend dat die verwantskappe tussen die funksionaliteit van die mikrobiese gemeenskappe binne die verskeie gronde verandering ondergaan het deur die verskillende stadia van "vrotpootjie" wat op sy beurt verskillende substraatverbruikingskapasiteite vir die onderskeie teenwoordige grond gemeenskappe impliseer. Diversiteitsindekse is bereken as die Shannon-Weaver indeks (H') en subtraat-gelykheid / "substrate equitability" (J) indeks. Albei indekse het in die hoër diversiteitsgrense geval van 3.6 en 0.8, onderskeidelik, wat aandui dat baie hoë substraat diversiteit waardes behaal is in die verskeie gronde. 'n Aansienlike persentasie van die koolstofbronne is verbruik, wat bygedra het tot die baie hoë Shannon-Weaver substraat verbruikingsindeks. Verkrygde substraat-gelykheids-indekse (J) het ook 'n hoë bestaande funksionele diversiteit aangetoon. Die funksionele diversiteit soos waargeneem tydens wisselbou, het statisties betekenisvol van mekaar verskil ($p < 0.05$), wat impliseer dat verskillende substraatverbruikingsvermoëns voorgekom het, wat moontlik toegeskryf kan word aan die uitskeiding van verskillende

wortel-eksudate deur sonneblomme en sojabone. Deur gebruik te maak van die Sorenson indeks, kon 'n duidelike onderskeid gemaak word tussen die vlakke van substraatverbruik tussen mikrobiese populasies in gronde wat aanleidend, onderdrukkend en neutraal tot die ontstaan van "vrotpootjie" is, sowel as tydens wisselbou. Die verskillende gronde kon ook onderskei word op grond van die mikrobiese gemeenskapstruktuur soos bepaal deur fosfolipied vetsuur (FLVS) analises. Grond wat onderdrukkend is tot "vrotpootjie" van koring het statisties betekenisvol verskil van gronde van neutraal was tot "vrotpootjie", wat 'n verskuiwing in die verhoudings tussen die strukturele diversiteit van die mikrobiese gemeenskappe in die gronde impliseer. 'n Positiewe assosiasie is waargeneem tussen die mikrobiese fosfolipied vetsuur profiele en dominante omgewingsveranderlikes in gronde aanleidend, ondrukkend en neutraal tot "vrotpootjie". Gronde wat aanleidend en neutraal was tot "vrotpootjie", is gekenmerk deur hoë konsentrasies van magnesium, asook verhoogde konsentrasies mono-onversadigde vetsure, terminaal vertakte versadigde vetsure, en poli-onversadigde vetsure wat aanduidend is van Gram negatiewe bakterieë, Gram positiewe bakterieë en mikro-eukariote (hoofsaaklik fungi), onderskeidelik. Hierdie gronde is ook gekenmerk deur lae konsentrasies van fosfor, kalium, persentasie organiese koolstof, persentasie organiese stikstof, sowel as lae grond pH. Gronde wat onderdrukkend was tot "vrotpootjie" is gekenmerk deur verhoogde vlakke van normale versadigde vetsure, wat alomteenwoordig is by mikro-organismes. Die konsentrasie van normale versadigde vetsure in onderdukkende gronde is aanduidend van lae strukturele diversiteit. Gronde onderdrukkend tot "vrotpootjie" is ook gekenmerk deur hoë konsentrasies fosfor, kalium, persentasie organiese koolstof, persentasie organiese stikstof, asook 'n verhoogde grond pH. Die verwantskap tussen FLVSe en landboukundige grond, was ondersoek deur gebruik te maak van hoof-komponent analises (KHA) / "principal component analysis (PCA)", "redundancy" analises (RDA), en diskriminante analises (DA). Gronde onderdrukkend tot "vrotpootjie" het betekenisvol verskil ($p < 0.05$) van gronde aanleidend en neutraal tot "vrotpootjie", wat impliseer dat 'n verskuiwing in verwantskappe tussen die strukturele diversiteit van microbiese gemeenskappe in die verskeie gronde plaasgevind het. 'n Positiewe assosiasie was merkbaar tussen mikrobiese FLVS profiele, en die dominante omgewingsveranderlikes van aanleidende, onderukkende, en neutraal gronde tot "vrotpootjie" van koring. Hiërargiese groep analises van die hoof FLVS groepe het getoon dat die strukturele diversiteit betekenisvol verskil het tussen gronde aanleidend, onderdrukkend, en neutraal tot "vrotpootjie" van koring wat veroorsaak

word deur *Gaeumannomyces graminis* var. *tritici*. Resultate toon aan dat mikrobiese gemeenskapsfunksionaliteit, sowel as mikrobiese gemeenskapstruktuur, betekenisvol beïnvloed was deur die teenwoordigheid van "vrotpootjie" van koring wat veroorsaak word deur *Gaeumannomyces graminis* var. *tritici*, en dat die karakterisering van die mikrobiese funksionaliteit en strukturele diversiteit deur die analisering van gemeenskapsvlak fisiologiese profiele en fosfolipied vetsuur analises, onderskeidelik, suksesvol gebruik kan word as evalueringskriteria vir die evaluering van landboukundige grond aanleidend, onderdrukkend, en neutraal tot "vrotpootjie" van koring, sowel as in wisselbou-sisteme. Hierdie metodologie kan van betekenisvolle waarde wees as 'n ondersteuning in die bestuur en evaluering van landboukundige grond onderwerp aan die voorkoms van ander grondgedraagde siektes.

Kernwoorde: Grond mikrobiese gemeenskappe; gemeenskapsvlak fisiologiese profiele (GVFP); *Gaeumannomyces graminis* var. *tritici*; "vrotpootjie" by koring; Wisselbou; Diversiteitsindeks; Fosfolipied vertsure (FLVS).

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AWCD	average well colour development
Bmonos	branched monounsaturated fatty acids
C	carbon
%C	organic carbon content
Ca	calcium
$Ca(NO_3)_2$	calciumnitrate
CF	canonical function
Cl	chloride
CLPP	community level physiological profile
CO_3^{2-}	carbonate ion
DA	Discriminant Analysis
DCA	Detrended Correspondence Analysis
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
E	Shannon-Weaver index of substrate evenness
EC	electrical conductivity
FAME	fatty acid methyl esters
FISH	fluorescent *in situ* hybridisation
Ggg	*Gaeumannomyces graminis* var. *graminis*
Ggt	*Gaeumannomyces graminis* var. *tritici*
H'	Shannon-Weaver substrate diversity index
HSD	Tukey's Honest Significant Difference test
J	Shannon-Weaver index of substrate equitability
K	potassium
KCl	potassium chloride
MBsats	mid-branched saturated fatty acids
Mn	manganese
Monos	monounsaturated fatty acids
N	nitrogen
NH_4^+	ammonium ion

Take-all is the name given to the disease caused by a soil-borne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*) which is responsible for the cause of crown and root rot in wheat, barley, rye and triticale (Rothrock and Cunfer, 1991). The fungus is an ascomycete of the family Magnaportheaceae (Cook, 2003). The fungus is not seed-borne (Hershman and Bachi, 1994), survives in crop stubble (Collins, 1995), is most common in regions where wheat is grown without adequate rotation (Hershman and Bachi, 1994), and when plants undergo nitrogen stress. The most damage to wheat, barley, rye and triticale is caused during early infections, when both roots and culms are affected (Collins, 1995). Infections of roots result in losses in crop yield and quality because of a lowering in nutrient uptake (Monsanto Company, 1998).

Several means to control take-all infection have been suggested. These mainly include: the application of crop rotation (McMullen and Lamey, 1999), the use of ammonium nitrogen fertilisers (Collins, 1995), and biological control (Cook, 2003). Biological control can be achieved with cultural practices that match up with introduced and resident antagonists, but success, in many cases, would involve a combination of introduced and resident antagonists. Pseudomonads have mainly been associated with the suppression of take-all (Weller and Cook, 1983). Pseudomonads are among the main colonisers of wheat residue that may reduce the ability of *Ggt* to survive through their competition with it and the production of antibiotic substances, siderophores (which deprives deleterious rhizosphere microorganisms of iron), and toxic secondary products such as cyanides (Katsuwon et al., 1990). Take-all decline has also been attributed to populations of *Trichoderma* spp. (especially *T. koningii*), with the ability to suppress the saprophytic and parasitic activity of take-all disease of wheat (Duffy et al., 1997) by the production of antibiotics and lytic enzymes (Simon and Sivasithamparam, 1988). *Gaeumannomyces graminis* var. *graminis* (*Ggg*) from wheat and other grasses has also been reported to suppress take-all disease in Australia and Europe. *Gaeumannomyces graminis* var. *graminis* might compete directly with virulent *Ggt* for the same substrates

NO_3^-	nitrate ion
Nsats	normal saturated fatty acids
P	phosphorous
PCA	Principal Component Analysis
PCR	polymerase chain reaction
PDA	potato dextrose agar
PHA	poly-β-hydroxyalkanoic acids
Phe	phenazine-type antibiotics
Phl	2,4-diacetylphloroglucinol
PLFA	phospholipid fatty acid
Polys	Polyunsaturated fatty acids
RDA	Redundancy Analysis
RRNA	ribosomal ribonucleic acid
S	Shannon-Weaver index of substrate richness
SRF	sterile red fungus
TBSats	terminally branched saturated fatty acids
TGGE	temperature gradient gel electrophoresis
UPGMA	unweighted pair group method with arithmetic mean
YIB	yield-increasing bacteria

and favoured sites in and on roots. It is assumed that *Ggt* might increase leakage of root exudates, thus increasing populations of other antagonistic rhizosphere microorganisms such as fluorescent *Pseudomonas* spp., that are especially well adapted to utilise root exudates very rapidly (Duffy and Weller, 1995).

Soil suppressiveness to plant disease occurs naturally as an inherent characteristic of physical, chemical, and/or biological structure of a particular soil, or it might be induced by some practices and activities such as planting of crops, or the addition of organisms or nutritional amendments, which cause a change in the microfloral environment (Larkin et al., 1993). Induced suppressive soils have been exemplified by occurrences of take-all decline of wheat that have resulted after several years of continuous monoculture of wheat (Andrade et al., 1994). Take-all decline could develop quickly in a field with no wheat culturing history since a suppressive factor could also be transferred from soil to soil, where it could multiply (Wildermuth, 1982).

Concerns about environmental effects of intensive agriculture have shifted the focus more towards ecologically sustainable systems that included the use of reduced tillage, inputs of organic materials and nutrient cycling strategies based on crop rotations (Pankhurst et al., 1996). During crop rotation, time is allowed between the cultivation of susceptible crops for the lowering of inoculum potential of soilborne plant pathogens below some economic threshold by resident mycoparasites, competitors, predators, and antibiotic-producing microorganisms (Cook, 1994). Take-all is, in most cases, also reduced by the application of an ammonium (NH_4^+) source as fertiliser (Sarniguet et al., 1992).

Key roles in functional processes carried out by soil microorganisms and soil microbial communities play a significant role in the productivity and health of agricultural systems (Pankhurst et al., 1996). When two different microbial populations are found in the same habitat, important beneficial or detrimental interactions within a single microbial population, or between diverse microbial populations are inevitable (Davies and Whitbread, 1989b; Cloete, 1999).

Microorganisms also exhibit these beneficial or detrimental interactions with plants (Atlas and Bartha, 1993). Considering the fact that plants are the major source of organic matter on which microorganisms are dependent (Cloete, 1999), a close relationship exists between plant and microorganism where microbial communities tend to influence plants in many direct and indirect ways. Reduced capability of plants to survive and maintain its ecological niche, is the result of malfunctioning caused by

microbial diseases of plants, resulting in death or a low growth yield of the plant (Atlas and Bartha, 1993; Cloete, 1999).

A large obstacle in the characterisation, evaluation and comparison of soil microbial communities has traditionally been the lack of effective methods to deal with community-level characteristics (Cavigelli et al., 1995). Since only a small percentage of all soil microorganisms are culturable (White et al., 1996), traditional culture-based assays of microbial populations or gross estimates of microbial biomass or activity provide important, yet very limited, information on these complex soil communities.

Recently, several useful community-level characterisation techniques have been developed that do not rely on culture-based assays. These quantitative, more representative and differentative assays overcome the limitations of conventional microbiological techniques and contribute substantially to the *in situ* characterisation of soil microbial communities.

For a better understanding of the actions of microbes in natural environments, microbial ecologists have developed numerous techniques to measure microbial community biomass, structure, metabolic status, and activity under *in situ* conditions, thus attempting to reveal more closely the functional role that microbial communities play in nature (Vestal and White, 1989). One approach to translate the information in a microbial ecosystem would be to determine the metabolic diversity (functional diversity) within the system. The metabolic diversity of heterotrophic microbial communities can be assayed by determination of community level physiological profiles (CLPP) based on tetrazolium violet dye reduction as an indicator of sole carbon source utilisation (Garland and Mills, 1991). Commercially available Biolog® microtiter plates allow for the simultaneous testing of 95 separate carbon sources, and the direct incubation of whole environmental samples (aquatic, soil, and rhizosphere) in Biolog® microtiter plates could thus produce information-rich data concerning bacterial functional biodiversity (Garland and Mills, 1991; Garland, 1996). The obtained data are especially pliable to multivariate analyses and other commonly used statistical procedures to look at taxonomic diversity in macroorganisms (Zak et al., 1994). Community level physiological profiles are highly reproducible, easy to use and are thus feasible for use in large-scale field studies (Bossio and Scow, 1995; Haack et al., 1995). This approach has been used extensively in monitoring various soil processes such as bioremediation (Lawley and Bell, 1998),

the release of genetically engineered microorganisms, flooding (Bossio and Scow, 1995), farming practices, and pollution. (Bossio and Scow, 1995).

The structural diversity of microbial ecosystems can also be characterised by the analysis of signature lipid biomarker fatty acid methyl esters (FAME). Fatty acid methyl esters (FAME) profiles, and specifically the phospholipid fatty acid methyl esters (PLFA) profiles are unique "signature" chemicals that are restricted to specific subsets of a microbial community or bacterial group (Zelles et al., 1992) that could be used as basis to identify microorganisms (Pankhurst et al., 1996) and to act as a fingerprint of the structural diversity of a microbial community (Petersen and Klug, 1994). Measurements of phospholipid fatty acid profiles in soils have been used extensively to estimate microbial biomass, and to examine community structure (Haack et al., 1994). This culture-independent technique provides a more comprehensive view of microbial communities than conventional culture-dependent techniques (Waldrop et al., 2000).

Molecular approaches have also been developed as a more effective method for studying the diversity, distribution, and behaviour of microorganisms in soil habitats to assist in the broader understanding of soil health (Hill et al., 2000). The most useful of the various nucleic acid techniques, is the determination of the sequences of 16S ribosomal RNA (rRNA) genes in prokaryotes and 5S or 18S rRNA in eukaryotes. Other molecular techniques used in microbial ecology studies, include temperature gradient gel electrophoresis (TGGE) / denaturing gradient gel electrophoresis (DGGE), fluorescent in situ hybridisation (FISH), and the analysis of soil microbial communities based on rRNA as opposed to rRNA genes that have been encoded by rDNA (Felske et al., 1996).

Although no single technique provides a complete representation of soil microbial characteristics, each of these techniques provides a slightly different perspective. A more complete presentation of soil microbial characteristics could thus be achieved with the use of multiple techniques (a polyphasic approach).
Due to the development of microbial communities antagonistic to *Ggt* infection in soils conducive to take-all disease of wheat, it is hypothesised that the microbial community function and structure in soils conducive and suppressive to take-all disease of wheat will differ significantly. Should significant differences be observed between the microbial

communities, the application of techniques independent of cultivation could be utilised to assess the effect of management practices applied to suppress take-all disease.

The aim of this study was thus to gain insight into aspects of both the functional and structural attributes of soil microbial communities related to the induction / suppression of take-all disease of wheat as caused by *Ggt* with the use of community level physiological profiles (CLPP) and phospholipid fatty acid methyl ester (FAME) profiling, respectively. This information could enable a more comprehensive assessment and characterisation of changes in multiple aspects of soil microbial community characteristics (Larkin, 2003), and could possibly assist in the management of take-all disease of wheat.

Specific objectives for this study were therefore: (1) the physico-chemical characterisation of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat, (2) the isolation, characterisation and comparative evaluation of certain culturable microorganisms using conventional microbiological techniques, the (3) characterisation and comparative evaluation of the functional diversity of the microbial communities within the various agricultural soils based on the statistical analysis of community level physiological profiles (CLPPs), (4) the evaluation of the effect of crop rotation on the functional diversity of microbial communities within agricultural soils conducive to take-all disease of wheat, (5) the characterisation and comparative evaluation of the structural diversity of the microbial communities within the various agricultural soils based on the statistical analysis of phospholipid fatty acids, and (6) the recommendation of some management criteria for the control / suppression of take-all disease of wheat in South Africa.

REFERENCES

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Andrade, O.A., Mathre, D.E., Sands, D.C. 1994. Natural suppression of take-all disease of wheat in Montana soils. Plant and Soil 164, 9-18.

Atlas, R.M., Bartha, R. 1993. Microbial Ecology: fundamentals and applications. 3rd ed. Benjamin/Cummings Publishing Company, Inc., Redwood City, 563 pp.

Bossio, D.A., Scow, K.M. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied and Environmental Microbiology 61, 4043-4050.

Cavigelli, M.A., Robertson, G.P., Klug, M.J. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99-113.

Cloete, T.E. 1999. Microbial Ecology. In: Principles in Microbiology '99. [CD-ROM.]

Collins, D.J. 1995. Diseases of barley, rye, and triticale in Alabama. [Web:] http://www.aces.edu/department/extcomm/publications/anr/anr-903/anr-903.html [Date of access: May 2001].

Cook, R.J. 1994. Problems and progress in the biological control of wheat take-all. Plant Pathology 43, 429-437.

Cook, R.J. 2003. Review: Take-all of wheat. Physiological and Molecular Plant Pathology 62, 73-86.

Davies, K.G., Whitbread, R. 1989b. Factors affecting the colonisation of a root system by fluorescent pseudomonads: the effects of water, temperature and soil microflora. Plant and Soil 116, 247-256.

Duffy, B.K., Weller, D.M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Disease 79, 907-911.

Duffy, B.K., Ownley, B.H., Weller, D.M. 1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopathology 87, 1118-1124.

Felske, A., Engelen, B., Nubel, U., Backhaus, H. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Applied and Environmental Microbiology 62, 4162-4167.

Garland, J.L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology and Biochemistry 28, 213-221.

Garland, J.L., Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351-2359.

Haack, S.K., Garchow, H., Klug, M.J., Forney, L.J. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Applied and Environmental Microbiology 61, 1458-1468.

Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.J. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60, 2483-2493.

Hershman, D., Bachi, P. 1994. Take-all of wheat. [Web:] http://www.ca.uky.edu/agcollege/plantpathology/PPAExten/PPFShtml/ppfagsg1.htm [Date of access: March 2000].

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15, 25-36.

Katsuwon, J., Anderson, A.J. 1990. Catalase and superoxide dismutase of root-colonizing saprophytic fluorescent pseudomonads. Applied and Environmental Microbiology 56, 3576-3582.

Larkin, R.P. 2003. Characterisation of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilisation, and fatty acid profiles. Soil Biology and Biochemistry 35, 1451-1466.

Larkin, R.P., Hopkins, D.L., Martin, F.N. 1993. Effects of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83, 1097-1105.

Lawley, T., Bell, C. 1998. Kinetic analysis of BIOLOG community profiles to detect changes in inoculum density and species diversity of river bacterial communities. Canadian Journal of Microbiology 44, 588-597.

McMullen, M.P., Lamey, H.A. 1999. Crop rotations for managing plant disease. [Web:] http://www.ext.nodak.edu/extpubs/plantsci/pests/pp705w.htm [Date of access: September 2002].

Monsanto Company. 1998. A guide to take-all – a disease in cereal grains. [Web:] http://www.takeall.com/english/default.htm [Date of access: July 2002].

Pankhurst, C.E., Ophel-Keller, K., Doube, B.M., Gupta, V.V.S.R. 1996. Biodiversity of soil microbial communities in agricultural systems. Biodiversity and Conservation 5, 197-209.

Petersen, S.O., Klug, M.J. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Applied and Environmental Microbiology 60, 2421-2430.

Rothrock, C.S., Cunfer, B.M. 1991. Influence of small grain rotations on take-all in a subsequent wheat crop. Plant Disease 75, 1050-1052.

Sarniguet, A., Lucas, P., Lucas, M., Samson, R. 1992. Soil conduciveness to take-all of wheat: Influence of the nitrogen fertilisers on the structure of populations of fluorescent pseudomonads. Plant and Soil 145, 29-36.

Simon, A., Sivasithamparam, K. 1988. The soil environment and the suppression of saprophytic growth of *Gaeumannomyces graminis* var. *tritici*. Canadian Journal of Microbiology 34, 865-870.

Vestal, J.R., White, D.C. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

Waldrop, M.P., Balser, T.C., Firestone, M.K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry 32, 1837-1846.

Weller, D.M., Cook, R.J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73, 463-469.

White, D.C., Ringelberg, D.B., Macnaughton, S.J. 1996. Review of PHA and signature lipid biomarker analysis for quantitative assessment of *in situ* environmental microbial ecology. 1996 International Symposium on Bacterial Polyhydroxyalkanoates 161-170.

Wildermuth, G.B. 1982. Soils suppressive to *Gaeumannomyces graminis* var. *tritici*: effects on other fungi. Soil Biology and Biochemistry 14, 561-567.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology and Biochemistry 26, 1101-1108.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology and Biochemistry 24, 317-323.

Soil provides an overall nutrient-rich environment to soil microorganisms which tend to be found in microcolonies on particles or in pores between soil particles for protection from predatory protozoa (Delisle et al., 1999). Soil microbial communities can be divided into two categories: autochthonous (mostly Gram-negative rods and actinomycetes capable of utilising refractory humic substances) and zymogenous (opportunistic soil organisms, exhibiting high levels of activity and rapid growth on easily utilisable substrates, incapable of utilising humic compounds) (Cloete, 1999). Depending on the soil microhabitat, individual soils may favour microbial populations with certain types of metabolisms – abiotic parameters of some soils restrict microbial populations that are not adapted to develop there (Atlas and Bartha, 1993). Microaerophiles and obligate anaerobes such as sulphate-reducers and Clostridium, are often present in flooded soils where water has displaced the air in the soil; providing an anoxic environment (Delisle et al., 1999).

1. Microbial Ecology

Microbial ecology can be defined as the study of interrelationships existing between organisms and their biotic and abiotic environment (Atlas and Bartha, 1993). When studying species composition of microbial communities, insight could be provided into ecological function of these communities, as well as the effects of environmental stresses on ecosystems (Van Heerden et al., 2000). Øvreås and Torsvik (1998) described diversity as the range of significantly different kinds of organisms and their relative abundance in natural entirety and habitat - the amount and distribution of genetic information in a natural community. A representative estimate of microbial diversity is a prerequisite for understanding the functional activity of microorganisms in such ecosystems (Zak et al., 1994). Microbial communities are involved in the acquisition and recycling of nutrients required for plant growth, maintenance of soil structure, degradation of pollutants and the biological control of plant and animal pests, as well as an integral part of the sustainability of agricultural systems productivity and

health of agricultural and other systems over long periods of time (Bossio and Scow, 1995; Delisle et al., 1999; Hill et al., 2000).

Profound impacts on ecosystem dynamics are caused by changes in the soil microbial diversity resulting from agricultural practices, ecosystem management, and global change (Bossio and Scow, 1995), indicating detrimental- or beneficial effects of any amendments or management strategies in agriculture (Pankhurst et al., 1996; Sharma et al., 1998). Communities with the ability to adapt to changing environments could be an indication of the capacity of the ecosystem to respond, which might be directly related to the diversity of the organisms present (Pankhurst et al., 1996).

1.1 Microbial Interactions

Interactions within a single microbial population, or between diverse microbial populations found in a habitat, are inevitable (Atlas and Bartha, 1993). Although several beneficial and / or detrimental interactions could occur within microbial populations, emphasis will only be placed on interactions relevant to this study.

Competition and amensalism are recognised as negative interactions that act as feedback mechanisms to prevent overpopulation, destruction of habitats and extinction. Competition between microbial species plays an important role in affecting colonisation and population levels (Cloete, 1999).

Competition is a detrimental interaction that occurs within and / or between microbial populations when all the members of the microbial population utilise limited environmental factors for growth and hence, grow at sub-optimal rates because they must share the same growth-limiting resource, whether space, or a limiting nutrient. This could bring about the ecological separation of closely related populations, known as the competitive exclusion principle, since two populations are precluded from occupying exactly the same niche; one will survive, while the other is eliminated. Coexistence can only be achieved if populations can avoid absolute direct competition by using different resources at different times (Atlas and Bartha, 1993).

Amensalism (antagonism) is another negative interactive association that is detrimental to one population while not adversely affecting the other population (Richards, 1994; Cloete, 1999), e.g. the incidence where one population produces an inhibitory substance (e.g., antibiotics). Microbial antagonism has been stimulated, and induced

suppression promoted, when soil treatments made conditions favourable for the proliferation of antagonists resident in the soil (Sturz and Bernier, 1991).
Parasitism is quite a specific interactive relationship which requires a relatively long contact-period between two organisms or populations, during which one population is harmed (the host) and the other benefits (ectoparasites, remaining outside the cells of the host population, or endoparasites, penetrating host cells) (Atlas, 1997).
Beneficial and detrimental interactions can be studied in complex natural biological communities between different populations, with positive interactions being likely to be more developed in established autochthonous (indigenous) population communities, whereas invaders of these established communities (allochthonous) would encounter negative interactions – in many cases severe (Cloete, 1999).

Microorganisms also exhibit beneficial or detrimental interactions with plants, since plant surfaces provide important habitats for microorganisms (Atlas and Bartha, 1993).

1.2 Interactions with plant roots

Plants are the major source of organic matter which microorganisms depend on. Because of the close relationship between plant and microorganism, microbial communities tend to influence plants in many direct and indirect ways. The rate at which organic matter such as inhibitors, stimulants and a wide range of potential microbial substrates are released from plant roots by oxidation, are increased in the presence of microorganisms (Cloete, 1999).
Plant roots are surrounded by a mucilaginous layer varying in composition from a simple oligosaccharide, to a complex pectic acid polymer (Richards, 1994). Although the plant root structure determines the size of the rhizosphere, the contact area with soil is usually very large. Despite this, only a small percentage of the actual rhizoplane is in direct physical contact with microorganisms; the rest of the root-associated microorganisms occur in the surrounding rhizosphere (Richards, 1994).
Atlas (1997) described the rhizosphere as the part of the plant that consists of the root-surfaces, as well as the region of surrounding soil that has an important effect on microbial populations. High numbers of characteristic microbial populations, quite distinct from the general soil population, surround the roots. The microbial communities present in the rhizosphere greatly affect crop production, soil fertility, energy flow and nutrient cycling (Pelczar et al., 1993; Brock et al., 1994; Richards, 1994). A general

selection of Gram-negative, non-spore forming, rod-shaped bacteria have been identified in the rhizosphere, with strains of *Pseudomonas*, *Agrobacterium* and *Achromobacter*, as major constituents (Garland, 1996b).

1.3 Effects of plant roots on microbial populations

Roots can modify the rhizosphere soil physically, chemically and microbiologically (Richards, 1994) since it has a direct influence on the density and composition of the soil microbial community. The structure of plant root systems promotes and leads to the establishment of microbial populations in the rhizosphere (Atlas and Bartha, 1993). Processes such as the release of organic chemicals to the soil by plant roots (Hodge et al., 1998), the uptake of water by plant systems, microbial production of plant growth factors and the availability of mineral nutrients mediated by microorganisms, are responsible for the interactive modification of the soil environment because of interactions between plant roots and the rhizosphere microorganisms (Richards, 1994; Atlas, 1997). Clear discrimination can be found between carbon sources utilised by microbial communities from different plant rhizospheres, resulting in the selection of different organisms in these rhizospheres (Grayston et al., 1998). The type of plant and its physiological maturity also plays an important role in the extent of this rhizosphere effect (Atlas and Bartha, 1998).

1.4 Effects of rhizosphere microbial populations on plants

Just as plant roots have a great influence on the rhizosphere microorganisms, just as great an influence do rhizosphere microorganisms have on plant roots and therefore, on the growth of the plant (Atlas, 1997).

The incident of growth stimulation of plants by rhizosphere microorganisms, as well as the impairment of plant growth, has been recognised in the absence of appropriate microbial populations in the rhizosphere (Davies and Whitbread, 1989a).

Interactive modification of the soil chemical environment by processes such as water uptake by the plant system, release of organic chemicals to the soil by plant roots, microbial production of growth factors, and microbially mediated availability of mineral nutrients, play important roles in the effects that rhizosphere microbial populations have on plants (Atlas, 1997). Rhizosphere microorganisms also remove hydrogen sulphide which is toxic to plant roots, and increase recycling and solubilisation of mineral

nutrients such as phosphate, iron, manganese and calcium needed by the plant for growth by increased rates of seed germination and root hair development. Synthesis of vitamins, amino acids, auxins and gibberellins to stimulate plant growth (Shen, 1997), as well as antagonism towards potential plant pathogens through competition and by producing antibiotics, are other ways in which rhizosphere microorganisms benefit plants (Cloete, 1999). Contrary to the benefits named above, a deficiency of minerals required by a plant can be created by the abundance in microbial populations in the rhizosphere (Atlas, 1997).

1.5 Microbial Diseases of Plants

Microbial diseases of plants do not only have major impacts on a country's ecology, but also on its economy (Cloete, 1999). Reduced capability of the plant to survive and maintain its ecological niche, is the result of malfunctioning caused by microbial diseases of plants. Immobility and the health of the plant, the period of time in which the plant grows, effectiveness of nutrient supply, and the protective measures of the plant, are all factors that largely influence the plant pathogen-host relationship. Other factors such as temperature, moisture and soil pH also play an important role in the development of plant diseases (Cloete, 1999).

Plant diseases develop with the initial contact and entry of the pathogen through natural openings or existing wounds. The growth of the pathogen advances in the plant until disease symptoms develop with the disruption of normal plant function by the production of degradative enzymes that result in the degeneration of the structure, toxins, and growth regulators of the plant (Atlas and Bartha, 1993). Penetration of the plant by pathogens, on the other hand, may lead to a morphological response of the plant to form modified structures in an attempt to block the spread of the pathogen (Atlas, 1997). In some cases, plants that are under attack by pathogens, might react by synthesising phytoalexins - antimicrobial substances used as a defence mechanism to slow or even stop the infection process, leaving the plant with increased systematic resistance against further attacks by pathogens (Atlas and Bartha, 1998).

Once disease symptoms, due to the invasion of primary plant pathogens are exhibited, the plant is subject to additional invasion by opportunistic secondary plant pathogens due to the loss of surface structure integrity, and the cell wall allows the invasion of opportunistic pathogens (Atlas and Bartha, 1993).

2. Take-All

Take-all disease was first described in Australia in 1852, and has ever since been recognised as a problem on small grains. The severity of the attack and devastation of take-all, caused to Australian wheat farmers' crops gave the disease its name around 1870 (Cook, 2003). Although the actual cause of the disease was unknown for almost 50 years, French researchers finally, during 1890, ascribed the disease correctly to a root rot fungus. Take-all was originally known as *Ophiobolus graminis* from 1881, until *Gaeumannomyces graminis* was shown in 1952 to be the correct name. (Bockus and Tisserat, 2000; CAB International, 2000.)

2.1 Pathogen biology

The take-all fungus, *Gaeumannomyces graminis* (*Gg*), is an exclusive soilborne root-rot pathogen of cereals and grasses, and is most damaging to intensively grown wheat and barley crops when crop rotation is not practised in a given site (Hershman and Bachi, 1994). Surviving in the infected residues of one crop, and then invading the roots of the following crop, it progressively destroys the root system. In exceptional cases it could kill an entire crop – hence the name "take-all" (Deacon, 2001). The mycelium initially infects the roots of the living hosts, and as the root dies, the fungus saprophytically colonises the decaying tissue (Bockus and Tisserat, 2000). Infested soilborne debris could be transported by farm machinery, animals, wind and water. The fungus is usually most productive under circumstances when host roots are plentiful and when relatively short saprophytic periods (weeks or a few months) prevail between the availability of susceptible roots. It is clear that the fungus is a relatively poor saprophyte and does not compete well with native soil microbial communities. It is also susceptible to heat-inactivation during heating of soil by solar energy (Bockus and Tisserat, 2000).

The *Gaeumannomyces graminis*-species is sub-divided into three varieties. These varieties do not only differ in pathogenicity, but also in minor physiological features:

1. *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*) is an aggressive soil-borne pathogen causing root rot of wheat (primary host), barley and rye crops (secondary host). The flowering, seedling, and vegetative growing stages can be affected by the infection of the whole plant, leaves, roots, and stems

(Rondon and Cunfer, 1991). The fungus is an ascomycete of the family Magnaportheaceae (Cook, 2003).

2. *Gaeumannomyces graminis* var. *avenae* is a soilborne pathogen causing root rot of *Avena*, oats species and turf grasses
3. *Gaeumannomyces graminis* var. *graminis* is usually a weak soilborne parasite found on maize and rhizomatous or stoloniferous grasses. It also causes a sheath rot of rice (Deacon, 2001; Bockus and Tisserat, 2000.)

The above-mentioned varieties are specialised parasites of the Gramineae grass family (Deacon, 2001).

2.2 Disease cycle

Following maturity and harvest of a wheat crop affected by take-all, the seminal and coronal roots and the culm bases, invaded by *G. graminis* var. *tritici* while the plant was still alive, become the source of inoculum for infection of the next cereal crop. During survival in infested remnants of host plants, the fungus exists as a saprophyte on the cellulose, proteins and other carbon and nitrogen sources available in the dead plant material, or even as ectoparasite on the roots of volunteer hosts, perennial hosts, or grassy weeds (Cook, 2003). It is important to note that no ascospores are produced during this latter stage (Bockus and Tisserat, 2000).

Take-all first becomes apparent on small grain near the time when the seed head emerges (Collins, 1995), giving rise to perhaps the most diagnostic field symptom: prematurely-ripe tillers, called "Whiteheads" (Rane et al., 1997; Monsanto Company, 1998). Other diagnostic field symptoms include yellow leaves and stunted plants; circular patches, although it could also be fairly uniform throughout a field (Hershman and Bachi, 1994). In the case of closely mowed bentgrass turf, take-all might cause roughly circular dead patches ranging up to more than a meter in diameter, with bronze to yellow-orange margins. In successive years of monocropping, patches may reappear in same locations (Hershman and Bachi, 1994; Bockus and Tisserat, 2000). *Gaeumannomyces graminis* grows along the root surface as darkly pigmented runner hyphae. The root cortex is penetrated at intervals by branches arising from these runner hyphae (Colbach et al., 1997; Monsanto Company, 1998). The ability of the fungi to cause an infection is determined by the nutrient reserves of the fungus (e.g. the size of the organic food base from which it infects), and the degree of host tissue resistance. The invading hyphae grow through the root cortex to invade and destroy the root

phloem (responsible for sugar-transport) to provoke a successful infection by a pathogenic strain. Thereafter, the xylem (responsible for water-transport) is invaded, causing discoloration and blockage of the xylem vessels – effectively cutting off and decaying the root at this point (Sarniguet et al., 1992b). Roots have initial black lesions that expand and eventually coalesce, extending existing lesions and/or producing new secondary infections, resulting in the remaining of the brittle and heavily rotted root system observed when the plants are pulled out of the soil (McMullen and Lamey, 1997; Bockus and Tisserat, 2000). A shiny black basal stem discoloration is highly diagnostic for take-all when the leaf sheaths are pulled away from the stem (Hershman and Bachi, 1994; Bockus and Tisserat, 2000). Dark brown "runner hyphae" (ectotrophic growth) responsible for invasion, progress to more roots, more of each root, the subcrown internode, the base of the mainstem, the region where culms are attached as tillers to the mainstem, and finally as mycelium 2-3 cm or more up the culm bases, with a slight grey colour with subtle black mottling on a small zone of internal tissue where the tiller bases are attached to the main stem (Sarniguet et al., 1992b). "Black stocking" – prominent grey-to-black crown tissue with the lower 2-4 cm of the culm tissue beneath the leaf sheath being enclosed by a sheath of shiny black mycelium (Hershman and Bachi, 1994). The stem base is eventually colonised, killing the plant. After the death of the plant, the fungus, once again survives saprophytically in the plant tissues it colonised during its parasitic phase, completing the cycle (Bockus and Tisserat, 2000). Remnant culm or root tissue smaller than 0.15-0.25 mm usually represent the remains of infested residue after many months of decomposition and fragmentation by cultivation of the soil (Chakraborty and Warcup, 1983). The distance take-all grows to reach and infect roots, is directly proportional to the mass of infested crop residue in the soil, but only under ideal soil conditions, and with no interference from competing microorganisms in the infested particles (CAB International, 2000).

Time and severity of infections and environmental conditions play a significant role in whole-plant symptoms of take-all (Cotterill and Sivasithamparam, 1987). Nitrogen, phosphorous, or nutrient deficiency symptoms, as well as small or spindly leaves, a progressive yellowing of the leaves, failure to develop tillers, retarded growth rate, small heads, and stunting of adult plants are all characteristic of the infection of seminal roots early in the growing season (CAB International, 2000).

The actual longevity of the saprophytic take-all is determined by soil conditions favourable to soil microorganisms with potential to supersede take-all within and/or

destroy its food base, resulting in a decrease in inoculum potential of take-all. Since the fungus is not seed-borne (Hershman and Bachi, 1994) and survives in crop stubble (Collins, 1995), it could survive through a year of fallow, resulting in take-all severity on wheat after a dry fallow (Cook, 2003; Cook, 1994). The fungus survives saprotrophically in the infected roots and stem bases. In suitable conditions, the fungus might produce its sexual stage - dark, flask-shaped fruiting bodies called perithecium, and each containing many asci, but these perithecia rarely or never form in the field (CAB International, 2000). Each ascus accommodates eight elongated, thin spores - ascospores – which are ejected forcibly to dissipate the fungus (Bockus and Tisserat, 2000; Deacon, 2001). It is clear that the sexual stage (ascospores) has no importance to the development of the disease (Bockus and Tisserat, 2000).

2.3 Epidemiology

Take-all occurs virtually throughout the temperate world – both in its northern and southern parts wherever wheat is grown without the benefit of crop rotation - and even at higher elevations in tropical areas wherever small grains are grown (Bockus and Tisserat, 2000). It also commonly occurs in the Western Cape (Verwoerd, 1929), Eastern Free State and all wheat-growing areas under irrigation in South Africa. Although take-all is traditionally accepted to occur in irrigated fields, infections have been found to occur in nonirrigated wheat fields during years of high rainfall and wet soils (McMullen and Lamey, 1999). In the Western Cape, take-all survives on grasses such as barley and rye, which often occurs among legumes used in rotation with wheat or barley. Take-all survives the time period between successive crops on volunteer wheat and crop residue. Except for only intermediate type cultivars found in the Eastern Free State that are more tolerant to take-all than early-planted winter types, all wheat cultivars are susceptible to take-all (Scott, 1990).

"Text book" take-all, also called "Wetland" take-all (Cook, 2003), develops in the presence of a nearly continuous supply of abundant moisture in the top 15-20 cm of soil where the pathogen inhabits and infects wheat (CAB International, 2000; Cook, 2003). High rainfall areas or areas under irrigation during most or all of the growing season, give rise to primary infections that result in secondary infections as the pathogen spreads through the soil. Patches of diseased plants usually develop under these conditions, with the shortest plants having the fewest tillers, being in the centres of the

patches (CAB International, 2000; Cook, 2003). Since infected plants are young and solely dependent on its seedling root system, symptoms of nutrient deficiency, stunting, yellowing of lower leaves, and failure to produce tillers are observed (Cook, 2003).
Lands with irrigation or high rainfall conditions are typically subject to substantial tillage for weed control and to eradicate straw from the soil surface. Tillage distributes primary inoculum uniformly in the top 15-20 cm soil, thus repeatedly infecting wheat plants growing in these lands. Colonisation and decomposition of this crop residue by microorganisms reduces the availability of the primary inoculum while host plants grow under these conditions, but secondary infections steadily become more important to the epidemiology of take-all. (CAB International, 2000.)

"Dryland" take-all typically develops on individual wheat plants rather than in patches in areas with an annual rainfall too low to support full development of the disease (Cook, 2003). Instead of a full display of all symptoms, disease development only persists as long as moisture permits, where after symptoms cease to progress further – rarely or never extending as black mycelium up culms. Wheat plants continue to grow by obtaining moisture from deeper in the soil, unless the culm bases are infected. Under these conditions, primary infections, and not secondary infections, play major roles in the epidemiology of this disease (CAB International, 2000; Cook, 2003).

Nitrogen-deficiency in soil gives rise to severe take-all infections (Hershman and Bachi, 1994). However, it has been reported that the application of ammonium sulphate at the time of sowing will reduce the occurrence of take-all in wheat. The application of ammonium fertiliser during summer is less effective when compared to the application of fertiliser during winter, since ammonium nitrogen is easily converted into nitrate nitrogen under warm and moist conditions. With the application of phosphorus and potassium, development of secondary roots and vigorous growth will be promoted, giving rise to the sustainability of less serious damage from take-all (Scott, 1990). High applications of lime always increases take-all severity, whatever the nutrient status of infested soil may be – elevated soil pH is even likely to give rise to deficiencies of minor nutrients such as copper and manganese, thus weakening the plant, and favouring take-all development (Scott, 1990). Take-all severity usually increases under reduced-tillage regimes, at soil pH between 6.0 and 8.5, moist soil conditions, soil temperatures between 5°C and 15°C, and coarse-textured soils with good aeration, as well as heavier soils (Bockus and Tisserat, 2000).

Damage resulting from soilborne diseases typically progress up to some limit in incidence or severity. At this stage, the disease usually does neither decrease nor worsen in importance with continuous cropping of susceptible hosts. Take-all of wheat follows the same pattern and finally declines with the continuous cropping of wheat (Cook, 2003). Take-all decline has been observed almost everywhere wheat is continuously cultivated with favourable soil conditions for take-all development. The level of disease control achieved over time between wheat monoculture and crop rotation is almost similar, but monoculture is usually not as complete or effective as crop rotation (Shipton, 1981; Cook, 2003). The decline can be ascribed to a change in populations of soil microorganisms – specifically higher numbers in rhizosphere microorganisms antagonistic to *G. graminis* var. *tritici* (CAB International, 2000; Cook, 2003). Since it is well known that the repeated exposure of soil microbiota to the same substrate increases microbial populations with the ability to decompose that substrate, it can also be concluded that the repeated exposure of soil microbial communities to roots with take-all disease, would increase the populations of microorganisms adapted to the kinds of nutrients and habitats provided by these diseased roots. (CAB International, 2000.)

2.4 Take-all disease management

Crop rotation is considered to be the most effective method of controlling take-all of small grains (McMullen and Lamey, 1999; Cook, 2003). It has been reported that management practices such as the planting of barley instead of wheat in years in which severe take-all is anticipated, resulted in the limiting of crop loss until soil suppressiveness is induced (Rondon and Cunfer, 1991). In order of susceptibility to take-all root rot, wheat is most susceptible, followed by barley, oats, triticale, and rye being considered the least susceptible (Collins, 1995). Because take-all is more severe where wheat follows wheat, it is recommended that wheat be rotated with maize or soybeans every 2-4 years (Hershman and Bachi, 1994), since wheat is a winter crop while maize and soybean are summer crops. Since *Gaeumannomyces graminis* var. *tritici* is a poor saprophytic competitor, rotations to non-host species, a weed-free fallow, or a one-year period without production, is considered sufficient to give almost complete control of take-all disease (Bockus and Tisserat, 2000). Care should be taken against grassy weeds that can harbour the fungus during the fallow or rotation period (Collins,

1995; Monsanto Company, 1998) – no fresh inoculum can be introduced in the absence of host crops to replace the disappearing inoculum from the soil and, depending on soil conditions, the pathogen ceases to be a threat to susceptible cereals planted in the field (Bockus and Tisserat, 2000).

- During tillage, grassy weeds and volunteer plants harbouring the take-all fungus, are killed, while infested debris is broken into smaller fragments that are exposed to more rapid decay and colonisation by saprophytic soil microorganisms, weakening the fungus (Bockus and Tisserat, 2000), resulting in a suppression of the pathogen. Fragmentation and stirring of soil also tends to accelerate microbial activity in the soil for the time being. By burying the crop residue, the soil shading effect is eliminated, giving rise to higher soil temperatures killing the fungus (Bockus and Tisserat, 2000, CAB International, 2000). There have also been reports of a higher incidence of more take-all on wheat planted into a seed bed prepared with conventional tillage than when direct drilling (Yarham, 1981). This was observed in the southeastern USA for wheat double-cropped with soybeans (Rondon, 1987). It can therefore be concluded that less tillage would result in more inoculum, or longer inoculum survival, thus favouring a higher survival and prevalence of take-all (CAB International, 2000). The cleaning of the plow from wheat residue after harvest, could also be used as a control measure to reduce the incidence of take-all disease, since a more rapid decomposition of residue is provided (Hershman and Bachi, 1994).

- Bockus and Tisserat (2000) reported that a firm seed bed is preferable to a loose one, since it reduces the spread of take-all along the roots. However, soil drainage and aerification also produce environments less conducive to take-all root rot (Monsanto Company, 1998; Bockus and Tisserat, 2000). Another way of controlling take-all is by means of flooding – the pathogen cannot survive in infested residue under flooded soil for more than a few weeks (CAB International, 2000).

- A decrease in take-all incidence has been observed with the use of the ammonium form of fertiliser. This fact was attributed to antagonistic microflora, i.e., fluorescent pseudomonads which are stimulated by the ammonium form of nitrogen; consequently limiting the development of the disease (Colbach et al., 1997). The effect of ammonium might thus, act indirectly by decreasing the soil pH and increasing manganese availability, therefore, having an impact on take-all (Huber and McCay-Buis,

1993). Care should be taken against high pH, low levels of phosphorous, and the use of nitrogen in the nitrate form (fast release form of nitrogen), since it could increase the severity of the disease, i.e. the pathogen (Rane et al., 1997), and its antagonistic microflora (Colbach et al., 1997). Sarniguet et al. (1992a) reported that antagonistic fluorescent pseudomonads might need a period prior to take-all infection to develop. Early infection would thus stimulate early fluorescent pseudomonad development, where the high nitrogen levels would then accentuate the development. The increase in fluorescent pseudomonad populations would later interfere with pathogen expansion (Sarniguet et al., 1992a).

- Cook (1994) reported soil disturbance in the seed row to be another control measure, since significant lower rates of root-infections were reported with soil disturbance in the seed row by helping to control rhizoctonia root rot and take-all.

- Cook (1994) suggested that in contrast to the usual uniform spacing of rows, the planting of the rows should rather be in pairs. This author also reported take-all to occur on fewer crown roots when the rows were paired, and concluded that an open canopy, created by pairing the rows, allowed for greater drying and more rapid and frequent warming of the top soil where the take-all fungus is active and where infection of crown roots occurs (Monsanto Company, 1998; Rane et al., 1997).

- Since the take-all fungus spreads short distances by mycelial growth, it is important to consider plant density (Colbach et al., 1997). Seeding rates could be reduced to avoid dense cropping (Monsanto Company, 1998). This fact is only important in the early stages at which the factor is positively correlated to take-all. The probability of contact between the take-all fungus and the plant roots will consequently be higher at a high plant density, because of the shorter distance between fungus and host. As the roots begin to grow, the probability of contact with the fungus increases proportionally. Because of this, a high root number per plant will increase early take-all infection. As soon as root density becomes established through growth, the fungus could readily reach new host plants. At this stage, plant density no longer influences the disease, since it is no longer a limiting factor.

- A short fallow and a delay in the planting date has also been reported to prolong the saprophytic phase of the fungus, weakening it by exposure to antagonistic microbial

activity needed to reduce the inoculum potential of the pathogen under suitable soil conditions, provided the volunteer cereals and grass hosts are also controlled (Colbach et al., 1997; Bockus and Tisserat, 2000).

- Nitrogen fertilisation supports root growth and, under limiting conditions to secondary infections, assist the plant in 'outgrowing' primary take-all infections (CAB International, 2000). Ammonium (e.g. ammonium sulphate) forms of nitrogen reduce root surface pH, limiting the severity of take-all in some soils (Taylor et al., 1983). Similarly, chloride applied as ammonium chloride, has also been reported to reduce take-all severity in some soils (Taylor et al., 1983). Take-all is inclined to be more severe on wheat deficient in essential nutrients (CAB International, 2000), and severity has been shown to be reduced by the foliar application of manganese sulphate on bentgrass turf (Bockus and Tisserat, 2000). In a similar manner, it could be beneficial to place relatively immobile nutrients (e.g., phosphorous, copper, zinc, manganese) within trouble-free reach of roots. This approach does not necessarily help the plant tolerate culm infections which results in the development of "whiteheads" (Rengel et al., 1996). Saprophytic take-all in dead culm bases of wheat tend to be displaced more rapidly by other saprophytic colonists in the absence of an external nitrogen source (CAB International, 2000).

- Although stubble burning could provide some benefit against take-all, it is clear that it might not help in areas or periods of high rainfall or under irrigation. This approach could however, have a significant controlling effect in semi-arid and sub-humid regions where relative humidity is low and exposed soils dry relatively quickly (CAB International, 2000).

- The application of water during irrigation should be as infrequent and deep as possible to allow the soil to dry out between irrigations. This procedure slows down the rate of root colonisation and succeeding colonisation of the crown (Bockus and Tisserat, 2000).

- Monoculture or the long-term continuous production of hosts, usually leads to a state of natural suppressiveness to take-all that can build up in the soil (Cook, 2003), but this suppressiveness is eliminated with rotation to non-grass crops. Bockus and Tisserat (2000; Cook, 2003) recently reported that the treatment of seed with

suppressive bacteria on small grains has also delivered great results during research, with the future promise of commercial application of this technology.

- Although no highly resistant cultivars of small grain or bentgrass are available, some level of resistance to take-all may be transferable into cultivated species from identified wild grass species with some level of resistance (Bockus and Tisserat, 2000). Differences in the incidence of "Whiteheads" may be observed among wheat varieties grown in field plots with natural inoculum of take-all, but these differences more often result from differences in development or maturity, relative to the epidemiology of take-all, and is therefore not a useful resistance trait to take-all (Scott, 1981). The formation of a barrier by the host in an effort to stop penetration by the fungus, is one mechanism for increased resistance. (Bockus and Tisserat, 2000.)

It has been reported that several plants produce saponins as resistance compounds against plant pathogens. These compounds are membrane-active agents that lyse wall-less cells. Avenacin A is one of the main saponins that occurs either as pre-formed resistance compounds in the roots of oats (Avena species), or is rapidly produced from inactive precursors when the roots are damaged (Deacon, 2001). Avenacin A has a definite effect on *Gaeumannomyces graminins* var. *tritici* even in low concentrations, but has only a temporary inhibitory effect on var. *avenae*. *Gaeumannomyces graminins* var. *avenae* rapidly overcomes inhibition by detoxifying the saponin with the production of a glycosidic enzyme, avenacinase, which renders the toxin inactive by cleaving some of the terminal sugars from avenacin. (Deacon, 2001.)

- Although Difenoconazole and Triadimenol are products available for seed-treatment to suppress take-all (McMullen and Lamey, 1997), take-all cannot be controlled or prevented with the application of current fungicides (Monsanto Company, 1998). Only certain systemic seed-treatment fungicides have been found to reduce take-all severity when incorporated in the soil or when added with fertiliser, but complete control has to date not yet been provided. The application of one or two fungicides is usually made in the fall for several consecutive years. No effect is observed on take-all of small grains with the application of foliar fungicides. (Bockus and Tisserat, 2000, CAB International, 2000.)

Since it is unlikely that a single management method by itself could provide adequate control of take-all disease (Yarham, 1981), it is evident that a combination of the above-mentioned methods are also needed to control other root diseases that tend to arise under the same weather and management conditions favourable to take-all. The need for integrated pest management is, however, questionable in agricultural fields subjected to adequate crop rotation (CAB International, 2000).

3. Suppressive / Conducive Soils And Take-All

Soil health is an ecological characteristic. Responses of soil microbial communities to the application of different stress factors at various intensities could be used as indicators of soil health. Soil health could then be measured by looking at the amplitude of a response and the time to return to the current state before application of stress. The suppression of diseases such as root pathogens that play an integral part of soil microbial communities, could be viewed as a manifestation of ecosystem health and stability. Since a link exists between soil health, the ability of the biological community to suppress plant pathogens, the population density of plant pathogens in soil, and ultimately disease incidence and severity, disease suppression could function as an indicator for a stable and healthy soil ecosystem (Van Bruggen and Semenov, 2000).

Soil health, which is primarily determined by ecological characteristics, is almost synonymous to soil quality and is defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran et al., 1996). Soil quality is encompassed by biological, chemical, and physical properties.

Plant disease suppression cannot be considered an indicator of soil health, but disease suppression could be an important function of a healthy soil - stable soil with resilience to stress, high biological diversity, and high levels of internal cycling of nutrients. Once again various physical, chemical and biological factors contribute to disease suppression (Van Bruggen and Semenov, 2000). Disease suppressive soils can be characterised by the changes in microbial community structure and the time required to return to the initial state after the application of various stresses (Van Bruggen and Semenov, 2000).

Ecosystem stability has been related to biodiversity and resilience in response to stress. Soil resilience has been defined in terms of tolerance against stress, buffering capacity, and the ability to regenerate (Van Bruggen and Semenov, 2000). Biodiversity defines the range of significant different types of organisms and their relative abundance in a community (Torsvik et al., 1998). Soil microbial diversity is normally considered as species or genetic diversity, rather than structural and functional diversity – the last two diversity measures might be more relevant to soil health, based on the assumption that there is functional redundancy in a healthy soil so that the soil ecosystem will recover from a stress factor that eliminates part of the microbial community (Van Bruggen and Semenov, 2000). There are three kinds of soil stress factors: physical (extreme temperatures, extreme drying and wetting cycles, osmotic potentials, high pressure), chemical (pH, anoxia, salinity, biocides such as heavy metals, radioactive pollutants, pesticides, hydrocarbons) and biological (eutrophication or oligotrophication, introduction of exogenous organisms with high competitive abilities, uncontrolled growth of pathogens or predators). It is important to note that individual stress factors seldom operate in isolation. (Van Bruggen and Semenov, 2000.)

Soil disturbance could lead to a succession in bacteria and fungi and the associated food web, leading to an initial decrease and then increase in biodiversity (Torsvik et al., 1998). In cases of short-term perturbations, biological communities in healthy soils would recover relatively quickly to initial conditions. In contrast, long-term (chronic) stresses would result in long-term succession leading to a new dynamic equilibrium among ecosystem components (Van Bruggen and Semenov, 2000). Lupwayi et al. (1998), for example, showed metabolic diversity (evenness as well as richness) to have decreased after tillage. This resulted in an increase in available carbon compounds from necromass. Staddon et al. (1997) found scarification of forest soil to result in temporary lower metabolic diversity than in control plots. Consideration of microbial succession resulting from various stress factors, could likely provide a means to determine health status of soil (Van Bruggen and Semenov, 2000).

Bulk density, water infiltration and holding capacity, total organic carbon and nitrogen, electrical conductivity, pH, plant-available nutrients, and measures of microbial biomass and activity are all factors affecting soil ecological functions and quality (Van Bruggen and Semenov, 2000). These properties might be used as indicators for soil quality, but they are not necessarily associated with soil health and the maintenance of essential soil ecological functions. The sensitivity of these measurements to the time of sampling

in relation to significant management or environmental events like tillage, irrigation, rainfall, etc. might be a reason for inconsistencies (Van Bruggen and Semenov, 2000). Soil health could also be indicated by finding universal and specific indicator-species or higher taxonomic groups associated with healthy or deteriorated soil – specific indicators are dependent on properties such as geographic zones, climate, soil type, and soil history, while universal indicators include properties such as stability, self-recovery from stress, and biodiversity. (Van Bruggen and Semenov, 2000.)

Disease suppression is described as the general - or specific suppression of the pathogen that grows parasitically in the host (Simon and Sivasithamparam, 1989). General suppression can be described as the antagonism -, nutrient - and energy supply available for growth of the pathogen through the soil and on the root surface that could be manipulated by practices such as crop rotation and cultivation. Specific suppression occurs due to a suppression resulting from the antagonism of the pathogen within the soil by specific groups of microorganisms or by populations of individual microorganisms (Van Bruggen and Semenov, 2000). The specific disease suppression that could occur after monocropping is called disease decline. A decline in take-all disease of cereal crops caused by *Gaeumannomyces graminis* var. *tritici* because of the increase in populations of specific antagonists, is a well-known example (Sturz and Bernier, 1991; Andrade et al., 1994). Take-all decline soils have specific antagonism (referring to the induction of suppression specifically by *Ggt*), in contrast to general antagonism that occurs in all cultivated soils characterised by a higher microbial activity in soil associated with organic amendments and fertility build-up (Wildermuth, 1982). Suppression in other soils could be transferred to infected soils to inhibit *Ggt* – transferable (specific) suppression. Transferable suppression (induced by live inoculum of *Ggt*) inhibits both wheat pathogens and fungi producing runner hyphae on wheat roots. Slightly suppressive soil, induced by dead inoculum of *Ggt*, inhibits some wheat pathogens, but no inhibition of fungi that produce runner hyphae on wheat roots (Wildermuth, 1982). Pathogen suppression occurs when the pathogen is growing saprophytically, surviving in the soil, or when suppression is directly affecting saprophytic growth or survival of the pathogen (Simon and Sivasithamparam, 1989).

Suppressive soils - defined as soils in which the pathogens cannot establish, or they establish but fail to produce disease, or they establish and cause disease at first, but diminish with continued culture of the crop - can occur naturally as inherent

characteristic of physical, chemical, and/or biological structure of a particular soil, or it might be induced by some practices and activities such as planting of crops, or the addition of organisms or nutritional amendments, which causes a change in the microfloral environment (Larkin et al., 1993). Induced suppressive soils have been exemplified by occurrences of take-all decline of wheat through long-term monoculture of cereals (Larkin et al., 1993; Andrade et al., 1994). Soils conducive to take-all have also been induced to become suppressive through the use of crop sequence treatments. Varying degrees of disease suppression are recognised through reduced numbers of *Ggt* recovered in wheat root tissues and by a reduction in root disease. (Sturz and Bernier, 1991.) Directional growth of the pathogen towards the root, and growth of fewer *Ggt*-hyphae from infested debris in the wheat rhizosphere, are less apparent in soil with take-all decline compared with conducive soil. These results clearly indicate that suppression can act to limit prepenetration growth of the pathogen. In contrast, Cook et al. (1986) found microorganisms in suppressive soil associated with wheat take-all decline not to limit the number of lesions produced by *Ggt*. More lesions was found per unit mass of inoculum in suppressive than in conducive soil and he thus concluded that the limitation of *Ggt* in suppressive soil resulted from suppression after initial infection, and not during its prepenetration and penetration stages (Cook et al., 1986).

4. Suppression of Take-All

"*Biological control cannot be separated from the whole subject of disease control, which involves eventually a complete knowledge of the biology and epidemiology of a disease, and of the ecology of the crop plant.*" – Garrett, quoted by Cook (1994).

Biological control can be brought about by either the introduction or augmentation in numbers of one or more controlling organisms, or a change in environmental conditions to favour the multiplication and activity of such organisms, or a combination of both procedures (Cook, 2003).

Antibiosis against *Ggt* in vitro has been the main criterion in the selection of organisms to test for their role in suppression of take-all, but the lack of correlation between in vitro antibiosis and tests in the field was the common denominator in most of the research undertaken. Mechanisms other than antibiosis, e.g. mycoparasitism, induction of

phytoalexins in roots, and competition, could also be involved in the suppression of *Ggt*. It should be considered that many of the mechanisms discussed in microbial antagonism are presumptive since proof is also difficult to obtain (Andrade et al., 1994).

Crop sequence treatments influence relative proportions and frequencies with which major-, minor- and nonpathogen groups comprise the mycofloral community (Sturz and Bernier, 1991). Fungal antagonists of *Ggt*, such as *Trichoderma* and *Penicillium* form a small percentage of root mycofloral communities. The possibility exists that a type of general or non-specific disease suppression can be attributed to noncereal crop rotations that resulted in lower levels of take-all (Sturz and Bernier, 1991). The low incidence of serious root disease coinciding with the recovery of *Ggt*, *Fusarium* (W. G. Sm.) Sacc., and *Cochliobolus sativus* (Ito and Kuribayashi) Drechs. ex Dastur in barley and oats sequences, could be attributed to a mutual antagonism and (or) cross-protection response between these major pathogens (Sturz and Bernier, 1991).

Bacterial root colonisation of wheat occurs in two phases (Howie et al., 1987). The first phase concerns the distribution of the bacteria by passive carriage downward with root extension through soil. The second phase concerns the multiplication and survival of the colonising bacteria. Root colonisation occurs during and after the first phase, whereby an increase to the limit of the ecological niche occurs in the population (Howie et al., 1987). The displacement of antagonists from root tissues, as well as the precolonisation of root cortices by avirulent or minor pathogenic fungi and (or) postinfection inhibited by Gram-negative bacteria, are all important mechanisms responsible for reduced take-all disease in so-called take-all decline soils. Competitive pressure of enhanced bacterial populations can also give rise to the suppression of the disease (Sturz and Bernier, 1991).

Organisms from different taxonomic groups are associated with take-all suppression, involving different mechanisms in different wheat growing areas to suppress take-all (Andrade et al., 1994).

4.1 Organisms responsible for the suppression of take-all

4.1.1 *Pseudomonas*

Fluorescent pseudomonads can be used to inoculate annual crops to control plant pathogens or to promote plant growth, but their effectivity is greatly affected by poor colonisation of the roots by plant growth promoting rhizobacteria (non-symbiotic bacteria capable of stimulating plant growth) as a result of environmental factors (Davies and Whitbread, 1989b). The rhizosphere and plant roots in solarised soil, on the other hand, are rapidly colonised by fluorescent pseudomonads. Its sensitivity towards heat is counterbalanced by beneficial factors such as short generation time and rapid growth as compared with other colonisers - it might eventually benefit from the reduction in population densities of competing microorganisms by solarisation (Gamliel and Katan, 1991). Several bacterial characteristics are involved in the attachment of bacterial cells to plant roots, such as the presence of pili, a root-adhesive protein, and surface charge properties (Glandorf et al., 1994).

Barnett et al. (1999) found a quantitative and qualitative difference in bacterial populations between lesioned and non-lesioned sections on take-all infected wheat roots. Fluorescent pseudomonads are selectively stimulated by infected roots in cases of take-all disease of wheat caused by *Ggt*. Numbers of fluorescent pseudomonads were found to increase in parallel to the development of necrosis because of the introduction of *Ggt* to the soil (Sarniguet and Lucas, 1992). The probable involvement of these bacteria in specific suppression are responsible for take-all decline which develops within a few seasons in the presence of severely infected host (Sarniguet and Lucas, 1992). The application of combinations of biocontrol agents might be one possible approach to increase the amount and consistency of disease control, since significantly better control of take-all was achieved by combining two to four strains of fluorescent *Pseudomonas* spp., than using one strain individually (Duffy and Weller, 1995). Sarniguet and Lucas (1992) found populations of rhizobacteria, including antagonistic fluorescent pseudomonads, to increase closer and on the site of an infection – this involved the qualitative and quantitative change in the rhizosphere populations. As the nodal roots grew over the seminal roots from the crown, *Ggt* infected the roots, but the colonisation of roots by hyphae was limited by the more

numerous and still present antagonistic bacteria. This resulted in the limiting of the rates of extension of the necrosis.

Melanisation of the take-all hyphae was found to be a way of resisting lysis by microbial enzymes during *Ggt*'s saprophytic existence in infected host residues (Chakraborty and Warcup, 1983). The growth of *Ggt* was initiated by hyaline hyphae that might have become pigmented later on. It was during this stage before root colonisation, that competition with certain soil microflora took place and where *Pseudomonas fluorescens* restricted *Ggt*'s growth. These antagonistic microflora arose as a consequence of wheat monoculture. As previously mentioned, *Pseudomonas* spp. are widely recognised to be involved in the suppression of take-all disease of wheat through a number of mechanisms, but the lack of consistent evidence under various conditions has hindered acceptance of this group as being the sole cause of suppression (Cook and Rovira, 1976; Andrade et al., 1994). Reports have been found on the increase in the proportion of fluorescent pseudomonads antagonistic to the pathogen or disease, in take-all suppressive soils (Weller, 1983; Barnett et al., 1999). Proposed characteristics that might enhance the establishment and / or survival of introduced bacteria included a higher growth rate than that of indigenous microbial population, resistance to adverse environmental conditions, cell motility, resistance against starvation, the production of substances to aid in adherence to plant roots, and the production of antibiotics (Mazzola et al., 1992).

4.1.1.1 *Production of antibiotics*

Production of phenazine-type antibiotics in the rhizosphere by *Pseudomonas fluorescens* seems to be the primary means to protect wheat roots against infection by *Ggt* (Mazzola et al., 1992; Duffy and Weller, 1995). Two specific sub-groups of fluorescent pseudomonads are mainly responsible for disease decline – those producing phenazine-type antibiotics (Phe), and the other sub-group producing 2,4-diacetylphloroglucinol (Phl) which is a broad-spectrum antibiotic that might be toxic to plant roots too when over-produced in the root zone. Phl strain population levels have been found to be markedly higher in suppressive soils, colonising emerging wheat roots, and attaining high population levels (Mazzola and Cook, 1991). Seed applications of bacterial isolates of fluorescent pseudomonads that produced the antibiotics 2,4-diacetylphloroglucinol or phenazine-1-carboxylic acid, increased grain yield about 10-20% in fields where take-all was the predominant limiting factor (Duffy et al., 1997).

Sarniguet and Lucas (1992) reported that populations of antibiotic-producing pseudomonads in soil were higher during the monoculture of wheat than in the case where wheat was rotated with other crops, inhibiting colonisation as well as further infection by *Ggt* in the lesions on wheat roots (Andrade et al., 1994).

4.1.1.2 *Production of siderophores*

Suppression to take-all has also been induced by the addition of siderophore-producing *Pseudomonas* spp. to a conducive soil, since certain root colonising fluorescent pseudomonads promoted plant growth by producing extracellular siderophores with the ability to deprive deleterious rhizosphere microorganisms of iron required for growth (Fuhrmann and Wollum, 1989; Andrade et al., 1994).

Andrade et al. (1994) reported that pseudomonads isolated from suppressive soils had a greater ability to inhibit the take-all fungus, than those isolated from conducive soils, resulting in higher populations of pseudomonads in suppressive soils than conducive soils whilst suppression of take-all could also be induced by applying pseudomonads to seed.

4.1.1.3 *Production of other toxic products*

Pseudomonads have also been reported to produce other toxic secondary products (e.g. cyanides) that are involved in the control of black root rot of tobacco (*Thielaviopsis basicola*) in spite of reports that cyanide might have a deleterious effect on plant growth (Sarniguet et al., 1992a). Young wheat and barley plantlets, on the other hand, were found to be resistant to cyanide in distilled water. All the highly cyanogenic NH_4-strains were highly antagonistic *in vivo* – it also increased root growth in the presence of *Ggt*, because substrates for cyanogenesis (e.g. glycine and cyanogenic glucosides) were produced in wheat root exudates. Sarniguet et al. (1992a) reported that cyanides possibly act as toxic metabolites directly against *Ggt*, but could also modify the plant physiology and enhance plant defence reactions.

4.1.2 *Gaeumannomyces graminis* var. *graminis*

Gaeumannomyces graminis var. *graminis* (*Ggg*) from wheat and other grasses, is a fungus with the ability to suppress take-all in Australia and Europe, by competing directly with virulent *Ggt* for the same substrates and favoured sites in and on plant roots (Duffy and Weller, 1995). These fungi are responsible for the probable induction of

host resistance in wheat and barley, since they colonise the root cortex, causing greater lignification and suberisation of the endodermis and xylem vessels. Probable increased leakage of root exudates caused by *Ggg*, might attract increasing populations of other antagonistic rhizosphere microorganisms such as fluorescent *Pseudomonas* spp., that are especially well adapted to utilise root exudates very rapidly (Duffy and Weller, 1995). The composition of plant root exudates and rhizosphere microflora differ between plant types, while the amount and composition of carbon loss from roots, or rhizodeposition, change with the age of the plant, resulting in changes in rhizosphere microbial community structure (Garland, 1996b).

4.1.3 *Trichoderma* and *Gliocladium*

Trichoderma and *Gliocladium* spp. contribute to biocontrol activity with a remarkable diversity of mechanisms depending on the strain, environmental conditions, and pathosystem, including the production of antibiotics and lytic enzymes; mycoparasitism and competitiveness in the rhizosphere, bulk soil, or crop residue (Duffy et al., 1997; Vey et al., 2001). Except for secondary metabolites, *Trichoderma* and *Gliocladium* also produce different classes of fungal cell-wall-hydrolytic enzymes such as chitinases and proteases which play an important role in mycoparasitism (Vey et al., 2001). *Trichoderma* spp. (especially *T. koningii*), suppresses take-all's saprophytic and parasitic activity, since a greater proportion of *Trichoderma* has been found in the suppressive than in the conducive soil to be antagonistic to *Ggt* in pure culture. (Simon and Sivasithamparam, 1988b; Duffy et al., 1997).

It has previously been mentioned that nitrogen plays an important role in the suppression of *Ggt*. Ammonium-nitrogen fertilisers reduce the rhizosphere pH of wheat and increase the proportion of rhizosphere bacteria antagonistic to *Ggt*; the reverse is true for nitrate-nitrogen fertilisers. The take-all suppressive activity of indigenous *Trichoderma* spp., as well as competitiveness and biocontrol activity, is increased by soil acidification with ammonium fertilisers - acidic conditions enhance conidia production and germination, mycelial growth, and the production and activity of antimicrobial compounds such as antibiotics and lytic enzymes (Duffy et al., 1997). These effects are lost with liming which results in an increase in soil pH (Simon and Sivasithamparam, 1988b). Mineral nutrition is essential for growth and, to a lesser extent, stimulatory of fungal secondary metabolism. Total nitrogen availability increases

sporulation, hyphal growth rate, and production of antifungal anthroquinone pigments of *Trichoderma* spp. against a wood rot fungus. Soil nitrate levels are positively correlated with cellulase production that might favour competitiveness against the pathogen (Duffy et al., 1997).

A negative interrelationship has been reported to exist between boron, copper, iron, soluble magnesium, nitrate-nitrogen, percent clay, and take-all severity in the presence of *Trichoderma koningii*, but take-all severity is positively interrelated with soil pH and available phosphorous in the presence of *T. koningii*. It is thus important to keep in mind that soil textural components, such as clays, play an important role in water flow, oxygen availability, and the matrix for fungal growth and dispersal. Some of the key soil factors favourable to *Trichoderma* (e.g., low pH and high iron) contrast those factors favourable to fluorescent pseudomonads (e.g., high pH and low iron). Mixtures of such diverse biocontrol agents might thus provide control across a wider range of soil types, provided that the fungal and bacterial agents are compatible in the field (Duffy et al., 1997).

4.1.4 *Sterile Red Fungus*

A basidiomycete sterile red fungus (SRF) with the ability to effectively suppress infection of wheat roots by *Ggt*, was isolated from wheat and rye grass roots by Aberra et al. (1998). The SRF was reported to colonise wheat seeds without affecting their viability, therefore suggesting the application of pre-colonised wheat seeds in the field. Some of the mechanisms, through which the SRF suppresses *Ggt* infection, include competition for space and nutrients; antibiosis, and plant growth promotion which could allow for escape, compensation for loss, or the masking of the disease through improved plant growth. Another mechanism concerns the induction of the host's defence protein synthesis (e.g. pathogenesis-related proteins, hydroxyproline-rich glycoproteins); an increase in defence enzyme activity (e.g. phenylalanine ammonia-lyase, peroxidases); phytoalexin synthesis, and cell wall strengthening (e.g. lignification). (Aberra et al., 1998.)

4.1.5 *Amoebae*

Soil amoebae producing cellulase and chitinase to enable the lysis of melanised fungal propagules in soil, have been isolated from ecologically diverse agricultural and forest

soils. Chakraborty and Warcup (1983) reported the isolation of mycophagous amoebae have been isolated from take-all conducive- and suppressive permanent-pasture soil, whose mycophagy has been demonstrated on the take-all fungus with the lysis of the hyaline hyphae of *Ggt.*

4.2 Crop rotation and take-all of wheat

During crop rotation, sufficient time is allowed between the planting of susceptible crops for the lowering of the inoculum potential of soilborne plant pathogens below some economic threshold by resident mycoparasites, competitors, predators, and antibiotic-producing microorganisms (Cook, 1994).

Soil microorganisms mediate processes essential to agricultural productivity of soil, like the decomposition of soil organic matter (Frey et al., 1999), the recycling of plant nutrients, maintenance of soil structure, degradation of agrochemicals and pollutants, and the control of plant and animal pests (Lupwayi et al., 1998; Wardle et al., 1999). Concerns about environmental effects caused by intensive agriculture, the loss of soil organic matter and contamination of ground water by agrochemicals in particular, have recently shifted the focus of research more towards ecologically sustainable systems: the use of reduced tillage, inputs of organic materials and nutrient cycling strategies based on crop rotations (Pankhurst et al., 1996). Beneficial effects of crop rotation have been ascribed to agronomic inputs such as improved soil management and fertilisation (Maas and Kotze, 1990), but soil tillage, on the other hand, might have a detrimental effect on soil microbial dynamics through deterioration of soil structures, and other factors (Lupwayi et al., 2001). Zelles et al. (1992) reported reduced soil microbial diversity in the fallow phase of crop rotation, while soil microbial diversity was enhanced when reduced tillage was practised (Lupwayi et al., 1998). Soil disturbances might result in reductions in soil organism diversity due to desiccation, mechanical destruction, soil compaction, reduced pore volume and disruption of access to food resources (Lupwayi et al., 1998). Zero tillage, on the other hand, leaves crop residues from preceding years' growth at the soil surface, resulting in high diversity in habitats with high and varied production of litter. Crop rotations under zero tillage with litter from several crops in preceding years, results in a greater variety of substrates than under conventional tillage where litter did not accumulate. Soil-mixing actions of tillage could even make substrates in soil more homogeneous (Lupwayi et al., 1998). Different bacterial community structures have been found between soils under conventional

tillage and under zero tillage, with greater bacterial diversity under zero tillage and larger soil aggregates (Lupwayi et al., 2001).

Crop rotation has been reported to stimulate soil microflora antagonistic to *Ggt*, thus reducing the chances of survival of *Ggt* by growing plant species such as lupins, field peas and rapeseed. These practices were reported to yield better results than just the maintenance of fallow conditions (Kollmorgen et al., 1983). The application of crop rotation is considered an effective method to reduce yield losses of wheat due to take-all until soil suppressiveness is induced (Rondon and Cunfer, 1991), but caution should be taken against crop rotation when certain crops such as legumes, lucerne and pasture are considered, since these crops tend to increase take-all in subsequent wheat crops (Maas and Kotze, 1990). Soil fertility and crop production, on the other hand, are enhanced with the use of legumes in crop rotation (Walters et al., 1992).

Diseases have been found to increase after fallowing (i.e., a land plowed, but left unplanted) or double-cropping with soybeans, but take-all has been reduced after double-cropping with tobacco. Maize has also been reported to decrease disease and to be an effective break crop in comparison with soybeans, sunflower or fallowing (Maas and Kotze, 1990). Sharma et al. (1998) reported an increase in the functional diversity of soil bacterial communities for about 32 weeks, where soils were amended with maize litter placed on the soil surface. Oats, which is resistant to *Ggt*, is the only small grain that could be effectively substituted for wheat in cropping systems to manage take-all (Rondon and Cunfer, 1991). Detectable disease levels were found in cases where wheat followed oats on a rotation system, but the build-up of the pathogen on the oats crop itself was not responsible (Rondon and Cunfer, 1991). Wheat, barley, and triticale are effective in the carryover of the take-all fungus. These crops allow selective herbicides to be used to control grass weeds that are hosts of *Ggt* (Kollmorgen et al., 1983).

Possible explanations for the reduced survival of *Ggt* under various crops could be the antifungal compounds exuded by the roots of the plants, depleted levels of soil nitrate, or stimulation of microflora antagonistic to *Ggt* during crop rotation (Kollmorgen et al., 1983; Sturz and Bernier, 1991). Prior knowledge of how the crop and root exudates affect microbial antagonism and virulence of the pathogen is very important before any crop can be recommended for inclusion in a rotation system (Maas and Kotze, 1990). Soybean and sunflower root exudates significantly increase the pathogenicity of *Ggt* in comparison with exudates from crops such as wheat, maize and tobacco. Soil with tobacco root exudates has been found to have the lowest disease levels (Maas and

Kotze, 1990). Rondon and Cunfer (1986) ascribed one of the mechanisms involved in the negative effect of double-cropping on take-all to the disturbance of antagonistic microorganisms which normally develop with wheat monoculture. It is known that take-all naturally declines after continuous cropping of wheat for several seasons. This decline can be attributed to a selection process occurring in the rhizosphere of infected wheat plants, and the gradual increase in populations of antagonistic fluorescent *Pseudomonas* spp., resulting in the suppression of the pathogens' parasitic activity (Simon and Sivasithamparam, 1988c; Duffy and Weller, 1995). Different crops have significant effects on the pseudomonad populations in soil, as well as on individuals antagonistic to *Ggt*. Tobacco and maize soils have been found to harbour the highest concentration of pseudomonads, but that only those in tobacco soil showed a high degree of antagonism towards *Ggt*. (Maas and Kotze, 1990.)

4.3 The role of nitrogen

The nitrogen cycle is exclusively carried out by a small number of microorganisms in nature (Brock et al., 1994), with nitrogen gas, ammonia, and nitrate, being the more easily available forms of nitrogen playing an important role in the biological nitrogen cycle. These nitrogen forms are interconverted by means of three processes that make up the nitrogen cycle:

Nitrogen fixation concerns the conversion of nitrogen gas to ammonia. Only a small amount of nitrogen is "fixed" by prokaryotes, either alone or in symbiosis with certain plants, in the form of nitrate, nitrite, ammonium or organic nitrogen.

Nitrification concerns the conversion of ammonia to nitrate. It is an aerobic process that occurs in well-drained soils at neutral pH.

Denitrification concerns the regeneration of nitrogen gas from nitrate and is the main means by which gaseous nitrogen is formed biologically (Ingraham et al., 1997; Delisle et al., 1999).

4.3.1 *Nitrogen and take-all*

In most cases, take-all disease has been reported to reduce by the application of an ammonium (NH_4^+) source (Christensen et al., 1987). The uptake of NH_4^+ by the plant roots resulted in a decrease in the pH of the rhizosphere, consequently resulting in the indirect inhibition of take-all because of changes in the rhizosphere microflora between

pH 5-7. A direct effect was found on the fungus for a pH lower than 5 (Sarniguet et al., 1992a). Take-all severity on crown roots of winter wheat have also been reduced by management practices leading to high NH_4^+ to NO_3^- ratios in the topsoil (Christensen et al., 1987). The application of NH_4^+ - and Cl-containing fertilisers at low soil pH (5.5) resulted in the highest NH_4^+ to NO_3^- ratios in soil, highest grain yield and lowest disease severity. Liming the soil to pH 6.6 and the application of ($Ca(NO_3)_2$) fertiliser resulted in lowest NH_4^+ to NO_3^- ratios, highest disease severity and lowest grain yield. The addition of an NH_4^+ source results in a higher proportion of rhizosphere pseudomonads, which are naturally antagonistic to *Ggt*, than the application of NO_3^-, thus lowering the intensity of the disease for ammonium-treated soil (Sarniguet and Lucas, 1992). The effect of the nitrogen source must be considered to influence the growth (saprophytic- and preparasitic phases) of *Ggt*. The nitrogen source has an impact on the parasitic phase (the extension of the lesions) of *Ggt*, since the disease severity is lower in NH_4^+-treated soil in comparison with NO_3^--treated soil (Sarniguet et al., 1992a). Ammonium chloride has been found to be superior to ammonium-sulphate, since the chloride lowers the plant water potential, resulting in greater suppressive-activity of antagonistic rhizosphere bacteria than those occurring on roots of wheat fertilised with ammonium-sulphate (Duffy et al., 1997).

Adequate supplies of micronutrients such as copper, zinc, iron, and manganese can also alleviate take-all, primarily because of the increased availability of the micronutrients under acidic soil conditions due to metabolic activities of certain indigenous microorganisms. The oxidation of manganese to Mn^{4+} by the pathogen, decreases availability of the micronutrient to wheat, leading to increased levels of take-all disease. This effect is once again reversed by soil acidification. This reaction favours rhizosphere bacterial populations that reduce manganese to Mn^{2+} which is more available to the plant (Duffy et al., 1997).

Plant roots in soil environments are able to affect nitrate dissimilation, especially denitrification (essentially through nitrate uptake), carbohydrate exudation and changes in the aeration status. Plant roots are also able to discriminate organisms on the basis of their nitrogen dissimilative abilities, thus selecting specific populations of microorganisms for significantly improving rhizosphere competence. The selection seems to be plant- and soil type-dependent, but no clear relationship between the distribution of metabolic types (functional diversity) and the distribution of bacterial species has yet been found (Clays-Josserand et al., 1999).

Soil, continuously cropped with wheat for nine years, was found to be disease suppressive but not pathogen suppressive, but with the annual soil treatment with ammonium sulphate, it became suppressive to both the disease and the pathogen - the addition of lime to ammonium sulphate treated soil, increased disease suppression, but reduced pathogen suppression (Simon and Sivasithamparam, 1988a).

Simon and Sivasithamparam (1988a) reported *Trichoderma* spp., as a proportion of the total number of filamentous fungi counted, to be greater in suppressive than in conducive soils. These results suggest that *Trichoderma* spp. might play an important role in the transferable suppression of saprophytic growth of *Ggt*.

The evolution of soil conduciveness between sowing and tillering was found to be parallel to the evolution of fluorescent pseudomonads when take-all occurs – qualitatively and quantitatively. Low levels of conduciveness was found to correspond to high frequencies of in vitro antagonistic bacteria (Sarniguet and Lucas, 1992). The frequency of antagonistic pseudomonads increased markedly in NH_4^+-treated soil after tillering compared to the same soil after sowing. It became higher than in NO_3^--treated soil at tillering (Sarniguet et al., 1992a).

Numerous microbiological techniques can be used to measure the response of a microbial community to stresses, and although quantitative yields and specific information on biodiversity can be obtained by conventional microbiological techniques, it is recognised that these techniques are incapable of estimating the true biodiversity of a sample (Øvreås and Torsvik, 1998). Recent studies have indicated that less than 1% of all microbes can be cultured on artificial media with viable counts of microorganisms from environmental samples accounting for 0.1 - 10% of the total community (Sharma et al., 1998; Vestal and White, 1989). Numerous alternative techniques to overcome the limitations frequently associated with conventional techniques have subsequently been developed and applied to characterise microbial ecosystems. Some of these techniques include the analysis of signature lipid biomarkers, DNA hybridisation or DNA/RNA fingerprinting techniques (Bossio et al., 1998), and community level physiological profiles by using Biolog® microtiter plates (Bossio and Scow, 1995). Despite significant improvement, it should be remembered that these alternative techniques are also subject to limitations and, in order to overcome these limitations, it is advisable that more than one technique be used in order to characterise microbial succession in soil (Van Bruggen and Semenov, 2000).

Responses of soil microbial communities to the application of different stress factors could be used as indicators of soil health. Disease suppression of root pathogens play an integral part of soil microbial communities and could be viewed as a manifestation of ecosystem health and stability. Since a link exists between soil health, the ability of the biological community to suppress plant pathogens, the population density of plant pathogens in soil, and ultimately disease incidence and severity, disease suppression could function as an indicator for a stable and healthy soil ecosystem (Van Bruggen and Semenov, 2000). Once again various physical, chemical and biological factors contribute to disease suppression. Disease suppressive soils can be characterised by the changes in microbial community structure and the time required to return to the initial state after the application of various stresses (Van Bruggen and Semenov, 2000). A decline in take-all disease of cereal crops caused by *Gaeumannomyces graminis* var. *tritici* because of the increase in populations of specific antagonists, is a well-known example (Andrade et al., 1994). Analyses of communities at microbial level have not been possible until recently, but numerous new assays and procedures have been developed to study microbial communities *in situ* that show promise for use in complex substrates. Most of these recently developed techniques are independent of cell culturability, thus circumventing numerous problems associated with conventional microbiological techniques.

5. Methods Used To Detect Changes In Microbial Communities

5.1 Analysis of functional diversity based on community level physiological profiles (CLPP)

The functional importance of diversity for the maintenance of soil biological processes is largely unknown; not to mention the extent of microbial diversity (Rondon et al., 1999). The analysis of soil microbial communities has been hampered by the inability of microbiologists to isolate and culture all of the species present, or even a reasonably representative subset (Buyer and Drinkwater, 1997). Technical difficulties in sampling and quantification of soil microorganism-diversity, and the changes in functional processes of soil microbial communities by agrochemicals and mechanical tillage, makes it even more difficult to analyse soil microbial communities in agricultural systems (Pankhurst et al., 1996). Microbial populations and their responses to stresses have been traditionally studied at the level of process in terms of the total number of

microorganisms, biomass, respiration rates and enzymatic activities, with little attention being paid to responses at the community or functional diversity level (Zak et al., 1994).

Bacterial functional diversity refers to the ability of these communities to utilise the large spectrum of different compounds occurring in plant tissue and root exudates, etc., whose proportional abundance changes during decomposition. Insight into ecological functioning of communities can be obtained by analysis of the presence and distribution of different operational taxonomic units within communities (e.g., diversity), since the analysis of the taxonomic structures of communities alone, limits insight into the ecological relevance of community structure (Garland and Mills, 1991). Microorganisms influence decomposition, nutrient transformations, plant growth promotion / suppression and miscellaneous soil physical processes. Microbial community functional diversity also includes the range and relative expression of activities involved in above-mentioned functions (Giller et al., 1997). Profiles of cellular constituents or phenetic characteristics can be used in numerical taxonomic studies (Van Heerden et al., 2000). Knowledge of microbial diversity is currently being incorporated into the improvement of agriculture (Shen, 1997): microbial products have been rapidly developed and commercialised for agricultural purposes; bioremediation of environmental pollutants; biological control of pests and plant diseases; probiotics of animals and humans. Likewise, a mixture of several bacteria isolated from natural environments using specific techniques, has been found to exalt crop growth, and suppress some plant diseases (yield-increasing bacteria (YIB)) (Shen, 1997). The mechanism of action of yield-increasing bacteria is associated with the production of hormones, antifungals and enzymes, hence giving rise to competition for ecological niches (Shen, 1997).

Highly structured communities composed of microorganisms of distinct morphology and/or nutritional strategies exist, but microbial ecologists have been unsuccessful at the identification and classification of microbial communities due to the small size and morphological similarity of the constituent members (Garland and Mills, 1991). Biodiversity is considered to consist of three interrelated elements: genetic, functional, and taxonomic diversity. For this reason, the full understanding of microbial communities can only be achieved with the integration of taxonomy information with genetic and metabolic / functional potential of communities (Dobranic and Zak, 1999).
One approach to translate the information in a microbial ecosystem would be to determine the metabolic diversity within the system (Garland and Mills, 1991; Van

Heerden et al., 2000). Garland and Mills (1991) subsequently proposed a community-level approach to assay microbial function to provide more sensitive and ecologically meaningful measures of heterotrophic microbial community structures. They hypothesised that sole carbon source utilisation exerted a strong influence on the classification of isolates and might therefore be a useful, community-level, functional measure in the characterisation and classification of heterotrophic communities. With this technique, advantage is taken of traditional methods of bacterial taxonomy in which bacterial species are identified based on their utilisation of different carbon sources (Hill et al., 2000). Commercially available Biolog® microtiter plates allow for the simultaneous testing of 95 separate carbon sources (Table 1).

The specific meaning of differences in community level physiological profiles (CLPP) is related to the rate of colour development (Garland and Mills, 1991) in a given well. It would appear to be related to the number of microorganisms that are able to use the substrate within the well as a sole carbon source and are concomitantly stained with tetrazolium violet dye. An observed correlation of average well colour development (AWCD) with inoculum density rather than activity was made by Winding and Hendriksen (1997). This problem can be dealt with by the determination of an AWCD value for each Biolog® microtiter plate by the calculation of the raw difference between each well's optical density as well as that of the control well. All the values are summed together and divided by 95. Every value for each substrate is divided by the AWCD for that microtiter plate to normalise comparisons between samples with different inoculum densities (Garland and Mills, 1991; Haack et al., 1995). Since the inoculation of bacteria into Biolog® microtiter plates is expected to result in growth, colour produced from the reduction of the tetrazolium violet dye is used as an indicator of respiration of sole carbon source utilisation because of growth of inoculated bacteria correlating positively with inoculum density (Winding and Hendriksen, 1997). The colour change is quantified spectrophotometrically (Hill et al., 2000), enabling the characterisation and classification of heterotrophic microbial communities (Garland and Mills, 1991) while multivariate statistical techniques are used to analyse community level physiological profile data (Hitzl et al., 1997). Communities with similar community level physiological profiles have been observed to cluster together with those from another community, whereas the profiles are segregated if the communities are considered functionally different (Hill et al., 2000).

Table 1. Carbon sources present in Biolog® GN2 microtiter plates (As adapted from Garland and Mills, 1991).

Carbohydrates	**Carboxylic acids**	**Amino acids**
N-Acetyl-D-galactosamine	Acetic acid	D-Alanine
N-Acetyl-D-glucosamine	cis-Aconitic acid	L-Alanine
Adonitol	Citric acid	L-Alanyl-glycine
L-Arabinose	Formic acid	L-Aspartic acid
D-Arabitol	D-Galactonic acid lactone	L-Glutamic acid
Cellobiose	D-Galacturonic acid	Glycyl-L-aspartic acid
i-Erythritol	D-Gluconic acid	Glycyl-L-glutamic acid
D-Fructose	D-Glucosaminic acid	L-Histidine
L-Fucose	D-Glucuronic acid	Hydroxy-L-proline
D-Galactose	α-Hydroxybutyric acid	L-Leucine
Gentiobiose	β-Hydroxybutyric acid	L-Ornithine
α-D-Glucose	γ-Hydroxybutyric acid	L-Phenylalanine
m-Inositol	ρ-Hydroxyphenylacetic acid	L-Proline
α-D-Lactose	Itaconic acid	L-Pyroglutamic acid
Lactulose	α-Ketobutyric acid	D-Serine
Maltose	α-Ketoglutaric acid	L-Serine
D-Mannitol	α-Ketovaleric acid	L-Threonine
D-Mannose	D,L-Lactic acid	D,L-Carnitine
D-Melibiose	Malonic acid	γ-Aminobutyric acid
β-Methyl-D-glucoside	Propionic acid	**Aromatic chemicals**
D-Psicose	Quinic acid	Inosine
D-Raffinose	D-Saccharic acid	Uronic acid
L-Rhamnose	Sebacic acid	Thymidine
D-Sorbitol	Succinic acid	Uridine
Sucrose	**Brominated chemicals**	**Polymers**
D-Trehalose	Bromosuccinic acid	Glycogen
Turanose	**Amides**	α-Cyclodextrin
Xylitol	Succinamic acid	Dextrin
Esters	Glucuronamide	Tween 80
Mono-methylsuccinate	Alaninamide	Tween 40
Methylpyruvate	**Amines**	**Phosphorylated chemicals**
Alcohols	Phenylethylamine	D,L-α-Glycerol phosphate
2,3-Butanediol	2-Aminoethanol	Glucose-1-phosphate
Glycerol	Putrescine	Clucose-6-phosphate

Direct incubation of whole environmental samples (aquatic, soil, and rhizosphere) in Biolog® microtiter plates thus produce patterns of metabolic response suitable for rapid classification of heterotrophic microbial communities (Garland, 1996a). Community level physiological profiles or "metabolic fingerprints" have been used to differentiate microbial communities from different soil systems (Garland and Mills, 1991), from the same soil type under different plant types (Zak et al., 1994), and from cultivated and noncultivated soils (Kelly and Tate, 1998). Community level physiological profiles have also been used to examine changes in microbial communities related to different carbon inputs and different moisture levels in agricultural plots (Bossio and Scow, 1995), while Kelly and Tate (1998) determined the impact of metal contamination of soils and the effect of reclamation of these soils on the soil microbial communities. Community level physiological profiles have proven to be highly reproducible (Haack et al., 1995), elucidating miscellaneous soil processes: bioremediation, the release of genetically engineered microorganisms, flooding, farming practices, and pollution (Bossio and Scow, 1995; Lawley and Bell, 1998). The determination of CLPP have also been shown to be effective for the detection of plant dependent differences in rhizosphere communities, and changes in response to plant developmental state (Garland, 1996b). This information allows the examination of natural variation and diversity of microbial communities, and the monitoring of changes in microbial diversity caused by environmental fluctuations, land management practices, pollution, and the impact of forestry practices on microbial diversity and community structure by using a subset of carbon substrates (Wünsche et al., 1995; Staddon et al., 1997).

Determination of CLPP depends on the ability to consistently characterise microbial communities based on profiles of sole carbon source utilisation and to define the cause of the community response. Some of the factors that need to be addressed to answer the latter question, include the percentage of the community members that responded under the Biolog® assay conditions, the degree of synergy and antagonism among community members, and the relative importance of community composition and physiological status (Garland, 1996b). The analysis of CLPPs using Biolog® has the important advantage that it is easy to use and is thus feasible for use in large-scale field studies (Bossio and Scow, 1995) as well as the provision of information-rich data concerning bacterial functional biodiversity (Zak et al., 1994). The obtained data is especially pliable to multivariate analyses and other commonly used statistical procedures to look at taxonomic diversity in macro-organisms (Zak et al., 1994). It is,

however, important to realise that active metabolism of microorganisms is a prerequisite to establish a metabolic fingerprint of the microbial community. It should therefore also be realised that the Biolog® method only allows the characterisation of the metabolic activities of aerobic or facultatively anaerobic, heterotrophic and copiotrophic microorganisms with the ability to grow at sufficient rates on the Biolog® media (Wünsche et al., 1995). The assay conditions are, however, quite different from the environmental conditions and the metabolic capabilities of entire soil communities can thus, not be reflected (Bossio and Scow, 1995).

In spite of the mentioned advantages, it is also important to realise that the metabolic fingerprinting of microbial communities using community level physiological profiles, has certain limitations concerning its sensitivity to inoculum densities, a selection of substrates biased towards simple carbohydrates, and its inability to determine fungal activity (Pankhurst et al., 1996). It is thus clear that, when using this method for community analysis, there are several important considerations that should be taken into account. Firstly, the density of the initial inoculum must be standardised due to the effect it has on the rate of colour development in the wells and thus the time at which colour development should be measured (Haack et al., 1995). Visible colour will not develop within a well until the total number of cells able to utilise that substrate reaches approximately 10^8 cells/ml. There may thus be a substantial lag phase while the cells grow within the wells; if the number of cells directly inoculated into the wells are below 10^8 cells/ml, it could lead to false negatives if wells were read too soon. Inaccurate community level physiological profiles may result if samples are to be dominated by only a few species capable of growing on particular substrates (Smalla et al., 1998). The period of microbial growth within wells can also lead to competition effects that again can bias community level physiological profiles (Haack et al., 1995). A second methodological consideration that should be kept in mind, is that functional diversity analysis is based on the assumption that colour development in each well is solely a function of the proportion of organisms present in the sample which are able to utilise a particular substrate (Garland, 1997). Community level physiological profiles obtained from Biolog® GN microtiter plates, does not necessarily reflect the functional potential of the numerically dominant members of the microbial community used as the inoculum (Smalla et al., 1998). Patterns measured with Biolog® systems also reflect the metabolic activities of only a certain proportion of the community, since certain organisms could not be separated from the soil matrix (Wünsche et al., 1995). Campbell et al. (1997)

included plant root exudate compounds as carbon sources in the analysis of community level physiological profiles of grassland sites and found these compounds to have had a greater differentiating ability since the substrates were biochemically more diverse, and selected for more slow-growing organisms that were usually present in the sample in smaller numbers.

Since fungi are the primary organisms responsible for the decomposition of organic matter in most terrestrial ecosystems, it is important to understand how fungal biodiversity and species richness relate to aspects of decomposition. It is for this reason that a FungiLog was recently developed to allow quantitative approaches to examining aspects of fungal diversity (Dobranic and Zak, 1999). Taxonomic data can be obtained in conjunction with functional diversity by using FungiLog; distinguishing patterns can also be obtained from differences in total activity and substrate richness in decomposition-environments (Dobranic and Zak, 1999). Other types of microtiter plates are also available; each yielding a specific pattern of activities representing the functional attributes of the inoculated sample with respect to a suite of substrates. MT Biolog® microtiter plates (empty Biolog® microtiter plates) contain no carbon source, but only the tetrazolium violet dye (Garland and Mills, 1991) which enables a researcher to study carbon sources of interest. GN Biolog® microtiter plates for the characterisation of Gram negative isolates, and GP Biolog® microtiter plates for the characterisation of Gram positive isolates. There are 62 substrates common to both GN and GP microtiter plates, but each has additional 33 substrates unique to each microtiter plate (Van Heerden et al., 2000). Although substrates in Biolog® GN microtiter plates might be of little use for strain identification, fewer substrates are sufficient for describing and distinguishing between different microbial communities (Hitzl et al., 1997). The Biolog® EcoPlate uses of a reduced set of discriminating carbon sources (e.g., reducing the number of observed substrates from 95 to 31 or less), but with a higher number of replicates on each plate. This might be a better approach, since statistical analysis could then be better applied (Hitzl et al., 1997).

5.1.1 *Diversity Indices*

Biodiversity can be determined by using several diversity measures, with the Shannon-Weaver index being the most common measure of biodiversity. The Shannon-Weaver index takes into account species richness and the proportion of each species within the

local soil microbial community. According to Magurran (1988), values of the Shannon-Weaver index fluctuate between 1.5 and 3.5, but rarely increase above 4.5. The functional diversity of soil microbial communities can thus be quantified with Shannon-Weaver's substrate diversity index (*H'*) by using the number of different substrates utilised by the microbial communities (Magurran, 1988; Lupwayi et al., 2001). The raw difference data can be incorporated into:

$$H' = -\Sigma [p_i \ln (p_i)]$$

where $p_i = a_i / \Sigma a$ = the proportional turbidity observed in the *i*th well and a_i = the turbidity of the *i*th well and Σa = the total turbidity observed in all sample wells (Lahav and Steinberger, 2001).

Bacterial functional diversity of substrate utilisation can also be determined by using substrate richness (*S*) and substrate evenness (*E*) (Lupwayi et al., 2001). *S* is the amount of different substrates used by the bacterial community, i.e. comparable to species richness in the soil, and was calculated by counting all positive optical density readings. *E* is a measure of the equitability of activities across all substrates, i.e. comparable to how equally abundant the species are in the soil and is calculated as (Magurran, 1988; Zak et al., 1994):

$$E = H' / \ln S$$

Where *E* is the Shannon index of evenness and *S* is the number of species.

The substrate equitability (*J*) of the microbial populations in the various soil samples can be determined by rescaling the substrate diversity index (Magurran, 1988; Zak et al., 1994):

$$J = H' / H'_{max}$$

where *H'* represents substrate diversity, and H'_{max} being the maximal substrate diversity index for the microtiter plates.

Similarity between samples can be calculated by using Sorenson's measure for quantitative data (Magurran, 1988):

$$C_N = 2jN / (aN + bN)$$

where C_N represents Sorenson's measure of similarity; *aN* is the sum of the turbidities for sample *a*; *bN* is the sum of the turbidities for sample *b*, and *jN* represents the sum of the lower of the two turbidities for each carbon source in the two samples compared. Magurran (1988) stated that a high evenness (equitability) would imply a high diversity.

In contrast to the Shannon-Weaver diversity index, Sorenson's index emphasises the degree of substrate utilisation (Magurran, 1988).

5.2 Analysis of structural diversity based on phospholipid fatty acid analysis

In situ analysis and characterisation of microbial communities presents a notable challenge (White et al., 1996). The analysis of signature lipid biomarkers is an analytical approach which is dependant of the culturing of organisms (Pankhurst et al., 1996). This approach is essential since it has been estimated that less than 1% of microorganisms that can be detected in stained microscopic preparations, can be cultured (White et al., 1996). The composition of communities can be characterised with the use of culture-independent techniques based on the extraction, identification, and quantification of signature lipid biomarkers which are specific to certain microorganisms or microbial groups, and is often referred to as chemical ecology (Hill et al., 2000). Detection of microorganisms in soils is very difficult, since many microorganisms are attached to soil particles and might thus, even be hidden. Agents used to detach microbes from soil particles are usually selective and do not release the microorganisms quantitatively (White et al., 1996). Furthermore, very little insight is given into the nutritional status or structure of a microbial community since the morphology of the microorganisms does not often reflect the activity or function of the community. Metabolic processes are also complicated by the fact that most soil-microorganisms are inactive until the necessary nutrients appear (White et al., 1996).

Phospholipid fatty acids (PLFAs) are exclusively found in cell membranes and not in other parts of the cell as storage products (White et al., 1996). Phospholipid fatty acids can therefore serve as important indicators of active microbial biomass as opposed to non-living microbial biomass (Hill et al., 2000). This technique tends to depict the present living community, qualitatively and quantitatively, thus enabling living biomass to be accurately measured (Vestal and White, 1989). The phospholipid component in the cell membranes of bacteria contains ester-linked fatty acids which serves as a useful tool to primarily identify prokaryotes (Pankhurst et al., 1996). Archaea are not taken into account, since they contain ether-bound lipids (Sundh et al., 1997). Measurements of fatty acids in soils have been used extensively to estimate microbial biomass and to examine the microbial community structure (Haack et al., 1994). Frostegård et al. (1993b) detected changes in various proportions of individual bacterial

phospholipid fatty acids (PLFA) during experimentally contamination of soils with zinc. This indicated shifts within the community structure in the soils examined. Bååth et al. (1995) studied the effect of clear-cut, wood ash fertilised, or prescribed burned coniferous forests on microbial biomass and community structure by using PLFA analysis, while Frostegård et al. (1993a) studied the effect of lime and wood-ash on total microbial structures in two coniferous forest soils by analysing the PLFA composition of the microbial communities. Frostegård et al. (1991) evaluated the use of total phosphate as a measure of biomass in soils with different organic matter content. Laczko et al. (1997) used PLFA analysis to estimate critical pollutant loads in anthropogenically influenced and disturbed soil, by determining the amount of cadmium, acting as an indicator of anthropogenical impacts, present in the soil. Fatty acids are "signature" chemicals that are restricted to specific subsets of a microbial community (Zelles et al., 1992) and since some bacteria have their own unique 'signature' phospholipid fatty acids, this signature lipid could be used as basis to identify specific groups of microorganisms (Pankhurst et al., 1996). The phospholipids of a given sample can therefore be taken as a fingerprint of a microbial community (Petersen and Klug, 1994). Knowledge of such signature molecules arose from the use of fatty acid analysis in bacterial taxonomy where specific fatty acid methyl esters (FAMEs) have been used as accepted taxonomic discriminator for species identification (Hill et al., 2000). Since phospholipid fatty acids are easily extracted from microbial cells in soil, access is allowed to a greater proportion of the microbial community resident in soil than would otherwise have been accessed during culture-dependent methods of analysis (Zelles and Bai, 1993). Presence and abundance of particular or groups of organisms in which those signatures can be found, is revealed by the presence and abundance of these signature fatty acids in soil (Hill et al., 2000). This method has also been used to detect differences between communities by comparison of fatty acid profiles of the whole soil (Haack et al., 1994), to compare changes in PLFA patterns at two sites to determine the extent of heavy metal influence on microbial communities (Pennanen et al., 1996), to study the effect of suppressors of soil-borne plant diseases (Tunlid et al., 1989), and to study the ecology of soil microorganisms in agroecosystems by examining soil samples from plots with a long-term agricultural monoculture and rotation experimental plots for fatty acid profiles in phospholipid- or lipopolysaccharide fractions (Zelles et al., 1992). Since it is becoming essential to understand how reliably PLFAs represent microbial communities in soil, it is also important to determine to what extent environmental and edaphic factors influence microbial communities (Bossio et al., 1998). As it is not known

which environmental determinants play the most important role in determining the composition of soil microbial communities, the effects of agricultural management, season, and soil type on phospholipid fatty acid profiles are currently being studied. The determinants are hence ranked in the following order: soil type > time > specific farming operation (e.g., cover crop incorporation or sidedressing with mineral fertiliser) > management system > spatial variation in the field (Bossio et al., 1998). A clear discrimination of microbial communities in different soils were observed with the study of PLFA profiles, and a successful differentiation was made between communities that had developed under different agricultural management systems. But measures of microbial community and soil properties were seldom associated with variations in PLFA profiles (Bossio et al., 1998). The application of PLFA analysis in these studies demonstrated the utility of this method in determining gross community changes associated with soil management practices (Hill et al., 2000).

The increasing use of signature lipid biomarkers has also lead to the questioning of the reliability of the biomarkers, more specifically, its universalness to a particular group of organisms in the indigenous community (Bossio and Scow, 1998). It is also important to distinguish whether the fatty acids indicates the presence of specific taxa, or physiological changes in a community with the same taxonomic composition. Changes in microbial community structure due to agricultural management regime have been indicated by sensitive PLFA profiles as indicators. The more commonly occurring bacterial fatty acids were more indicative of changes in microbial communities, than markers specific to certain subgroups (White et al., 1996; Bossio and Scow, 1998). Quantitative analysis is done by using gas chromatography and gas chromatography-mass spectrometry (White et al., 1996). The metabolic state of the organism, environmental changes and the exposure of the organism to toxic substances, are all factors that influence the PLFA composition of cell membranes (Frostegård et al., 1993b). Temperature is the environmental factor that affects the composition of PLFA most directly, since it has a direct influence on membrane functions such as membrane permeability and transport systems across the membrane (Petersen and Klug, 1994), since the fluidity of the lipid layer will increase as temperature increases. This results in the formation of non-bilayer phases that, in turn, affects membrane permeability, and membrane transport systems. A lack of adaptation of the PLFA composition in order to modify membrane fluidity to compensate for such effects, influences the adaptation of

the organisms to dramatic temperature change because of starvation, which induces death or stress reactions (Petersen and Klug, 1994).

5.2.1 *Lipid functions*

Lipids are exceptionally efficient in the storage of energy (Mathews and Van Holde, 1996) and are also responsible for the structure of cell membranes (White et al., 1996). Apart from mentioned functions, lipids also play important roles in photosynthetic processes in plants and microorganisms, and the association of certain lipids provides red and yellow pigments of some microorganisms (Pelczar et al., 1993). Lipids partake in the organisation of bacterial cell envelopes as components of both the inner membrane of Gram-positive bacteria and the outer membrane of Gram-negative bacteria (Ratledge and Wilkinson, 1988a). Some bacterial extracellular lipids act as surfactants and emulsifiers which might be accompanied by extracellular enzymes (lipases, esterases and phospholipases) which assists the growth of microorganisms on fats or even hydrocarbons which might serve as external supplies (Ratledge and Wilkinson, 1988a).

5.2.2 *Lipid extraction and fractionation*

Lipids can be divided into two main fractions: Neutral lipid and polar lipid fractions. Neutral lipids do not contain any charged atoms, whereas polar lipids consist of a polar head group. The polar lipid fraction can be further divided into two fractions: glycolipid and phospholipid fractions. Fractionation into neutral lipid, glycolipid and phospholipid fractions can be accomplished with the use of silicic acid column chromatography and several solvents: chloroform, acetone and methanol, respectively (Findlay and White, 1987). Neutral lipids are typically regarded as the waxes and acylglycerols (Brennan, 1988). Acylglycerols are esters of alcohol, glycerol and fatty acids, while waxes are esters with constituent alcohol and fatty acid components, giving waxes with long hydrocarbon chains from C_{20} to C_{40} (Ratledge and Wilkinson, 1988b). In contrast, phospholipid fatty acids are lipid compounds that are esters of phosphoric acid. A phospholipid also contains one or two molecules of fatty acid, an alcohol, and sometimes a nitrogenous base (Atlas, 1997). These lipids are made up of glycerol and two fatty acids, and a phosphate functional group (Atlas, 1997). A saturated fatty acid on the first carbon, and an unsaturated fatty acid on the second carbon of the glycerol

backbone is usually characteristic of phospholipid fatty acids (Ratledge and Wilkinson, 1988b). Phospholipid fatty acids can be divided into different classes – each class having its own unique characteristics (Mathews and Van Holde, 1995).

The phospholipid contents of microorganisms vary across taxa and lipid and fatty acid profiles of individual taxa are also known to vary quantitatively and qualitatively, to a lesser extent – with changes in growth temperature or growth media (Haack et al., 1994). This has resulted in scepticism concerning the reproducibility and interpretation of fatty acid profiles from microbial communities. The application of multivariate statistical methods, however, strengthens the ability to discriminate between community level profiles (Haack et al., 1994). Palojärvi et al. (1997) used two multivariate methods to statistically analyse PLFA profiles: principal component analysis (PCA) and discriminant analysis (DA); more clear groupings were achieved with DA, in comparison to PCA.

There are a number of cautions and limitations that should be kept in mind when using fatty acid analysis in soil (Pankhurst et al., 1996). Haack et al. (1994) found bacteria and fungi to produce different amounts of PLFA at large, and the types of fatty acids to vary with growth conditions and environmental stresses. Lipid analysis was found not to provide taxonomic information, since widely different taxa had similar fatty acid profiles; taxonomic variation has been found in fatty acid yields. In general, the method cannot be used to characterise microorganisms to the species level. Any variation in signature fatty acids would give rise to false community estimates created by artefacts in the method, since the method relies heavily on these signatures to determine gross community structure. Signature PLFAs could be correlated with the presence of some groups of organisms, but they might not necessarily be unique to only those groups under all conditions, giving rise to false community signatures (Haack et al., 1994). The technique might be serviceable to look at shifts in community structure in soil (Frostegård et al., 1993a), but qualitative and quantitative information about species present might not be provided, and neither information on the functioning of the community nor genetic divergence between organisms (Pankhurst et al., 1996). Another limitation that should be considered, is the choice of buffer used in the extraction mixture, since different buffers give different amounts of lipid phosphate during lipid extraction in different soils. Frostegård et al. (1991) found the choice of buffer being less critical for sandy than for soils of a higher organic matter content.

5.2.3 *Lipid nomenclature*

Fatty acids are designated in terms of A:BωC, where A refers to the total number of carbon atoms, B refers to the number of double bonds, and C refers to the position of the double bonds from the methyl end (aliphatic ('ω') end) of the chain. *Cis* and *trans* configurations are indicated by 'c' and 't', respectively. The prefixes 'i', 'a', and '10Me' refer to methyl branching at the iso and anteiso positions and at the 10^{th} carbon from the carboxyl end of the molecule, respectively. Cyclopropane fatty acids have the prefix 'cy'. (Sundh et al., 1997; Bossio et al., 1998.)

5.2.4 *Phospholipid fatty acids in microbial ecology*

Microorganisms usually live in communities with other microbes, where they play a primary role in the recycling of biologically important elements (Vestal and White, 1989). For better understanding concerning the actions of microbes in natural environments, microbial ecologists have developed numerous techniques to measure and characterise microbial community biomass, structure, metabolic status, and activity under in situ conditions, thus attempting to reveal more closely the functional role the microbial community plays in nature (Vestal and White, 1989).

The physiological status and community structure can be inferred from lipid profiles (Steenwerth et al., 2003) since certain fatty acids are unique to specific groups of organisms (Table 2). Phospholipid fatty acid profiles can therefore signify changes in the microbial composition of a soil (Hill et al., 2000).

The composition of PLFAs in microorganisms is affected by the metabolic state of the organism, environmental factors, and exposure to toxic substances (Frostegård et al., 1997).

Table 2. Phospholipid fatty acids (PLFAs) and community structure groups associated with the membranes of various microorganisms (Olsson, 1999; Ponder and Tadros, 2002; Rütters et al., 2002; Steger et al., 2003).

PLFA structure group	General classification
Normal saturated	A general microbial biomarker found in both the prokaryotic and eukaryotic (polyenoic fatty acids) kingdoms; a relative increase has been shown to correlate with decreased diversity.
Terminally-branched saturated	Representative of Gram-positive bacteria, including Arthrobacter and Bacillus spp. Many of these types of bacteria can be spore formers and can exist in environments that are lower in overall organic carbon content.
Mid-chain branched saturated	Primarily indicative of Actinomycete type bacteria in surface soils. It has been hypothesised that since these bacteria grow hyphae, they are able to better survive in harsh environments due to their stability to span interstitial spaces to collect water and nutrient sources.
Monounsaturated	Indicative of predominantly Gram-negative bacteria, which are fast-growing, utilise many carbon sources, and adapt quickly to a variety of environments; may also be found in the cell membranes of obligate anaerobes such as sulphate or iron-reducing bacteria; an increase in the amount and type of carbon sources has been shown to increase this marker.
Polyunsaturated	Representative of fungi and other microeukaryotic organisms; this marker too shows significant differences due to land-use

5.2.4.1 *Microbial Biomass*

Although several methods have been used to estimate the amount of microbial biomass in soil, only few methods allow differentiation between different groups of microorganisms (Frostegård and Bååth, 1996). The determination of the total PLFAs provides a quantitative measure of the viable biomass of a microbial community, therefore providing an estimate of the amount of active microorganisms in a particular environment and, thus, the capability of the microbial community for metabolic transformations in that environment (White et al., 1979). The viable biomass of a

microbial community can be determined by measurement of phospholipid fatty acids. Upon cell death, the phosphate group is cleaved from the phospholipid, leaving the diglyceride with intact fatty acids (Vestal and White, 1989). The remaining diglyceride fatty acids (DGFA) thus contain the same signature fatty acids as the original phospholipid fatty acids. A comparison of the PLFA:DGFA ratio can thus be successfully used to determine or obtain an indication of the viable to non-viable microbes. Frostegård and Bååth (1996) used PLFA and ergosterol analysis to differentiate between bacterial and fungal biomass in soil, respectively. This enabled biomass estimation of bacteria and fungi with a single technique in the same soil sample. The quantity of 18:2ω6 was used as an indicator of fungal biomass, while the use of the sum of PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy 17:0, 18:1ω7 and cy 19:0) as an index of the bacterial biomass in soil.

5.2.4.2 *Metabolic Status*

The formation of poly-β-hydroxyalkanoic acids (PHA) relative to the PLFA provides a measure of the metabolic status of bacteria (White et al., 1996). When essential components become available, these bacteria catabolise PHA and form PLFA as they grow and divide. Specific patterns of PLFA can indicate physiological stress in certain bacterial species (Vestal and White, 1989). Exposure to toxic environments can lead to minicell formation and a relative increase in specific PLFAs. Increasing conversion of cis to trans PLFA has been reported in *Vibrio cholerae* and other microorganisms during microbial nutrient starvation, indicating that the shift in the fatty acid *trans:cis* ratio's was associated with starved or stressed bacteria in natural environments (Guckert et al., 1986). Knowing the specific metabolic processes available can be of great importance in bioprocess control and environmental practices.

Due to problems associated with the application of conventional microbiological methods for the enumeration and quantification of microorganisms in industrial and environmental samples, as well as with the analysis of both functional (Buyer and Drinkwater, 1997) and structural diversity (White et al., 1996) of microbial communities, molecular approaches have also been developed as a more effective method for studying the diversity, distribution, and behaviour of microorganisms in soil habitats to assist in the broader understanding of soil health (Hill et al., 2000). Methodological difficulties regarding sampling, identification, and available methods for environmental

monitoring have been hampering studies of the diversity of soil microbial communities as well as rhizobia (Coutinho et al., 1999). Øvreås and Torsvik (1998) estimated the genetic diversity of soil to be 200 times greater than the diversity among bacteria cultured from the same soil, indicating that soil microbial communities are much more complex than currently recognised. The analysis of DNA sequences might thus provide greater understanding of microbial diversities that exist in soil than could be gained from culture-dependent methods. Although no molecular methods were used during this study, the techniques will be discussed since they have been successfully applied in numerous studies to characterise microbial communities.

5.3 Molecular Techniques

Some of the most useful molecular techniques used to provide a new understanding of the phylogenetic diversity of microbial communities in soil, are those in which small subunit rRNA genes are amplified from soil-extracted nucleic acids (Hill et al., 2000). Microbial rRNA genes can be detected directly from soil samples and sequenced. The acquired sequences are then compared with sequences from other known microorganisms. Direct visualisation of microorganisms in soil habitats is made possible by the development of group- and taxon-specific oligonucleotide probes from the microbial rRNA sequenced from soil samples. New methods of assessing soil microbial diversity, allowing a more complete understanding of the potential impacts of environmental processes and human activities on response of soil microorganisms, are made possible with the use of these techniques. (Hill et al., 2000.)

The most useful of the various nucleic acid techniques, is the determination of the sequences of 16S ribosomal RNA (rRNA) genes in prokaryotes and 5S or 18S rRNA in eukaryotes (Rondon et al., 1999). The small subunits rDNA, which encodes rRNA, are especially suited for these studies since they are found universally in bacteria, archaea, and eukaryotes. It is also composed of highly conserved regions as well as regions with considerable sequence variation enabling the measurement of phylogenetic relationships at several hierarchical levels from comparative sequence analysis. The phylogenetic information held in the rDNA molecule is additionally enhanced by its relative large size and the presence of many secondary structural domains. The small subunit (SSU) rDNA can also be easily amplified using polymerase chain reaction (PCR) and rapidly sequenced (Hill et al., 2000). One great advantage of the analysis of

SSU rDNA is the study and characterisation of microorganisms from natural systems without culturing. RDNA from over 90% of the microorganisms observed microscopically *in situ*, can be extracted and analysed (Zhou et al., 1996). Individual amplicons must be separated prior to sequence analysis once the microbial community rDNA is amplified from soil samples using PCR. The most common-used methods for the separation of individual amplicons have been standard cloning procedures using a variety of *Escherichia coli* vectors (Hill et al., 2000).

Recent developments, however, allow the separation of individual amplicons with the use of denaturing gradient and temperature gradient gel electrophoresis (DGGE / TGGE). This technique allows the separation of mixtures of PCR products that are of the same length, but differ in sequence alone. DNA molecules are electrophoresed in an increasing gradient of denaturant or temperature, where it remains double-stranded until it reaches the concentration or temperature where the double-stranded molecule melts. Mobility in the gel is reduced by branching of the DNA as it melts, and since the melting behaviour is mostly dictated by nucleotide sequences, separation will resolve individual bands that correspond to a unique sequence (Heuer et al., 1997). RDNA amplicons cloned or separated by DGGE or TGGE can be sequenced and analysed for similarity to other known sequences. The identity of the microorganism from which the SSU rDNA gene was derived, can thus be determined by estimating phylogenetic relatedness to other sequences in other databases (Hill et al., 2000). The estimation of phylogenetic relatedness to other sequences has made it possible to determine the identity of the microorganism from which the SSU rRNA gene has been derived (Hill et al., 2000).

Recent analyses have been focusing on the characterisation of soil microbial communities based on rRNA as opposed to rRNA genes that have been encoded by rDNA (Felske et al., 1996). This technique offers three main advantages over rDNA techniques. Firstly, ribosomes are the sites of protein synthesis, which enables the direct correlation of cellular ribosome content (rRNA) with metabolic activity and growth rate (Felske et al., 1996). Secondly, rRNA is easier to detect since it is present in cells in higher copy numbers than rDNA sequences, and finally, only rRNA from active cells are detected when ribosomes are extracted directly from soil samples, since free nucleic acids and many dormant microorganisms are excluded (Hill et al., 2000).

The banding pattern obtained when rRNA amplicons are separated on a DGGE or TGGE gel, serves as a fingerprint of the soil microbial community, where the intensity of a given band indicates the abundance of the corresponding rRNA sequence in the soil community. A complicating factor is that rRNA sequences heterogeneity occurs within cells of the same species (Hill et al., 2000).

Although nucleic acid techniques are useful, they still suffer numerous limitations. Where samples have been stored aerobically, or at room temperature prior to measurement of metabolic activity, shifts in active functional groups of prokaryotes have been observed. Comparisons of activity among organisms or soil samples can also be confounded by several factors such as extraction efficiency and sequence amplification and detection (Hill et al., 2000).

Direct identification and quantification of specific and / or general taxonomic groups of microorganisms within their natural microhabitat have been made possible with the use of fluorescent *in situ* hybridisation (FISH). This technique has been used primarily with prokaryotic communities, but it has been able to detect microorganisms of all phylogenetic levels as well, and is more sensitive than immunofluorescence since non-specific binding to soil particles does not occur (Hill et al., 2000). The 16S or 23S rRNA of fixed whole cells is hybridised with fluorescently-labeled taxon-specific oligonucleotide probes. The labelled cells are then viewed by scanning confocal laser microscopy (SCLM). Scanning confocal laser microscopy outdoes epifluorescence microscopy in sensitivity and has the ability to view the distribution of various taxonomic groups simultaneously as a three-dimensional image (Kirchhof et al., 1997). Fluorescing microbes can be differentiated from autofluorescent soil particles and plant debris with the use of distinctive fluorescent dyes and corresponding filter sets (Macnaughton et al., 1996). Detection of metabolically active single cells, with cell walls sufficiently permeable to allow penetration of the probe (which is generated without prior isolation of the microorganism), within complex environments such as rhizosphere and bulk soils has been made possible by greatly improving the sensitivity of FISH (Macnaughton et al., 1996). Fluorescent *in situ* hybridisation can be used in combination with a few other techniques to gain information concerning the structure and function of microorganisms within complex microbial communities; it has also been used for studying population dynamics, tracking microorganisms released into the environment, epidemiology, and

microbial ecology of economically important plant pathogens in agricultural soils (Kirchhof et al., 1997).

Since only a small percentage of all soil microorganisms can be cultured (White et al., 1996), traditional culture-based assays of microbial populations have always provided valuable, but very limited, information on complex soil communities. It is therefore clear that a lack of effective methods for addressing community-level characteristics have always been a large obstacle to the evaluation and comparison of soil microbial communities (Cavigelli et al., 1995).

Due to the development of microbial communities antagonistic to *Ggt* infection in soils conducive to take-all disease of wheat, it is hypothesised that the microbial community function and structure in soils conducive and suppressive to take-all disease of wheat will differ significantly. Should significant differences be observed between the microbial communities, the application of techniques independent of cultivation could be utilised to assess the effect of management practices applied to suppress take-all disease. The aim of this study was, therefore, to evaluate the applicability of the assessment of polyphasic approach and compare soilborne disease suppressiveness / conduciveness with the analysis of microbial community dynamics. Since no single approach could provide a complete representation of soil microbial characteristics, a more complete presentation of soil microbial characteristics could be achieved with the use of multiple approaches. Insight could be gained into aspects of both functional and structural attributes of soil microbial communities with the use of CLPP and FAME profiling, respectively, thus enabling a more comprehensive assessment of changes in multiple aspects of soil microbial community characteristics.

Specific objectives for this study therefore included: (1) the physico-chemical characterisation of soils conducive-, suppressive-, and neutral with respect to take-all disease of wheat, (2) the characterisation of the soil microbial communities using traditional culture-based microbiological techniques using specific selective media, (3) the characterisation of the functional diversity of soil microbial communities associated with conducive-, suppressive-, and neutral agricultural soils with respect to take-all disease of wheat, based on statistical analysis of community level physiological profiles, (4) the evaluation of the influence of crop rotation (wheat with sunflower / soybeans) on the functional diversity soil microbial communities associated with agricultural soils conducive to take-all disease of wheat using community-level physiological profiles, (5)

the characterisation of the structural diversity of soil microbial communities associated with agricultural soils conducive-, suppressive-, and neutral with respect to take-all disease of wheat, based on the statistical analysis of phospholipid fatty acid analysis.

6. References

Aberra, M.B., Seah, S., Sivasithamparam, K. 1998. Suppression of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) by a sterile red fungus through induced resistance in wheat (*Triticum aestivum*) seedling roots. Soil Biology and Biochemistry 30, 1457-1461.

Andrade, O.A., Mathre, D.E., Sands, D.C. 1994. Natural suppression of take-all disease of wheat in Montana soils. Plant and Soil 164, 9-18.

Atlas, R.M. 1997. Principles of Microbiology. 2nd ed. WCB Publishers, Dubuque, 1298 pp.

Atlas, R.M., Bartha, R. 1993. Microbial Ecology: fundamentals and applications. 3rd ed. Benjamin/Cummings Publishing Company, Inc., Redwood City, 563 pp.

Atlas, R.M., Bartha, R. 1998. Microbial ecology. 4th Ed. Benjamin/Cummings Science Publishing, Menlo Park, California, 694pp.

Bååth, E., Frostegård, Å., Pennanen,T., Fritze, H. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry 27, 229-240.

Barnett, S.J., Singleton, I., Ryder, M. 1999. Spatial variation in populations of *Pseudomonas corrugata* 2140 and pseudomonads on take-all diseased and healthy root systems of wheat. Soil Biology and Biochemistry 31, 633-636.

Bockus, W.W., Tisserat, N.A. 2000. Take-all root rot. The plant health instructor. [Web:] http://www.apsnet.org/education/LessonsPlantPath/TakeAll/top.htm [Date of access: November 2002].

Bossio, D.A., Scow, K.M. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied and Environmental Microbiology 61, 4043-4050.

Bossio, D.A., Scow, K.M. 1998. Impacts of carbon flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265-278.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid analysis. Microbial Ecology 36, 1-12.

Brennan, P.J. 1988. *Mycobacterium* and other actinomycetes. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Volume 1. Academic Press, London, pp. 3-22.

Brock, T.D., Madigan, M.T., Martinko, J.M., Parker, J. 1994. Biology of Microorganisms. 7th ed. Prentice Hall, Inc., New Jersey, 909 pp.

Buyer, J.S., Drinkwater, L.E. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3-11.

CAB International. 2000. Crop Protection Compendium. CABI Publishing, Wallingford, Oxon, UK. [CD-ROM.]

Campbell, C.D., Grayston, S.J., Hirst, D.J. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods 30, 33-41.

Cavigelli, M.A., Robertson, G.P., Klug, M.J. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99-113.

Chakraborty, S., Warcup, J.H. 1983. Soil amoebae and saprophytic survival of *Gaeumannomyces graminis tritici* in a suppressive pasture soil. Soil Biology and Biochemistry 15, 181-185.

Christensen, N.W., Powelson, R.L., Brett, M. 1987. Epidemiology of wheat take-all as influenced by soil pH and temporal changes in inorganic soil N. Plant and Soil 98, 221-230.

Clays-Josserand, A., Ghiglione, J.F., Philippot, L., Lemanceau, P., Lensi, R. 1999. Effect of soil type and plant species on the fluorescent pseudomonads nitrate dissimilating community. Plant and Soil 209, 275-282.

Cloete, T.E. 1999. Microbial Ecology. In: Principles in Microbiology '99. [CD-ROM.]

Colbach, N., Lucas, P., Meynard, J.-M. 1997. Influence of crop management on take-all development and disease cycles on winter wheat. Phytopathology 87, 26-32.

Collins, D.J. 1995. Diseases of barley, rye, and triticale in Alabama. [Web:] http://www.aces.edu/department/extcomm/publications/anr/anr-903/anr-903.html [Date of access: May 2001].

Cook, R.J. 1994. Problems and progress in the biological control of wheat take-all. Plant Pathology 43, 429-437.

Cook, R.J. 2003. Review: Take-all of wheat. Physiological and Molecular Plant Pathology 62, 73-86.

Cook, R.J., Rovira, A.D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biology and Biochemistry 8, 269-273.

Cook, R.J., Wilkinson, H.T., Alldredge, J.R. 1986. Evidence that microorganisms in suppressive soil associated with wheat take-all decline do not limit the number of lesions produced by *Gaeumannomyces graminis* var. *tritici*. Phytopathology 76, 342-345.

Cotterill, P.J., Sivasithamparam, K. 1987. Intermittent wetting of soils at high temperature reduces survival of the take-all fungus. Plant and Soil 103, 289-291.

Coutinho, H.L.C., Oliveira, V.M., Lovato, A., Maia, A.H.N., Manfio, G.P. 1999. Evaluation of the diversity of rhizobia in Brazilian agricultural soils cultivated with soybeans. Applied Soil Ecology 13, 159-167.

Davies, K.G., Whitbread, R. 1989a. A comparison of methods for measuring the colonisation of a root system by fluorescent pseudomonads. Plant and Soil 116, 239-246.

Davies, K.G., Whitbread, R. 1989b. Factors affecting the colonisation of a root system by fluorescent pseudomonads: the effects of water, temperature and soil microflora. Plant and Soil 116, 247-256.

Deacon, J. The Microbial World: Biology and control of take-all disease. [Web:] http://helios.bto.ed.ac.uk/bta/microbes/takeall.htm [Date of access: July 2001].

Delisle, G.J., Tomalty, L.L., Jarrell, K.F. 1999. Microbial Ecology. In: Microbes in Motion II. WBC/McGraw-Hill. [CD-ROM.]

Dobranic, J.K., Zak, J.C. 1999. A microtiter plate procedure for evaluating fungal functional diversity. Mycologia 91, 756-765.

Doran, J.W., Sarrantonio, M., Liebig, M.A. 1996. Soil health and sustainability. Advances in Agronomy 56, 2-54.

Duffy, B.K., Weller, D.M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Disease 79, 907-911.

Duffy, B.K., Ownley, B.H., Weller, D.M. 1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopathology 87, 1118-1124.

Felske, A., Engelen, B., Nubel, U., Backhaus, H. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Applied and Environmental Microbiology 62, 4162-4167.

Findlay, R.H., White, D.C. 1987. A simplified method for bacterial nutritional status based on the simultaneous determination of phospholipid and endogenous storage lipid poly-β-hydroxyalkanoate. Journal of Microbiological Methods 6, 113-120.

Frey, S.D., Elliott, E.T., Paustian, K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climate gradients. Soil Biology and Biochemistry 31, 573-585.

Frostegård, Å., Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Frostegård, Å., Tunlid, A., Bååth, E. 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151-163.

Frostegård, Å., Bååth, E., Tunlid, A. 1993a. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723-730.

Frostegård, Å., Tunlid, A., Bååth, E. 1993b. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605-3617.

Fuhrmann, J., Wollum II, A.G. 1989. *In vitro* growth responses of *Bradyrhizobium japonicum* to soybean rhizosphere bacteria. Soil Biology and Biochemistry 21, 131-135.

Gamliel, A., Katan, J. 1991. Involvement of fluorescent pseudomonads and other microorganisms in increased growth response of plants in solarized soils. Phytopathology 81, 494-502.

Garland, J.L. 1996a. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology and Biochemistry 28, 213-221.

Garland, J.L. 1996b. Patterns of potential C source utilization by rhizosphere communities. Soil Biology and Biochemistry 28, 223-230.

Garland, J.L. 1997. MiniReview: Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiology Ecology 24, 289-300.

Garland, J.L., Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351-2359.

Giller, K.E., Beare, M.H., Lavelle, P., Izac A.-M.N., Swift, M.J. 1997. Agricultural intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology 6, 3-16.

Glandorf, D.C.M., Van Der Sluis, I., Anderson, A.J., Bakker, P.A.H.M., Schippers, B. 1994. Agglutination, adherence, and root colonization by fluorescent pseudomonads. Applied and Environmental Microbiology 60, 1726-1733.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Guckert, J.B., Hood, M.A., White, D.C. 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 794-801.

Haack, S.K., Garchow, H., Klug, M.J., Forney, L.J. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Applied and Environmental Microbiology 61, 1458-1468.

Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.J. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60, 2483-2493.

Hershman, D., Bachi, P. 1994. Take-all of wheat. [Web:] http://www.ca.uky.edu/agcollege/plantpathology/PPAExten/PPFShtml/ppfagsg1.htm [Date of access: March 2000].

Heuer, H., Krsek, M., Baker, P., Smalla, K., Wellington, E.M.H. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S RNA and gel-electrophoretic separation in denaturing gradients. Applied and Environmental Microbiology 63(8), 3233-3241.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15, 25-36.

Hitzl, W., Rangger, A., Sharma, S., Insam, H. 1997. Separation power of the 95 substrates of the BIOLOG® system determined in various soils. FEMS Microbiology Ecology 22, 167-174.

Hodge, A., Paterson, E., Grayston, S.J., Campbell, C.D., Ord, B.G., Killham, K. 1998. Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO_2 enrichment. Soil Biology and Biochemistry 30, 1033-1043.

Howie, W.J., Cook, R.J., Weller, D.M. 1987. Effects of soil matric potential and cell motility on wheat root colonisation by fluorescent pseudomonads suppressive to take-all. Phytopathology 77, 286-292.

Huber, D.M., McCay-Buis, T.S. 1993. A multiple component analysis of the take-all disease of cereals. Plant Disease 77, 437-447.

Ingraham, J.L., Schaechter, M., Neidhardt, F.C. 1997. Microbial Ecology. In: An Electronic Companion to Beginning Microbiology '97. [CD-ROM.]

Kelly, J.J., Tate III, R.L. 1998. Use of BIOLOG® for the analysis of microbial communities from zinc-contaminated soils. Journal of Environmental Quality 27, 600-608.

Kirchhof, G., Schloter, M., Abmus, B., Hartmann, A. 1997. Molecular microbial ecology approaches applied to diazotrophs associated with non-legumes. Soil Biology and Biochemistry 29, 853-862.

Kollmorgen, J.F., Griffiths, J.B., Walsgott, D.N. 1983. The effects of various crops on the survival and carry-over of the wheat take-all fungus *Gaeumannomyces graminis* var. *tritici*. Plant Pathology 32, 73-77.

Laczko, E., Rudaz, A., Aragno, M. 1997. Diversity of anthropogenically influenced or disturbed soil microbial communities. In: Insam, I., Rangger, A. (Eds.), Microbial Communities: Functional versus Structural Approaches. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 57-65.

Lahav, I., Steinberger, Y. 2001. Soil bacterial functional diversity in a potato field. European Journal of Soil Biology 37(1), 59-67.

Larkin, R.P. 2003. Characterisation of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilisation, and fatty acid profiles. Soil Biology and Biochemistry 35, 1451-1466.

Larkin, R.P., Hopkins, D.L., Martin, F.N. 1993. Effects of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83, 1097-1105.

Lawley, T., Bell, C. 1998. Kinetic analysis of BIOLOG® community profiles to detect changes in inoculum density and species diversity of river bacterial communities. Canadian Journal of Microbiology 44, 588-597.

Lupwayi, N.Z., Rice, W.A., Clayton, G.W. 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biology and Biochemistry 30, 1733-1741.

Lupwayi, N.Z., Arshad, M.A., Rice, W.A., Clayton, G.W. 2001. Bacterial diversity in water-stable aggregates of soils under conventional and zero tillage management. Applied Soil Ecology 16, 251-261.

Maas, E.M.C., Kotzé, J.M. 1990. Crop rotation and take-all of wheat in South Africa. Soil Biology and Biochemistry 22, 489-494.

Macnaughton, S.J., Booth, T., Embley, T.M., O'Donnell, A.G. 1996. Physical stabilisation and confocal microscopy of bacteria on roots using 16S rRNA targeted, fluorescent-labelled oligonucleotide probes. Journal of Microbiological Methods 26, 279-285.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey, pp. 179.

Mathews, C.K., Van Holde, K.E. 1996. Biochemistry. 2nd ed. The Benjamin/Cummings Publishing Company, Inc., New York, 1159 pp.

Mazzola, M., Cook, R.J. 1991. Effects of fungal root pathogens on the population dynamics of biocontrol strains of fluorescent pseudomonads in the wheat rhizosphere. Applied and Environmental Microbiology 57, 2171-2178.

Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M., Pierson, L.S. 1992. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Applied and Environmental Microbiology 58, 2616-2624.

McMullen, M.P., Lamey, H.A. 1997. Symptoms and controls of crop diseases. [Web:] http://www.ext.nodak.edu/extpubs/plantsci/crops/pp533w.htm [Date of access: September 2002].

McMullen, M.P., Lamey, H.A. 1999. Crop rotations for managing plant disease. [Web:] http://www.ext.nodak.edu/extpubs/plantsci/pests/pp705w.htm [Date of access: September 2002].

Monsanto Company, 1998. A guide to take-all – a disease in cereal grains. [Web:] http://www.takeall.com/english/default.htm [Date of access: July 2002].

Olsson, P. A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbial Ecology 29, 303-310.

Øvreås, L., Torsvik, V. 1998. Microbial diversity and community structure in two different agricultural soil communities. Microbial Ecology 36, 303-315.

Palojärvi, A., Sharma, S., Rangger, A., Von Lutzow, M., Insam, H. 1997. Comparison of BIOLOG® and Phospholipid Fatty acid patterns to detect changes in microbial community. In: Insam, H., Rangger, A. (Eds.), Microbial Communities - Functional versus Structural Approaches. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 37-48.

Pankhurst, C.E., Ophel-Keller, K., Doube, B.M., Gupta, V.V.S.R. 1996. Biodiversity of soil microbial communities in agricultural systems. Biodiversity and Conservation 5, 197-209.

Pelczar, M.J., Chan, E.C.S., Krieg, N.R. 1993. Microbiology: Concepts and Applications. 1st ed. McGraw-Hill, Inc., New York, 896 pp.

Pennanen, T., Frostegård, Å., Fritze, H., Bååth, E. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. Applied and Environmental Microbiology 62, 420-428.

Petersen, S.O., Klug, M.J. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Applied and Environmental Microbiology 60, 2421-2430.

Ponder, Jr. F., Tadros, M. 2002. Phospholipid fatty acids in forest soil four years after organic matter removal and soil compaction. Applied Soil Ecology 19, 173-182.

Rane, K., Ruhl, G., Sellers, P., Scott, D. 1997. Crop diseases in corn, soybean, and wheat. [Web:] http://www.btny.purdue.edu/Extension/Pathology/CropDiseases/Wheat/Wheat1.html [Date of access: February 2001].

Ratledge, C., Wilkinson, S.G. 1988a. An overview of microbial lipids. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Volume 1. Academic Press, London, pp. 3-22.

Ratledge, C., Wilkinson, S.G. 1988b. Fatty acids, related and derived lipids. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Volume 1. Academic Press, London, pp. 23-53.

Rengel, Z., Gutteridge, R., Hirsch, P. 1996. Plant genotype, micronutrient fertilization and take-all infection influence bacterial populations in the rhizosphere of wheat. Plant and Soil 183, 269-277.

Richards, B.N. 1994. The Microbiology of Terrestrial Ecosystems. Longman Scientific & Technical, New York, 399 pp.

Rondon, M.R., Goodman, R.M., Handelsman, J. 1999. The earth's bounty: assessing and accessing soil microbial diversity. Trends in Biotechnology 17, 403-409.

Rondon, C.S. 1987. Take-all of wheat as affected by tillage and wheat-soybean doublecropping. Soil Biology and Biochemistry 19, 307-311.

Rondon, C.S., Cunfer, B.M. 1986. Absence of take-all decline in double-cropped fields. Soil Biology and Biochemistry 18, 113-114.

Rondon, C.S., Cunfer, B.M. 1991. Influence of small grain rotations on take-all in a subsequent wheat crop. Plant Disease 75, 1050-1052.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J. 2002. Phospholipid analysis as a tool to study complex microbial communities in marine sediments. Journal of Microbiological Methods 48, 149-160.

Sarniguet, A., Lucas, P. 1992. Evaluation of populations of fluorescent pseudomonads related to decline of take-all patch on turfgrass. Plant and Soil 145, 11-15.

Sarniguet, A., Lucas, P., Lucas, M. 1992a. Relationships between take-all, soil conduciveness to the disease, populations of fluorescent pseudomonads and nitrogen fertilisers. Plant and Soil 145, 17-27.

Sarniguet, A., Lucas, P., Lucas, M., Samson, R. 1992b. Soil conduciveness to take-all of wheat: Influence of the nitrogen fertilisers on the structure of populations of fluorescent pseudomonads. Plant and Soil 145, 29-36.

Scott, D.B. 1990. Wheat Diseases in South Africa. Department of Agricultural Development, Pretoria, South Africa, 62 pp.

Scott, P.R. 1981. Variation in host susceptibility. In: Asher, M.J.C., Shipton, P.J. (Eds.), Biology and Control of Take-all. Academic Press, London, UK, pp. 219-236.

Sharma, S., Rangger, A., Von Lützow, M., Insam, H. 1998. Functional diversity of soil bacterial communities increases after maize litter amendment. European Journal of Soil Biology 34, 53-60.

Shen, D. 1997. Microbial diversity and application of microbial products for agricultural purposes in China. Agriculture, Ecosystems and Environment 62, 237-245.

Shipton, P.J. 1981. Saprophytic survival between susceptible crops. In: Asher, M.J.C., Shipton, P.J. (Eds.), Biology and Control of Take-all. Academic Press, London, UK, pp. 295-316.

Simon, A., Sivasithamparam, K. 1988a. Microbiological differences between soils suppressive and conducive of the saprophytic growth of *Gaeumannomyces graminis* var. *tritici*. Canadian Journal of Microbiology 34, 860-864.

Simon, A., Sivasithamparam, K. 1988b. The soil environment and the suppression of saprophytic growth of *Gaeumannomyces graminis* var. tritici. Canadian Journal of Microbiology 34, 865-870.

Simon, A., Sivasithamparam, K. 1988c. Interactions among *Gaeumannomyces graminis* var. *tritici*, *Trichoderma koningii*, and soil bacteria. Canadian Journal of Microbiology 34, 871-876.

Simon, A., Sivasithamparam, K. 1989. Pathogen-suppression: A case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. Soil Biology and Biochemistry 21, 331-337.

Smalla, K., Wachtendorf, U., Heuer, H., Liu, W-T., Forney, L. 1998. Analysis of BIOLOG® GN substrate utilization patterns by microbial communities. Applied and Environmental Microbiology 64, 1220-1225.

Staddon, W.J., Duchesne, L.C., Trevors, J.T. 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilisation patterns. Microbial Ecology 34, 125-130.

Steenwerth, K.L., Jackson, L.E., Calderón, F.J., Stromberg, M.R., Scow, K.M. 2003. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology and Biochemistry 35, 489-500.

Steger, K., Jarvis, Å., Smårs, S., Sundh, I. 2003. Comparison of signature lipid methods to determine microbial community structure in compost. Journal of Microbiological Methods 55, 371-382.

Sturz, A.V., Bernier, C.C. 1991. Fungal communities in winter wheat roots following crop rotations suppressive and nonsuppressive to take-all. Canadian Journal of Botany 69, 39-43.

Sundh, I., Nilsson, M., Borga, P. 1997. Variation in microbial community structure in two Boreal peatlands as determined by analysis of phospholipid fatty acid profiles. Applied and Environmental Microbiology 63, 1476-1482.

Taylor, R.G., Jackson, T.L., Powelson, R.L., Christensen, N.W. 1983. Chloride, nitrogen form, lime, and planting date effects on take-all root rot of winter wheat. Plant Disease 67, 1116-1120.

Torsvik, V., Daae, F.L., Sandaa, R-A., Øvreås, L. 1998. Review article: Novel techniques for analysing microbial diversity in natural and perturbed environments. Journal of Biotechnology 64, 53-62.

Tunlid, A., Hoitink, H.A.J., Low, C., White, D.C. 1989. Characterization of bacteria that suppress *Rhizoctonia* damping-off in bark compost media by analysis of fatty acid biomarkers. Applied and Environmental Microbiology 55, 1368-1374.

Van Bruggen, A.H.C., Semenov A.M. 2000. In search of biological indicators of soil health and disease suppression. Applied Soil Ecology 15, 13-24.

Van Heerden, J., Ehlers, M.M., Korf, C., Cloete, T.E. 2000. BIOLOG® for the determination of microbial species diversity and evenness in activated sludge systems. Final report to the Water Research Commission, WRC Report No. 933/1/2000.

Verwoerd, L. 1929. A preliminary checklist of diseases of cultivated plants in the winter rainfall area of the Cape province. Science Bulletin 88, 3-28.

Vestal, J.R., White, D.C. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

Vey, A., Hoagland, R.E., Butt, T.M. 2001. Toxic metabolites of fungal biocontrol agents. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Fungi as biocontrol agents. CAB International, pp. 311-346.

Walters, D.T., Aulakh, M.S., Doran, J.W. 1992. Effects of soil aeration, legume residue, and soil texture on transformations of macro- and micronutrients in soils. Soil Science 153, 100-107.

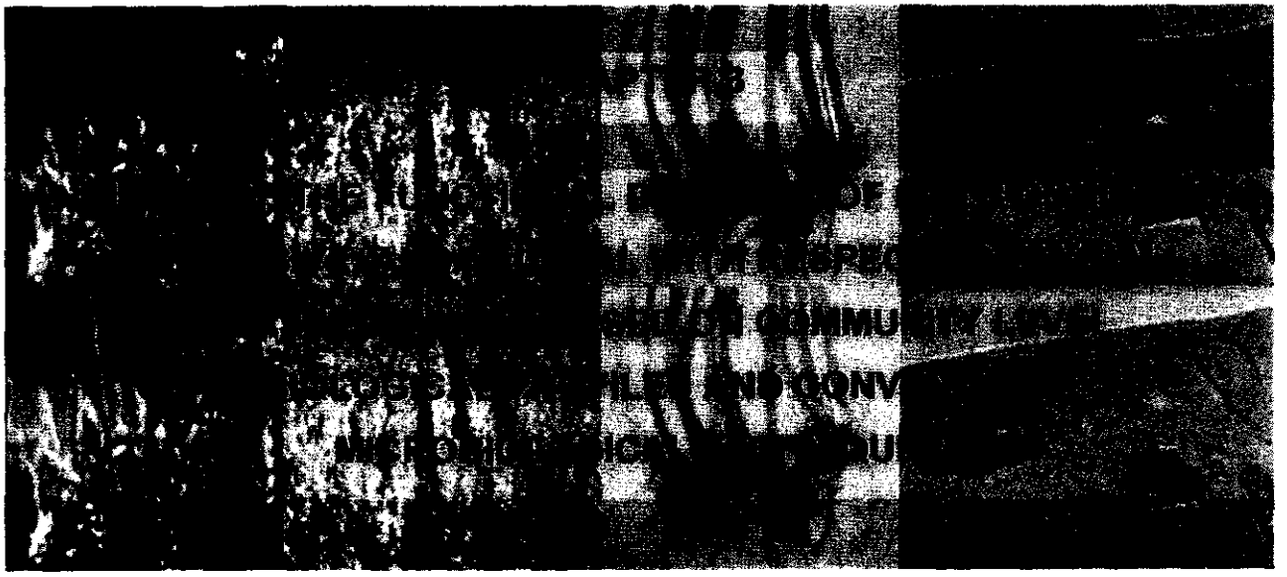
Wardle, D.A., Yeates, G.W., Nicholson, K.S., Bonner, K.I., Watson, R.N. 1999. Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period. Soil Biology and Biochemistry 31, 1707-1720.

Weller, D.M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology 73, 1548-1553.

White, D.C., Ringelberg, D.B., Macnaughton, S.J. 1996. Review of PHA and signature lipid biomarker analysis for quantitative assessment of *in situ* environmental microbial ecology. 1996 International Symposium on Bacterial Polyhydroxyalkanoates 161-170.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51-62.

Wildermuth, G.B. 1982. Soils suppressive to *Gaeumannomyces graminis* var. *tritici*: effects on other fungi. Soil Biology and Biochemistry 14, 561-567.



ABSTRACT

Community level physiological profiles (CLPP) have been reported to be useful in the characterisation of the functional diversity of soil microbial communities. During this study, the relationship between functional diversity of soil microbial communities, and physical and chemical characteristics of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker were evaluated. Profiles of utilisation capacity of carbon substrates were statistically analysed using principal component analysis (PCA), detrended correspondence analysis (DCA) and discriminant analysis (DA). The functional diversity of the soil microbial communities associated with soils suppressive or conducive to take-all disease of wheat differed significantly ($p < 0.05$) from each other, thus implying different substrate utilisation capabilities. No clear distinction could be made between the functional diversity of microorganisms associated with soils suppressive or neutral to take-all disease of wheat. Furthermore, the functional diversity of the soil microbial communities in conducive soils, but which had been subjected to crop rotation with sunflower and soybeans, differed significantly ($p < 0.05$) from each other. This could possibly be ascribed to the differences in the composition of the root exudates by sunflower and soybeans. The Shannon-Weaver substrate diversity (H') indices were overall within the higher diversity range (>3.5), indicative of a high degree of substrate utilisation and thus, functional diversity, within the various soils. Substrate evenness (equitability) (J) indices ranged between 0.826 and 0.899 for the various soils, indicating an even distribution of substrate utilisation. Since the Shannon index emphasises the amount of carbon sources utilised, a clear distinction could be made between the degree of substrate utilisation between soils

conducive to take-all disease of wheat, and soils suppressive and neutral to take-all disease of wheat. This could possibly be ascribed to the presence of antagonistic soil microbial communities towards the take-all fungus. Based on the Sorenson's index, a clear distinction could be made between the degrees of substrate utilisation of the microbial communities and crop rotation.

No significant difference ($p > 0.05$) could be observed between the various soils based on conventional microbial enumeration techniques using various selective media. *Fusarium solani* and *F. oxysporum* were found in approximately the same numbers in soils suppressive to take-all disease of wheat, and in conducive soils where crop rotation was practised. *Gliocladium* sp. was mainly isolated from soils suppressive to take-all disease of wheat. Results obtained, indicate that the microbial community functionality is significantly influenced by the presence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, and that the characterisation of microbial functional diversity by analysis of community level physiological profiles can be used as a suitable assessment criteria for the evaluation and management of agricultural soils conducive, suppressive, and neutral to take-all disease of wheat.

Keywords: Soil microbial communities; Community level physiological profiles (CLPP); *Gaeumannomyces graminis* var. *tritici*; Take-all disease; Crop rotation.

1. INTRODUCTION

Take-all is the name given to the disease caused by a soilborne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*). This fungus is responsible for the cause of crown and root rot in wheat, barley, rye and triticale (Rothrock and Cunfer, 1991). *Gaeumannomyces graminis* var. *tritici* is not a seed-borne fungus (Hershman and Bachi, 1994) and survives in crop stubble (Collins, 1995). The disease occurs in early seeded wheat during the growth season and when plants experience nitrogen stress. The most damage is caused during early infections, when both roots and culms are affected (Collins, 1995). When germinating seedlings from newly planted cereal crops come into contact with the take-all pathogen, colonisation of roots continue to occur as the season progresses. Infections of roots results in losses of crop yield and quality because of a lowering in nutrient uptake (Monsanto Company, 1998). Take-all is most common in regions where wheat is grown without adequate crop rotation (Hershman and Bachi, 1994).

Crop rotation and the use of ammonium nitrogen fertilisers to decrease soil pH, are currently the best applicable practices to control take-all infection (McMullen and Lamey, 1997). Crop rotation allows time between the planting dates of susceptible crops, which causes a decrease in the inoculum potential of soilborne plant pathogens to levels below the economic threshold by resident mycoparasites, competitors, predators, and antibiotic-producing microorganisms (Cook, 1994). Rothrock and Cunfer (1991) reported that oats was the only small grain to be effectively substituted for wheat in cropping systems to manage take-all. However, other studied substitutes include sunflower, soybeans, maize and tobacco.

Soilborne disease suppressiveness is an inherent characteristic of the physical, chemical, and/or biological structure of a particular soil which might be induced by agricultural practices and activities such as planting of crops, or the addition of organisms or nutritional amendments, causing a change in the microfloral environment (Larkin et al., 1993). Suppressive factors can be transferred from soil-to-soil where it could multiply.

Soil is a complex and heterogeneous ecosystem with a character still imposing a momentous challenge. Although the characterisation of the physical and chemical analysis of sand, clay and silt contents, soil pH, water holding capacity, total nitrogen-,

and total carbon contents are commonly applied, none of these above-mentioned techniques enables any detailed conclusions to be made concerning the biological structure and function of soils primarily due to methodological limitations (Sharma et al., 1998). Although conventional microbiological techniques have been used to quantify and identify cultured soil microbial populations, these techniques are restrictive due to the selective nature of the laboratory media and incubation conditions which excludes most other microorganisms, the unculturability of many microorganisms and the difficulty in determining microbial community function and structure *in situ*. (Kersters et al., 1997). Other major problems encountered with the quantification of microbial diversity, are the high numbers of individuals per sample, and the identification of different populations (Sharma et al., 1998). Although quantitative yields and specific information on biodiversity can be obtained by plate count populations, it should be kept in mind that plating studies are incomplete methods for estimating biodiversity (Øvreås and Torsvik, 1998). Recent studies have indicated that less than 1% of all microbes can be cultured on artificial media with viable counts of microorganisms from environmental samples accounting for 0.1 - 10% of the total community (Sharma et al., 1998; Vestal and White, 1989) and consequently thorough analyses of communities at microbial level have not been possible in the past. Primarily due to limitations of conventional microbiological techniques, numerous new assays and procedures to study microbial communities *in situ* have been developed. Most of these recently developed techniques are independent of cell culturability, and circumvent the numerous problems associated with conventional microbiological techniques.

One particular technique includes the characterisation of the functional diversity of microbial communities with the use of community level physiological profiles (CLPP), which can be illustrated by the numbers, types, activities and rates at which a suite of substrates are utilised. The numbers and types of substrates utilised form an integral part of the functional diversity of the population (Zak et al., 1994). Characterisation of community level physiological profiles (CLPP) among microbial communities are determined with the use of commercially available Biolog® microplates (Biolog® Inc., Hayward, USA). Although the Biolog® system was primarily developed for the identification of pure bacterial cultures, it is currently widely applied to the characterisation of soil microbial communities, resulting in a community level physiological profile which can be interpreted in relation to metabolic activities of specific communities in a sample (Palojärvi et al., 1997). The most frequently applied

Biolog® microplate is the Biolog® GN microplate for the identification of Gram-negative bacteria (Garland and Mills, 1991; Buyer and Drinkwater, 1997).

The application of community level physiological profiles with the use of Biolog® microplates has been extensively studied in various environments. This approach has been successfully used to characterise microbial succession after maize litter amendment (Sharma et al., 1998) and microbial communities in potato fields (Lahav, 2001), to classify and compare heterotrophic microbial communities (Garland and Mills, 1991), and for risk and efficacy testing in diverse soils after microbial inoculation of wheat roots (Gagliardi et al., 2001). Widmer et al. (2001) reported Biolog® microplates to yield highly reproducible results for three different soils and allowed the discrimination between soils based on community level physiological profiles.

Since the conservation of the microbial diversity in agricultural soils is essential to preserve the soil-fertility, all the various factors affecting the diversity and the conservation thereof are also an important field of study. With the determination of community level physiological profiles, the capacity to quickly characterise potential pathogens, access suppressive strategies, and evaluate subsurface communities and their recovery during and after perturbation, will only enhance agricultural technologies.

The aim of this study was therefore to characterise the community level physiological profiles of microbial communities present in agricultural soils conducive, suppressive, and neutral to take-all disease of wheat as caused by the soilborne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*).
The objectives of this research were: (1) the physico-chemical characterisation of the various agricultural soils; (2) the characterisation of certain culturable microorganisms using conventional microbiological techniques; (3) the characterisation of the functional diversity of the microbial communities within the various agricultural soils based on the statistical analysis of community level physiological profiles (Biolog®); and (4) the evaluation of the effect of crop rotation on the functional diversity of microbial communities within agricultural soils conducive to take-all disease of wheat.

2. MATERIALS AND METHODS

2.1 Preparation of sampling sites

Take-all disease increases over years, reaching a maximum 3 – 4 years under wheat monoculture. Following continued monoculture, disease incidence decreases to a level where crop yield is equal to that of the first year. In a joint venture with the Agricultural Research Council, Small Grain Institute (Bethlehem, South Africa) to breed for resistance against Gaeumannomyces graminis var tritici, new plots at the Agricultural Research Council, Grain Crops Institute (Potchefstroom, South Africa) were continuously cultivated with wheat to ensure the availability of diseased plots. Plots where no wheat had been planted for at least 30 years prior to sampling, were considered as a natural soil, as well as neutral with respect to take-all disease of wheat. Plots where wheat was grown in monoculture for 4 years, represented conducive soils, while plots under wheat monoculture for 7 years represented suppressive soils (P.S. van Wyk, personal communication).

2.2 Samples

Soil samples were aseptically obtained from adjacent plots of the Agricultural Research Council (Potchefstroom, South Africa) using a randomised plot design. These experimental plots were conducive, suppressive, and neutral with respect to take-all disease of wheat. Soil samples were collected from each experimental plot in the spring (post-harvest of wheat).

In order to evaluate the effect of crop rotation as a management practice to control take-all disease, soil samples were also obtained from an adjacent experimental plot, which was conducive to take-all disease and had been cultivated with sunflower and soybean in the summer prior to the cultivation of wheat. Soil samples for the analyses of the functional diversity of the microbial communities were also collected from each section in the spring (post-harvest of wheat).

Five sample sites were randomly chosen within each of the experimental plots. Soil samples were obtained using a soil auger rinsed with methanol:water (80:20 v/v) between each sampling site to prevent the occurrence of cross contamination. Soil samples consisted of ten sub-samples randomly taken from a 2 m^2 area. Each of the

sub-samples were consolidated and mixed thoroughly in order to create a composite sample, which was considered to be representative of the specific sample site within a plot (n = 5). The soil samples used for the determination of functional diversity were kept cool on ice and transported at 4°C. Analyses were performed in triplicate within 4 hours of collection, since short-term sample storage has been reported to result in less significant changes in soil microbial activity (Petersen and Klug, 1994).

The physico-chemical analyses and characterisation of the various soil samples were undertaken by the Agricultural Research Council, Grain Crops Institute (Potchefstroom, South Africa).

2.3 Determination of the Functional Diversity

The functional diversity of the microbial communities within each of the consolidated soil samples was determined according to the procedure as described by Buyer and Drinkwater (1997) using commercially available Biolog® Gram-negative (GN2) microtiter plates (Biolog® Inc., Hayward, USA).

Soil dilutions were prepared by shaking approximately 10 g of soil (wet weight) in 90 ml sterile deionised water for 50 minutes on a rotary shaker at 250 rpm. The suspension was allowed to settle for a period of 2 h to remove larger soil particles (Sharma et al., 1998), where after the supernatant was diluted with sterile deionised water to a final dilution of 1:3000. The soil suspension was poured into a reagent reservoir and the Biolog® microtiter plates were aseptically inoculated with 150 µl aliquots of each sample using a multi-channel pipettor. Community level physiological profiles (CLPP) analyses of each sample were performed in triplicate. After an initial reading (time = 0), the Biolog® microtiter plates were incubated at 25°C. The tetrazolium violet reduction in each well was spectrophotometrically quantified at 590 nm every eight hours for a period of 224 hours using a Powerwave microtiter plate reader (Winding and Hendriksen, 1997).

2.4 Statistical Analyses

All analyses were conducted in triplicate in order to determine the standard deviation and reproducibility. The optical density (OD) values obtained from each BIOLOG® microtiter plate were analysed using the average well colour development (AWCD)

technique as described by Garland (1996a). Standardised patterns were obtained by blanking the absorbance values for the wells with carbon sources against the absorbance value of the control well (A01) without carbon source. Any negative values were converted to zero, and any variance in the inoculum density was accounted for by dividing the absorbance of each well by the average absorbance for the whole microtiter plate, giving the standardised OD. Instead of using the absolute values, standardised patterns were subsequently compared. Garland (1996a) demonstrated a variation of 10-20% in the AWCD in Biolog® microtiter plates inoculated with identical inoculum densities and recommended the comparison of samples of equivalent AWCD in order to eliminate the variation in AWCD.

Substrate utilisation patterns, "metabolic fingerprints", were compared from the intermediate phase of the BIOLOG® incubation and an AWCD value of 0.25 absorbance units was used as the reference point for multivariate statistical analysis of the data. Profiles of carbon substrate utilisation were statistically analysed by three multivariate statistical analyses: principal component analysis (PCA), detrended correspondence analysis (DCA) and discriminant analysis (DA) (Palojärvi et al., 1997; Buyer and Drinkwater, 1997). Staddon et al. (1997) suggested that PCA analysis be routinely performed on AWCD-corrected data. The purpose of the PCA and DCA ordinations was to arrange samples of multidimensional space into a low-dimensional space such that similar samples are close by and dissimilar samples are far apart (Garland, 1996a). Each principal component (PC) extracts a portion of the variance in the original data, with the greatest amount of variance extracted by the first ordination axis. Relationships among samples are readily observed by plotting samples in two dimensions on the basis of their scores for the first two principal components (PCs). Such relationships are difficult to observe when examining the original variables in multidimensional space (95-dimensional space). PCA also calculates the proportion of variance explained by a given PC. By correlating PCs with the original variables, the axes can be "labelled" with a subset of the original variables. This statistical analysis was used by Garland and Mills (1991; Garland, 1996a) to reduce complex multidimensional data into a smaller number of interpretable variables, or principal components, that represent a subset of the original variables. PCA also attempts to maximise the amount of variance in the data set that can be explained by the first few principal components. DCA has been shown to be superior to PCA for analysis of community data sets (Garland, 1996a). DCA is used to efficiently project a multidimensional data set into fewer dimensions or axes. DCA

utilises weights for data points proportional to the total for the sample as opposed to equal weights in the derivation of these axes. Differences between observed samples in ordination space can be directly related to differences in carbon source utilisation by evaluating the correlation between ordination co-ordinate scores and absorbance values for the individual variables (Garland, 1996a). PCA of the Biolog® colour responses allow for comparison of microbial samples on the basis of differences in the pattern of sole-carbon-source utilisation. PCA determines how the samples are different, but does not test among samples for specific differences selected *a priori*. Since the substrate utilisation data was non-parametric, the variable loading scores obtained from the various PCA and DCA analyses of the AWCD (0.25 absorbance units) were analysed using the Mann-Whitney U test, and Box and Whisker plots were obtained. Statistical analyses were undertaken using the Multivariate Statistical Package 3.1 (Kovach Computing Services), NCSS 97 software (Statistical Solutions, Ireland), Statistica 6.0 (Statsoft, Inc.), and Canoco 4.5 (Biometris, Wageningen). The relationship amongst different samples on the basis of either the raw difference data or the transformed data was determined by PCA, using both the multi-variate Statistical Package 3.1 (Kovach Computing Services) software package as well as Canoco 4.5 (Biometris, Wageningen). These techniques project the original data onto new axis (principal components [PCs]) which reflect any intrinsic pattern in the multidimensional data swarm (Garland and Mills, 1991). Hypothesis testing and a more rigorous analysis of catabolic profiles was achieved using discriminant analysis, since discriminant analysis selects those variables that yield the best separation according to the given groups (Buyer and Drinkwater, 1997).

In order to further distinguish between the functional diversity of the various soil samples, the carbon sources as supplied in the Biolog® microtitter plate were divided into major groups – carbohydrates, carboxylic acids, amino acids, esters, alcohols, amines, amides, aromatic chemicals, polymers, and phosphorylated chemicals. Statistical analyses were also carried out on the different major groups to determine the patterns of substrate utilisation as an indication of functional diversity of microorganisms present in the soil samples.

The significance of the discriminant function was tested with Wilks' lambda, while the F value tested the significance of the Wilks' lambda. A Wilks' lambda value near zero is indicative of an accurate model, while a value near one is indicative of a poor model.

The significance level of the F-test is represented by p, where a value less than 0.05 is considered significant (Hintze, 1997).

2.5 Diversity Indices

The Shannon-Weaver substrate diversity index (*H'*) was measured and calculated by using the number of different substrates utilised by the microbial communities (Magurran, 1988; Lupwayi et al., 2001). The raw difference data were incorporated into:

$$H' = -\Sigma [p_i \ln (p_i)]$$

where $p_i = a_i / \sum a$ = the proportional turbidity observed in the *i*th well and a_i = the turbidity of the *i*th well and $\sum a$ = the total turbidity observed in all sample wells (Lahav and Steinberger, 2001).

Bacterial functional diversity of substrate utilisation was also determined by determination of the substrate richness (*S*) and substrate evenness (*E*) indices (Lupwayi et al., 2001). Substrate richness (*S*) is regarded as the amount of different substrates utilised by the bacterial community, i.e. comparable to species richness in the soil, and was calculated by enumeration of all positive optical density readings. Substrate evenness (*E*) is a measure of the equitability of activities across all substrates, i.e. comparable to how equally abundant the species are in the soil and is calculated as (Magurran, 1988; Zak et al., 1994):

$$E = H' / \ln S$$

Where *E* is the Shannon-Weaver index of evenness and *S* is the number of species.

The substrate equitability of the microbial populations in the various soil samples was also determined. The substrate equitability (*J*) is a rescaling of the Shannon-Weaver substrate diversity index (Magurran, 1988; Zak et al., 1994):

$$J = H' / H'_{max}$$

where *H'* represents the Shannon-Weaver substrate diversity index, and H'_{max} being the maximal substrate diversity index for the microtiter plate.

Similarity between samples was also calculated using Sorenson's measure for quantitative data (Magurran, 1988):

$$C_N = 2jN / (aN + bN)$$

where C_N represents the Sorenson's measure of similarity; *aN* is the sum of the turbidities for sample *a*; *bN* is the sum of the turbidities for sample *b*, and *jN* represents the sum of the lower of the two turbidities for each carbon source in the two samples compared. Using the matrix obtained, a dendogram was constructed with Ward's clustering algorithm (NCSS 97, Statistical Solutions, Ireland).

2.6 Determination of soil dry mass

The weight of each soil sample was determined in its field moist state and again after oven drying (105°C overnight). The difference attributed to the loss of water was calculated and related to the soil moist weight. The dry mass was determined as reported by Alef and Nannipieri (1995).

2.7 Conventional enumeration of specific microbial populations

The supernatant of soil suspensions used in the carbon utilisation were serially diluted (10^{-4}-10^{-9} for R2A agar, water yeast agar, and 10% plate count agar; 10^{-1}-10^{-4} for nitrite agar, ammonia agar, nitrogen fixing agar, and Kings B medium). 100 µl suspensions of the respective serial dilutions were spreadplated on to the following selective media: ammonia agar (Atlas and Parks, 1993) for the isolation, cultivation and enrichment of ammonia-oxidising bacteria from soil; Kings B medium (Glandorf et al., 1994) for the nonselective isolation, cultivation and pigment production of *Pseudomonas* species; nitrite agar (Aleem and Alexander, 1958) for the isolation of nitrification bacteria; nitrogen fixing agar (Garvin and Lindemann, 1986) for the isolation of nitrogen-fixing bacteria from soil; 10% plate count agar (Atlas and Parks, 1993) and R2A agar (Atlas and Parks, 1993) to enumerate heterotrophic bacteria; and water yeast agar (Crawford et al., 1993) for the isolation and cultivation of aerobic *Actinomycetes* from soil. *Fusarium* media was prepared for the selective isolation of *Fusarium* from the soil samples according to Van Wyk et al. (1986). For the isolation of *Fusarium*, soil debris was air dried overnight on sterile paper towels and finally dried for 12 hours in a desiccator. Approximately 10 pieces of debris were placed on the *Fusarium* agar plates, and incubated at 25°C for 6-10 days. Colonies which developed on the debris pieces on the *Fusarium* isolation plates were transferred to potato dextrose agar (PDA) containing streptomycin (50 mg l^{-1}), and then onto wateragar directly from the plate. PDA and wateragar plates were incubated at 25°C until plates were covered with growth from

transferred debris. The plates were taken to the Agricultural Research Council, Grain Crops Institute (Potchefstroom, South Africa) for identification. All spreadplates were analysed in duplicate and incubated at 25°C for 2 days (Kings B medium), 5 days (ammonia agar, nitrogen fixing agar, nitrite agar), 8 days (R2A agar, 10% Plate count agar, water yeast agar), and 10 days (*Fusarium* medium) until the development of colony forming units (cfu) could be observed.

characterised by high concentrations of sodium and calcium, and low ammonium-to-nitrate ratios. Concentrations of manganese were lower when compared to soils neutral (N) to take-all disease of wheat, but slightly higher when compared to soils suppressive (S) to take-all disease of wheat. These soils were also characterised by low concentrations of potassium and phosphorous, as well as a lower pH and lower total nitrogen when compared to soils suppressive (S) to take-all disease of wheat. Low

3. RESULTS AND DISCUSSIONS

3.1 Functional diversity of soil microbial communities in various soils

The physico-chemical characteristics of the various soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat are summarised in Table 1.

Table 1. Physico-chemical characteristics of the various consolidated soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat.

Variable	Conducive (C)	Suppressive (S)	Neutral (N)
K (mg kg^{-1})	87.40 ± 3.50	96.00 ± 2.21	97.00 ± 1.52
Ca (mg kg^{-1})	1304.20 ± 5.35	1286.00 ± 3.48	1141.20 ± 4.76
Mg (mg kg^{-1})	485.40 ± 6.68	527.20 ± 5.45	451.20 ± 3.22
Na (mg kg^{-1})	21.40 ± 2.14	9.20 ± 0.86	9.20 ± 0.86
Mn (mg kg^{-1})	7.00 ± 1.05	5.80 ± 0.66	8.20 ± 1.02
P (mg kg^{-1})	22.74 ± 0.48	30.19 ± 0.11	29.05 ± 0.05
pH (Water)	7.50 ± 0.07	7.66 ± 0.08	7.26 ± 0.09
pH (KCl)	6.57 ± 0.11	6.82 ± 0.04	6.38 ± 0.01
Ammonium-N (mg kg^{-1})	2.75 ± 0.02	2.01 ± 0.05	2.55 ± 0.05
Nitrate N (mg kg^{-1})	3.76 ± 0.07	2.23 ± 0.06	2.36 ± 0.05
Total Nitrogen (% N)	0.08 ± 0.00	0.09 ± 0.00	0.07 ± 0.00
Total Carbon (% C)	0.72 ± 0.00	0.81 ± 0.01	0.71 ± 0.00
Organic Carbon Fraction (% C*F)	0.94 ± 0.02	1.06 ± 0.02	0.93 ± 0.02
Electrical Conductivity (EC) (mS m^{-1})	27.42 ± 0.56	30.88 ± 0.12	25.94 ± 0.09
Clay (%)	26.12 ± 0.38	26.58 ± 0.21	25.18 ± 0.09
Silt (%)	15.00 ± 1.14	14.42 ± 0.22	15.22 ± 0.09
Sand (%)	57.56 ± 0.78	58.00 ± 2.30	58.26 ± 0.18

A PCA biplot illustrating the relationship between the physical and chemical characteristics of the various soils is presented in Figure 1.

The various soils were found to group in different quadrants of the ordination axes. Soils where wheat was cultivated in monoculture for four years, representing conducive (C) soils with respect to take-all disease of wheat, grouped together in the top left quadrant of the ordination diagram with respect to the first ordination axis. These soils were characterised by high concentrations of sodium and calcium, and low ammonium-to-nitrate ratios. Concentrations of manganese were lower when compared to soils neutral (N) to take-all disease of wheat, but slightly higher when compared to soils suppressive (S) to take-all disease of wheat. These soils were also characterised by low concentrations of potassium and phosphorous, as well as a lower pH and lower total nitrogen when compared to soils suppressive (S) to take-all disease of wheat. Low

levels of phosphorous have been reported to result in unhealthy plants, unable to withstand an infection by the take-all fungus, *Gaeumannomyces graminis* var. *tritici*

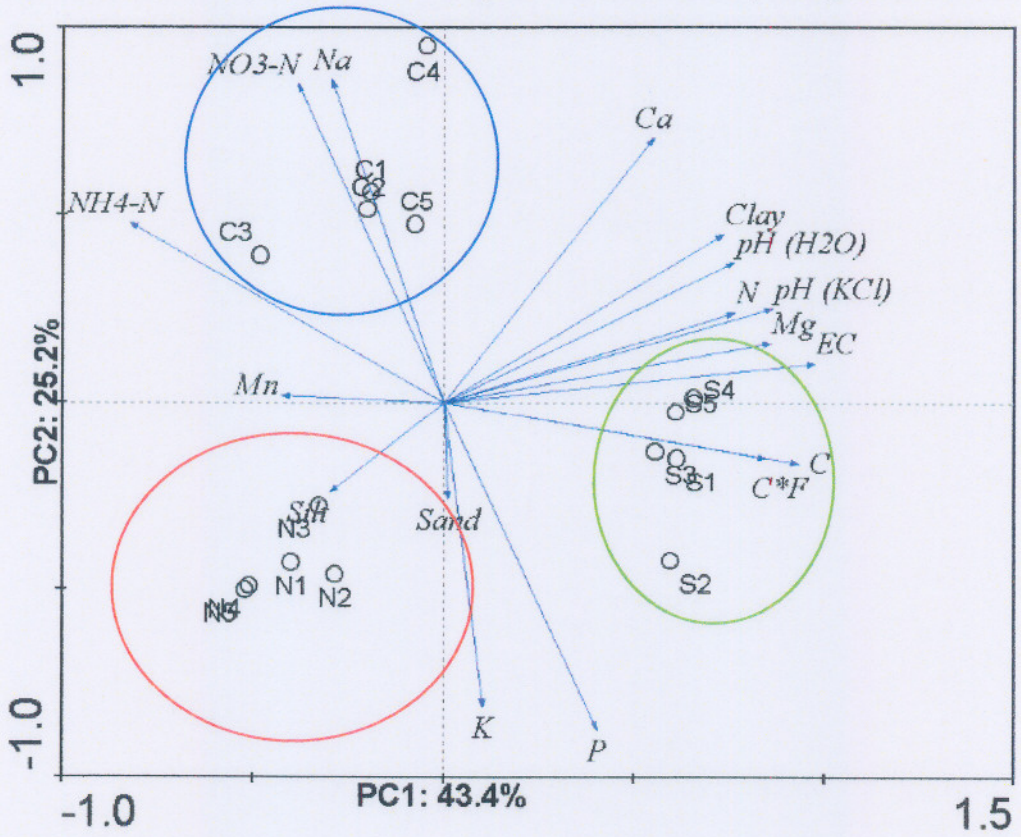


Figure 1. Principal Component Analysis (PCA) biplot of the physico-chemical characteristics of the various soil samples obtained from the experimental plots. The eigenvalues for the first two ordination axes of the PCA were 0.434 and 0.252, respectively. These two axes accounted for 68.5% of the total observed variance. Conducive (C); suppressive (S); neutral (N).

(*Ggt*) (Brennan, 1995). Brennan (1995) reported that phosphorous reduced the levels of take-all severity in soils conducive to take-all disease of wheat and where the levels of take-all severity were >65%. The presence of higher levels of nitrate, lower levels of total nitrogen, and high levels of calcium due to liming, could result in higher pH and increased severity of take-all disease of wheat (Brennan, 1993; Rane et al., 1997). Increased levels of ammonium in soils conducive (C) to take-all disease of wheat when compared to suppressive (S) soils, has been reported to enhance the proportion of rhizosphere pseudomonads, which are naturally antagonistic to *Ggt* (Sarniguet and Lucas, 1992). Adequate supplies of micronutrients such as copper, zinc, iron and manganese, combined with the continued addition of ammonium fertilisers, alleviates take-all due to the increased availability of the micronutrients under acidic conditions (Duffy et al., 1997). Oxidation of manganese to Mn^{4+} by *Ggt* is an important component

of the virulence of take-all disease of wheat (Huber and McCay-Buis, 1993; Rengel, 1997), since oxidation decreases the availability of the micronutrient to wheat, leading to increased take-all. Soil acidification favours rhizosphere bacterial populations with the ability to reduce manganese to Mn^{2+} which is more available to the plant (Duffy et al., 1997).

Soils where wheat was cultivated in monoculture for seven years represented suppressive (S) soils with respect to take-all disease of wheat. These samples all grouped in the bottom right quadrant of the biplot with respect to the first ordination axis. These soils were characterised by slightly higher clay content, high soil pH and electrical conductivity, as well as elevated concentrations of total nitrogen, magnesium, organic carbon and phosphorous. The soil of the experimental plots suppressive with respect to take-all disease of wheat were also characterised by lower concentrations of manganese and ammonium when compared with soils conducive (C) to take-all disease of wheat. Increased levels of phosphorous have been reported to enhance root formation, thus increasing the ability of some roots to "evade" being infected by *Ggt* (Brennan, 1995). Decreased levels of nitrate and ammonium result in a lower ammonium-to-nitrate ratio, and subsequent higher pH, and higher proportions of rhizosphere pseudomonads, which are naturally antagonistic to *Ggt* (Sarniguet and Lucas, 1992). Decreased levels of ammonium, when compared to soils conducive (C) to tale-all disease of wheat, resulted in higher soil pH which might inhibit rhizosphere bacterial populations with the ability to reduce manganese to Mn^{2+} which is more available to the plant (Duffy et al., 1997).

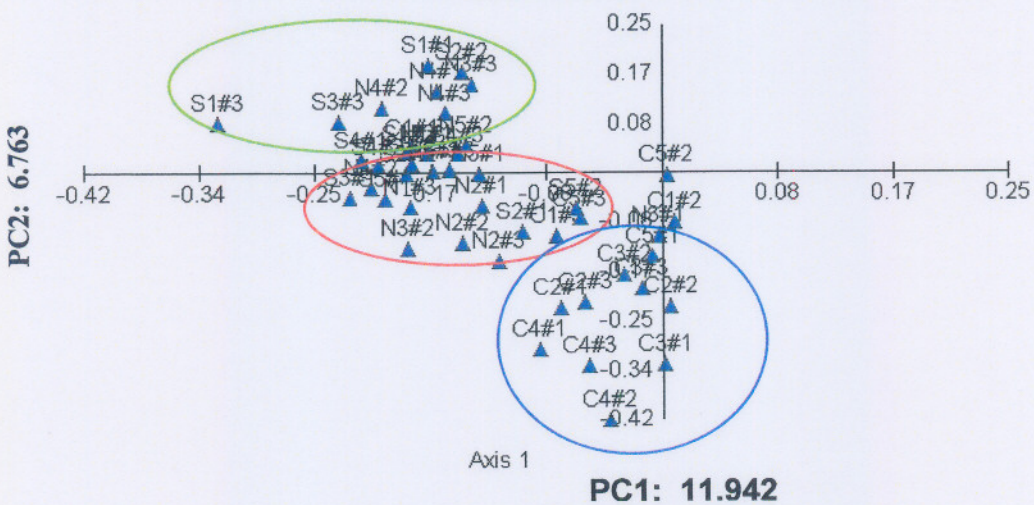
Soils where no wheat was cultivated in monoculture for thirty years represented neutral (N) soils with respect to take-all disease of wheat, and grouped together in the bottom left quadrant of the biplot with respect to the first ordination axis. These soils were characterised by high concentrations of potassium, phosphorous, and slightly higher silt and sand content when compared to soils conducive (C) and suppressive (S) to take-all disease of wheat. These soils were also characterised by elevated levels of manganese when compared to soils conducive (C) and suppressive (S) to take-all disease of wheat. The higher levels of manganese present in the soil of the experimental plots neutral (N) with respect to take-all disease of wheat might have reached toxic levels to the saprophytic survival of *Ggt* (Graham and Rovira, 1984). Since manganese nutrition affects photosynthesis which, in turn, controls the rate of exudation of soluble organic compounds by plant roots. These root exudates directly affect the rhizosphere

microorganisms while indirectly affecting the ectotrophic growth of *Ggt* (Cook and Rovira, 1976).

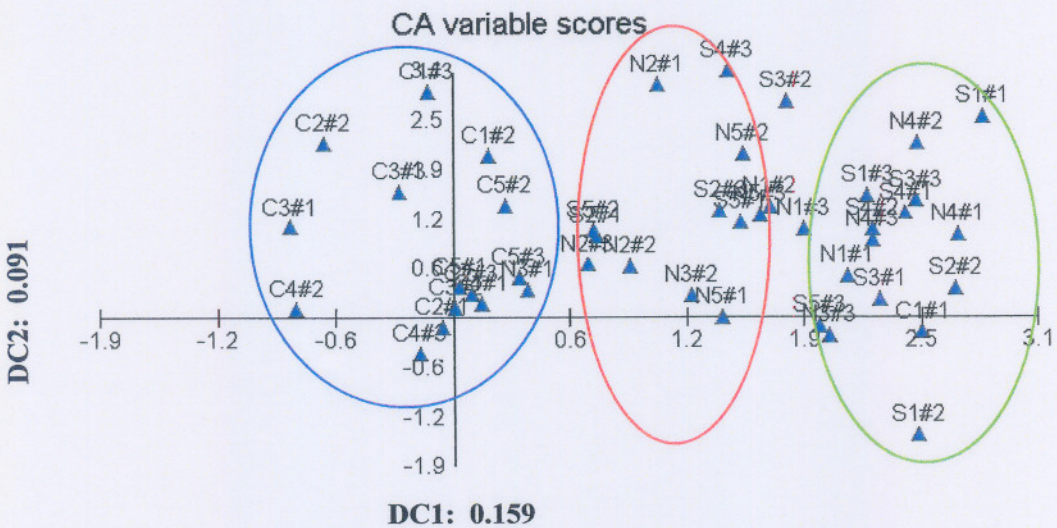
It is evident that the physico-chemical characteristics of the soil samples obtained from experimental plots varied markedly in terms of their chemical characteristics and it is assumed that this could have a significant impact on the functional diversity of the soil microbial communities present within the various soils.

Although PCA has generally been the most commonly applied multivariate analysis for community level physiological profiles (CLPPs), both PCA and DCA were undertaken. PCA and DCA ordination biplots, and box and whisker plots illustrating the relationship between the CLPPs of the various soils are shown in Figure 2a, 2b, and 2c, respectively.

(a)



(b)



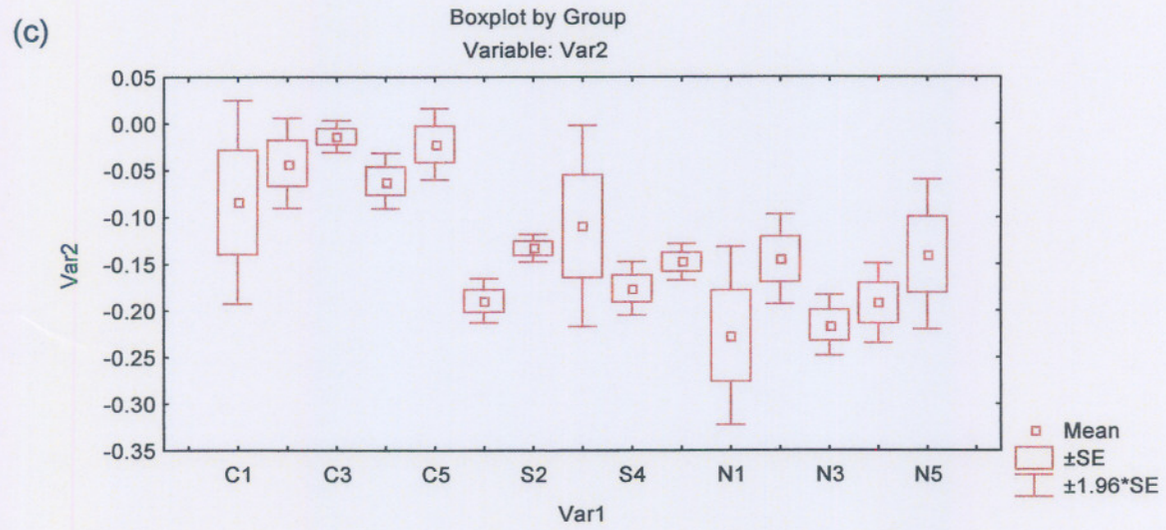


Figure 2. PCA (a) and DCA (b) ordination diagrams and box and whisker plot (c) of the community level physiological profiles obtained using GN2 Biolog® microplates for the microbial communities present in the various soil samlples. The eigenvalues for the first two ordination axes of the PCA (a) were 11.942 and 6.763, respectively. These two axes accounted for 20.8% of the total observed variance. The eigenvalues for the first two ordination axes of the DCA (b) were 0.159 and 0.091, respectively. These two axes accounted for 15.3% of the total observed variance. Conducive (C); suppressive (S); neutral (N).

Although no clear distinction could be made between soils suppressive (S) and neutral (N) to take-all of wheat, it is however, evident that the majority of the soils suppressive (S), and conducive (C) to take-all of wheat differed substantially in their CLPPs (Figures 2a, 2b, and 2c). With two exceptions, the functional diversity of the microbial communities present in the soils conducive (C), and suppressive (S) to take-all disease differed significantly from each other ($p < 0.05$). These results are illustrated in the box and whisker plot (Figure 2c). The exceptions could possibly be ascribed to natural variation in biological systems. The difference in functional diversity between the various samples, but especially the large difference between the soils conducive (C) and neutral (N) / suppressive (S) to take-all disease of wheat, could possibly be ascribed to the development of lesions and subsequent excessive leakage of root exudates due to take-all infection in soils conducive (C) to take-all disease of wheat. Probable increased leakage of root exudates caused by *Ggt* and other secondary soilborne pathogens, might attract increasing populations of antagonistic rhizosphere microorganisms such as fluorescent *Pseudomonas* spp., that are especially well adapted to utilise root

exudates very rapidly (Duffy and Weller, 1995), giving rise to soils suppressive (S) to take-all disease of wheat.

Garland and Mills (1991) related the separation of samples in PC space to differences in carbon source utilisation by examination of the correlation of the original variables to the PCs. Carbon sources which had at least half of their variance explained by PC1 or PC2 (Table 2), were defined as being the most important in differentiating among the communities. The degree to which a PC explains the variance in the colour response of an individual carbon source is related to differences in the response in samples with different co-ordinate values for that PC (Garland and Mills, 1991).

Table 2. Correlation of carbon source variables to PCs for analysis of CLPPs obtained from soils conducive (C), suppressive (S) or neutral (N) to take-all disease of wheat.

PC1		**PC2**	
Carbon source	***r***	**Carbon source**	***r***
Carbohydrates		Carbohydrates	
D-Galactose	-0.626	Adonitol	-0.803
α-D-Glucose	-0.666	D-Mannitol	-0.664
D-Mannitol	-0.678	D-Trehalose	-0.710
D-Mannose	-0.700	Turanose	-0.719
Sucrose	-0.658		
Carboxylic Acids		Carboxylic Acids	
cis-Aconitic acid	-0.859	*cis*-Aconitic acid	0.605
D-Galacturonic acid	-0.524		
D-Saccharic acid	-0.704	Phosphorylated chemicals	
		D,L-α-Glycerol phosphate	-0.680
Amino Acids			
L-Asparagine	-0.692		
L-Aspartic acid	-0.647		
L-Glutamic acid	-0.942		
Amines			
Phenylethylamine	0.54		
Alcohol			
2,3-Butanediol	0.501		

Analysis of PC1 (Figure 2a) indicates that microbial communities in soils suppressive (S) or neutral (N) to take-all disease of wheat utilised an amine (phenylethylamine) and an alcohol (2,3-butanediol) to a relatively lesser degree, and several carbohydrates (D-galactose, α-D-glucose, D-mannitol, D-mannose, and sucrose), carboxylic acids (*cis*-aconitic acid, D-galacturonic acid, and D-saccharic acid), amino acids (L-asparagine, L-aspartic acid, and L-glutamic acid) to a relatively greater degree than microbial communities in soils conducive (C) to take-all disease of wheat. On the basis of PC2

analysis, microbial communities in soils conducive (C) to take-all disease of wheat utilised three carbohydrates (adonitol, D-trehalose, and turanose), and D,L-α-glycerol phosphate to a relatively greater degree, and *cis*-aconitic acid to a relatively lesser degree than microbial communities in soils suppressive (S) or neutral (N) to take-all disease of wheat. Microbial communities in soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat, utilised D-mannitol to the relatively same degree.

Since the group membership during this study was known *a priori*, multivariate analysis using discriminant analysis (DA) was also applicable. Discriminant analysis selects those variables that yield the best separation according to the given groups. Hypothesis testing and a more rigorous analysis of catabolic profiles can be achieved using discriminant analysis (Buyer and Drinkwater, 1997).

In order to place the various community level physiological profiles (CLPPs), and thus the functional diversity, into perspective, an ordination plot of the canonical variate scores 1 and 2 as obtained from discriminant analysis of the CLPPs (functional diversity) of the soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat is shown in Figure 3.

Figure 3 illustrates the shift in the functional diversity of soil microbial communities within the various soils at different stages of take-all disease of wheat. The first canonical function (CF1) obtained from the discriminant analysis accounted for 91.7% of the total variability observed in the data set. This CF was significant in discriminating between the various soils. CF1 and CF2 in total, accounted for 100% of the total variability observed in the data set. It is therefore possible to discriminate between the three stages of take-all disease of wheat, which differed significantly (Eigenvalue = 37.44; Wilks' Lambda = 0.0059; F = 13.8; p = 0.0000) from each other (Figure 3) using discriminant analyis.

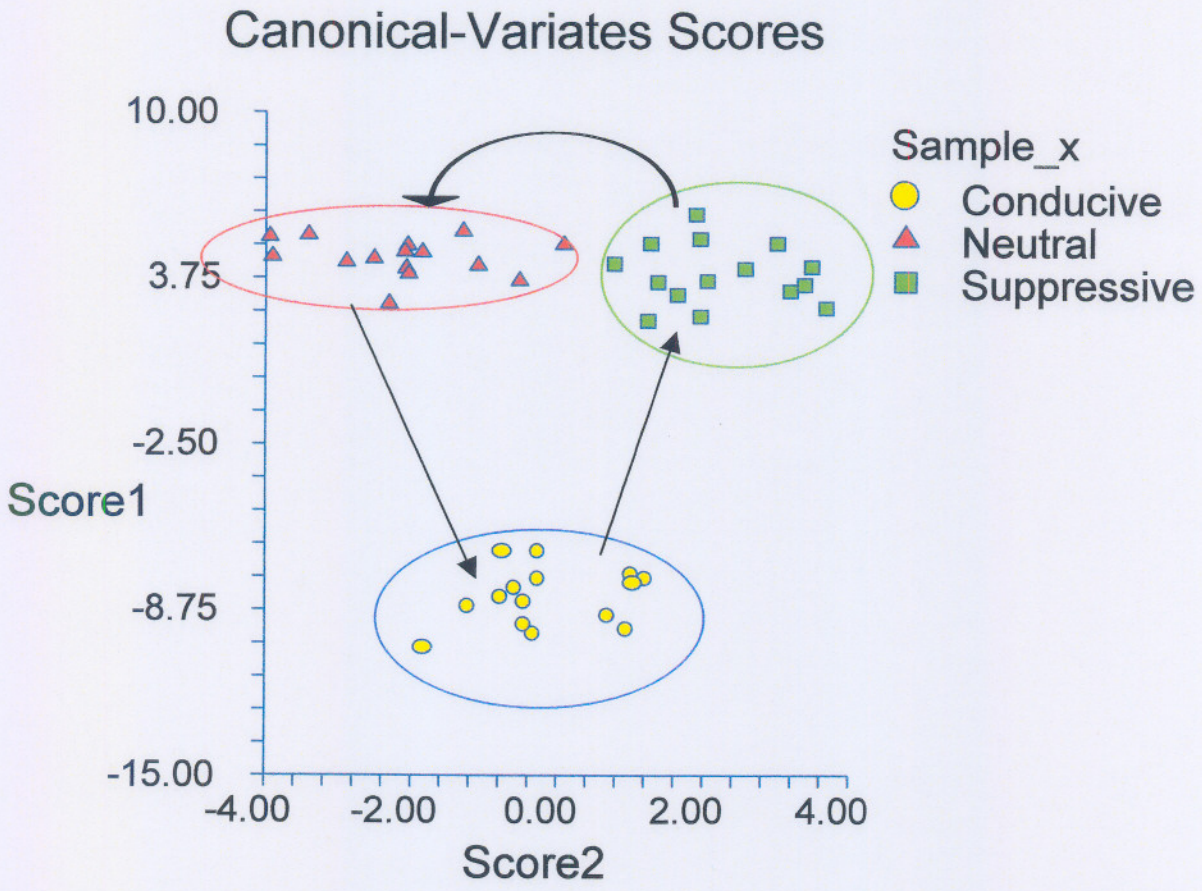


Figure 3. Ordination plot of the canonical variate scores 1 against 2 as generated by discriminant analysis of normalised GN2 community level substrate utilisation data obtained for the functional diversity of the microbial communities present in the various soils.

The statistically significant difference in community-level physiological profiles of soil microbial communities present in the various soils also implied different substrate utilisation capabilities of the microbial communities present in the soils.

The substrate utilisation capabilities (AWCD) of the microbial communities differed significantly ($p < 0.05$) in their ability to utilise 17 of the 95 different carbon sources supplied on the Biolog GN2 microtiter plate during the date of sampling (Table 3).

Some carbon sources may be better for monitoring community changes over time, while others are more suited to examine treatment effects. Staddon et al. (1997) considered carboxylic acids to be an example of the latter.

Table 3. The mean AWCD and standard deviation (STDEV) of the independent variables found to differ significantly between the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat.

		Mean AWCD ± STDEV		
Well Number	**Variable**	**Conducive**	**Neutral**	**Suppressive**
A06	Tween 80	1.27 ± 1.19	1.47 ± 1.38	1.61 ± 1.71
A08	N-Acetyl-D-glucosamine	0.84 ± 0.74	2.44 ± 2.71	1.42 ± 0.91
A12	Cellobiose	1.45 ± 1.12	1.31 ± 2.49	0.78 ± 1.19
B02	D-Fructose	0.79 ± 1.26	1.95 ± 1.61	1.42 ± 1.65
B03	L-Fucose	1.83 ± 1.71	0.66 ± 0.80	0.42 ± 0.40
B09	Lactulose	0.66 ± 0.64	1.44 ± 1.28	0.22 ± 0.22
C02	β-Methyl-D-glucoside	0.31 ± 0.54	0.82 ± 1.11	1.17 ± 1.77
E04	α-Ketoglutaric acid	0.44 ± 0.73	1.72 ± 2.35	1.27 ± 1.42
E06	D,L-Lactic acid	0.32 ± 0.62	0.85 ± 0.96	1.59 ± 1.37
E12	Succinic acid	0.32 ± 0.39	1.19 ± 0.93	1.98 ± 1.53
F02	Succinamic acid	0.71 ± 0.75	1.23 ± 1.69	0.29 ± 0.32
F03	Glucuronamide	0.49 ± 0.55	0.29 ± 0.38	0.41 ± 0.43
F08	L-Asparagine	0.45 ± 0.40	2.58 ± 2.36	1.93 ± 1.30
F10	L-Glutamic acid	1.56 ± 1.53	1.99 ± 1.78	2.89 ± 2.40
G03	L-Leucine	0.97 ± 0.95	0.89 ± 1.09	0.68 ± 1.10
G06	L-Proline	0.57 ± 0.64	1.24 ± 1.48	1.44 ± 1.04
H02	Inosine	0.30 ± 0.47	1.02 ± 1.35	0.77 ± 0.92

Differential utilisation of a polymer (tween 80), carbohydrates (N-acetyl-D-glucosamine, cellobiose, D-fructose, L-fucose, lactulose, β-methyl-D-glucoside), carboxylic acids (α-ketoglutaric acid, D,L-lactic acid, succinic acid), amides (succinamic acid, glucuronamide), amino acids (L-asparagine, L-glutamic acid, L-leucine, L-proline) and an aromatic chemical (inosine) enabled differentiation of the microbial communities present in the various soils (Table 3).

The Shannon-Weaver substrate diversity (*H'*) and equitability (*J*) indices were calculated during this study. On the basis of the substrate diversity values obtained using the Shannon-Weaver substrate diversity (*H'*) index, it was evident that a high substrate diversity existed in all the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat (Table 4). According to Magurran (1988), values of the Shannon index fluctuate between 1,5 and 3,5 and rarely increase above 4,5. Values obtained during this study were overall within the higher diversity range, thus indicating the achievement of very high substrate diversity values. The high substrate diversity values obtained were established by the fact that a substantial percentage of the carbon sources were utilised, contributing to the very high Shannon-Weaver substrate diversity indices.

Obtained substrate evenness (equitability) (*J*) indices ranged between 0.83 and 0.90 for all the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat, and did not show significant differences or trends (Table 5). According to Magurran (1988), substrate evenness (equitability) assumes a value between 0 and 1 with 1 being complete evenness, indicating a high diversity. It could therefore be concluded that a high functional diversity existed in all the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat.

Table 4. Shannon-Weaver substrate diversity index values (*H'*) obtained for all the various soils. Conducive (C); suppressive (S); neutral (N).

Sample	***H' Value***
C1	3.66 ± 0.18
C2	3.72 ± 0.03
C3	3.88 ± 0.08
C4	3.74 ± 0.09
C5	3.93 ± 0.09
S1	3.53 ± 0.14
S2	3.94 ± 0.14
S3	3.84 ± 0.07
S4	3.84 ± 0.06
S5	3.76 ± 0.09
N1	3.75 ± 0.12
N2	3.69 ± 0.13
N3	3.76 ± 0.14
N4	3.83 ± 0.02
N5	3.76 ± 0.04

Table 5. Substrate equitability indices (*J*) obtained for all the various soils. Conducive (C); suppressive (S); neutral (N).

Sample	*J* Value
C1	0.89 ± 0.02
C2	0.83 ± 0.01
C3	0.86 ± 0.02
C4	0.83 ± 0.02
C5	0.90 ± 0.02
S1	0.84 ± 0.02
S2	0.89 ± 0.02
S3	0.88 ± 0.01
S4	0.85 ± 0.01
S5	0.87 ± 0.02
N1	0.85 ± 0.01
N2	0.85 ± 0.02
N3	0.88 ± 0.03
N4	0.85 ± 0.01
N5	0.84 ± 0.01

Whilst the Shannon-Weaver substrate diversity index emphasises the amount of carbon sources utilised, Sorenson's index emphasises the degree of substrate utilisation. A dendogram constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA), illustrates the similarity in carbon source utilisation of the samples from the various soils (Figure 4). The clusters obtained could be distinguished based on their Sorenson's indices. Since the similarity of pairs of sites are studied by taking species abundance into account (Magurran, 1988), a clear distinction could be made between the degree of substrate utilisation between soils conducive (C) to take-all disease of wheat, and soils suppressive (S) or neutral (N) to take-all disease of wheat possibly due to the presence of antagonistic soil microbial communities towards the take-all fungus, *Ggt*.

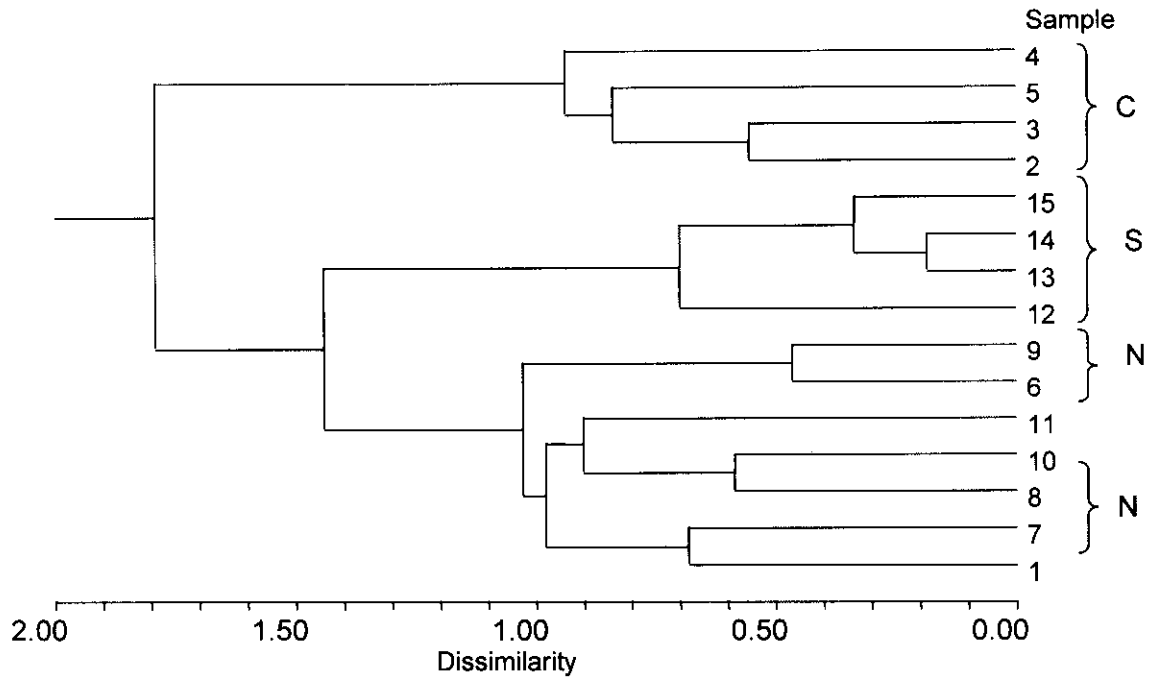


Figure 4. Dendogram constructed from unweighted pair group method with arithmetic mean (UPGMA), illustrating the similarity in carbon source utilisation patterns between the various soils. Conducive (C), suppressive (S), neutral (N).

3.2 Conventional enumeration of specific microbial populations

Microbial diversity (cfu g^{-1}) of the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat, obtained using conventional plate counting techniques on selective microbiological media, is summarised in Table 6. Despite the fact that differences in the microbial populations could be observed between the various soils using the various selective media, most of the differences were not statistically significant ($p > 0.05$).

Ammonia oxidising- and nitrifying bacteria were present in low numbers in soil conducive (C) to take-all disease of wheat due to the high percentage of NH_4^+ present in soils, which might have reduced the pH to control the take-all fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*). Low numbers of *Actinomycetes* were present in soil conducive (C) to take-all disease of wheat. Thus, could possibly be attributed to competition with abundant *Ggt* for nutrients and space. Low numbers of nitrogen-fixing bacteria in soils suppressive (S) to take-all disease of wheat might be attributed to the presence of excess nitrogen in soils due to the application of nitrogen fertilisers to assist in the suppression of *Ggt*. Low numbers of pseudomonads in soils

conducive (C) to take-all disease of wheat, might be due to competition with the abundant presence of *Ggt*, thus making the successful establishment of pseudomonads difficult. The high numbers of pseudomonads in soils suppressive (S) to take-all disease of wheat may be attributed to the selective stimulation of Pseudomonads by infected roots due to excretion of root exudates in cases of take-all disease of wheat caused by *Ggt* and confirms various reports in the literature (Duffy and Weller, 1995; Barnett et al., 1999). The presence of low numbers of heterotrophic bacteria in soils suppressive (S) to take-all disease of wheat might be attributed to the abundant presence of antibiotic-producing pseudomonads responsible for the suppression of take-all disease of wheat, confirming a previous report by Mazzola and Cook (1991). Plots where no wheat had been planted for at least 30 years prior to sampling were considered as a natural soil, as well as neutral with respect to take-all disease of wheat (P.S. van Wyk, personal communication). The highest diversity as obtained using conventional microbial techniques was apparent in soils neutral (N) to take-all disease of wheat.

Despite differences in the functional diversity of soils suppressive / neutral and conducive to take-all disease of wheat as determined by CLPPs, no significant difference ($p > 0.05$) in soil microbial community diversity between the various soils could be observed primarily due to the restriction of currently applied conventional techniques with the use of selective media, thus selection against the non-culturable and growth-dependent fraction of the microbial community.

Table 6. Colony Forming Units (cfu's) obtained from the various soils as determined by conventional plate counting technique using selective microbiological media. All cfu's have been standardised to ($x10^3$) enable comparison. Conducive (C), suppressive (S), neutral (N).

	R2Agar ($x10^3$)	Water Yeast Extract Agar ($x10^3$)	10% Plate Count Agar ($x10^3$)	Ammonia Agar ($x10^3$)	Nitrogen-Fixing Agar ($x10^3$)	Nitrite Agar ($x10^3$)	Kings B medium ($x10^3$)
C1	4650 ± 3930^{a}	610 ± 50^{a}	540 ± 340^{a}	0.23 ± 0.23^{a}	11.60 ± 3.13^{abc}	22.53 ± 2.17^{abc}	123.75 ± 12.25^{ab}
C2	23270 ± 630^{a}	640 ± 160^{a}	1400 ± 720^{ab}	0.08 ± 0.08^{a}	11.37 ± 0.33^{abc}	13.70 ± 0.67^{ab}	187.25 ± 8.25^{abcd}
C3	210 ± 70^{a}	10 ± 10^{a}	3920 ± 780^{abc}	0.27 ± 0.03^{a}	5.35 ± 1.08^{ab}	20.53 ± 1.50^{ab}	121.25 ± 19.75^{a}
C4	320 ± 140^{a}	240 ± 0^{a}	190 ± 130^{a}	0.10 ± 0.10^{a}	10.83 ± 1.60^{abc}	31.87 ± 5.83^{bc}	111.00 ± 4.50^{a}
C5	27590 ± 2090^{a}	200 ± 20^{a}	5850 ± 1490^{bc}	0.05 ± 0.05^{a}	5.75 ± 1.28^{ab}	5.25 ± 0.75^{a}	125.25 ± 13.75^{abc}
S1	2230 ± 290^{a}	160 ± 120^{a}	1420 ± 1060^{ab}	0.38 ± 0.35^{a}	13.00 ± 1.97^{abcd}	24.37 ± 1.33^{abc}	180.50 ± 12.00^{abcd}
S2	13790 ± 12670^{a}	1820 ± 300^{abc}	4080 ± 1340^{abc}	0.10 ± 0^{a}	9.20 ± 3.73^{ab}	17.00 ± 8.03^{ab}	182.25 ± 5.75^{abcd}
S3	990 ± 90^{a}	250 ± 90^{a}	2750 ± 170^{abc}	0.00 ± 0^{a}	3.57 ± 0.60^{a}	16.92 ± 0.88^{ab}	222.00 ± 36.00^{cde}
S4	4290 ± 3010^{a}	2680 ± 180^{bc}	1790 ± 1110^{ab}	0.77 ± 0.40^{a}	5.10 ± 2.60^{ab}	41.87 ± 4.17^{c}	296.25 ± 20.75^{e}
S5	26600 ± 22260^{a}	310 ± 90^{a}	470 ± 190^{a}	0.03 ± 0^{a}	6.25 ± 1.12^{ab}	8.20 ± 0.77^{a}	134.25 ± 29.75^{abc}
N1	3160 ± 2120^{a}	610 ± 270^{a}	3770 ± 1610^{abc}	1.08 ± 0.62^{a}	19.87 ± 3.43^{bcd}	13.87 ± 1.17^{ab}	164.50 ± 9.00^{abcd}
N2	613100 ± 209740^{b}	350 ± 10^{a}	2480 ± 300^{ab}	12.53 ± 0.17^{b}	25.03 ± 2.00^{cd}	21.87 ± 1.17^{ab}	250.50 ± 4.00^{de}
N3	9150 ± 3410^{a}	1440 ± 120^{ab}	3160 ± 100^{abc}	3.90 ± 3.80^{ab}	28.20 ± 4.57^{d}	21.53 ± 2.17^{ab}	179.50 ± 23.00^{abcd}
N4	8780 ± 2160^{a}	3460 ± 1200^{c}	5870 ± 650^{bc}	112.95 ± 5.25^{c}	130.53 ± 5.83^{e}	79.20 ± 6.83^{d}	219.75 ± 12.75^{bcde}
N5	21080 ± 13420^{a}	1330 ± 90^{ab}	7870 ± 1310^{c}	0.200 ± 0.17^{a}	12.73 ± 2.30^{abcd}	14.50 ± 1.00^{ab}	200.00 ± 4.50^{abcde}

‡ Microbiological media with the same combination of superscript alphabetic letters indicate no significant differences among media for each soil.

The relative percentage fraction of *Fusarium solani*, *F. equiseti*, *F. oxysporum*, and *Gliocladium* in soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat, as well as soils conducive to take-all disease of wheat cultivated with sunflower and soybeans during the crop rotation experiment as obtained using conventional techniques, are presented in Figure 5.

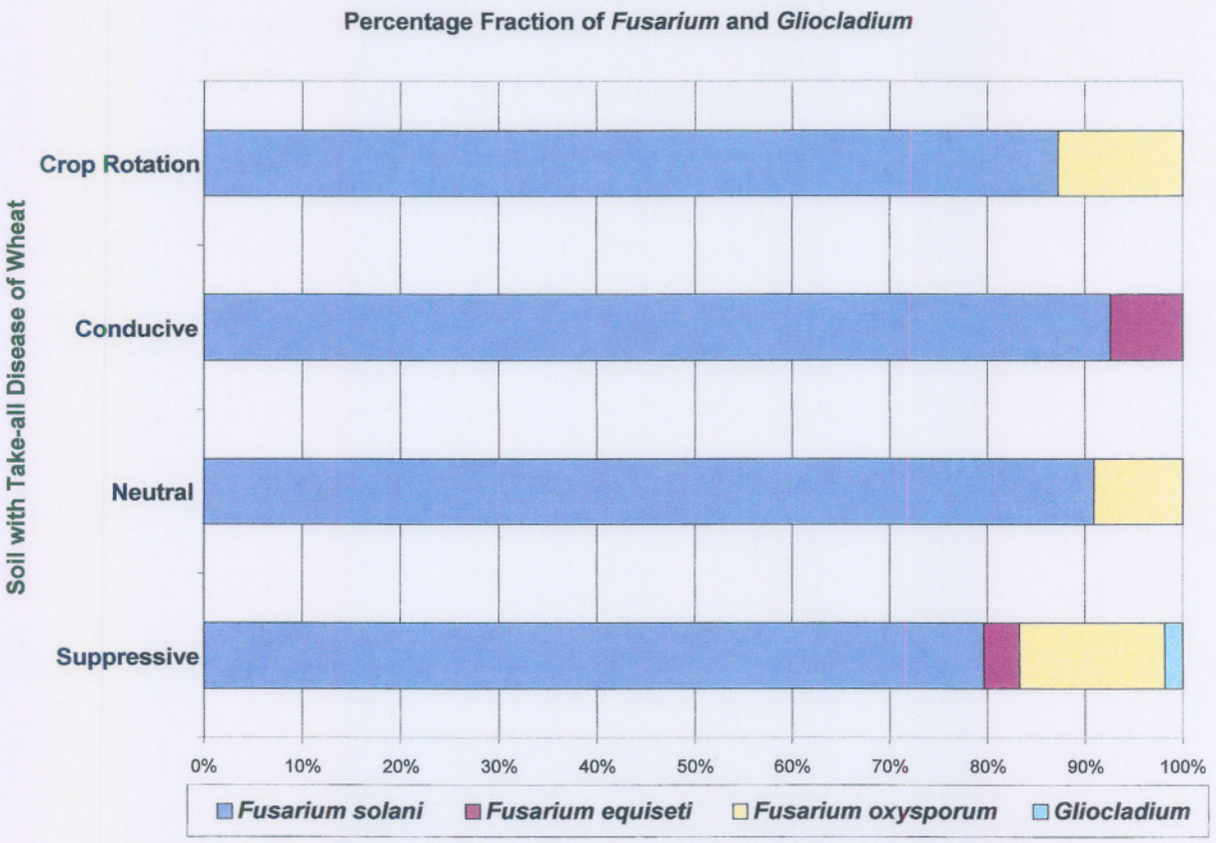


Figure 5. Relative percentage fraction of *Fusarium solani*, *F. equiseti*, *F. oxysporum*, and *Gliocladium* in the various soils, as well as soils cultivated with sunflower and soybeans to demonstrate the effect of crop rotation on soils conducive to take-all disease of wheat.

Based on the results obtained, it was evident that *Fusarium solani* was particularly abundant in soils conducive, and neutral to take-all disease of wheat. Lower numbers of *Fusarium solani* were present in soils suppressive to take-all disease of wheat. *Fusarium solani* is considered an especially troublesome secondary pathogen in take-all lesions and tends to pre-empt or overrun the growth of *Ggt* from roots plated on a non-selective medium. Many reports in the scientific literature of a '*Fusarium* root rot' of wheat caused by *Fusarium* species must, therefore, be evaluated cautiously as a possible misdiagnosis of take-all. *Fusarium* root rot is caused by *F. culmorum*, *F. graminearum* or *F. avenaceum*, with any one of these species usually accounted for 90-95% of the isolates obtained from any given population of infected plants (Cook,

1981). *Fusarium oxysporum* was isolated from soils neutral and suppressive to take-all disease of wheat, with slightly higher numbers present in soils suppressive to take-all disease of wheat. *Fusarium equiseti* was also isolated from soils conducive and suppressive to take-all disease of wheat. Slightly higher numbers of *Fusarium equiseti* was isolated from soils conducive to take-all disease of wheat, than in soils suppressive to take-all disease of wheat.
Fusarium solani and *Fusarium oxysporum* were found in approximately the same numbers in soils suppressive to take-all disease of wheat, and in soils where crop rotation was practised (Figure 5).

Gliocladium sp. are naturally occurring soil fungi. During this study primarily, *Gliocladium* sp. were isolated in soils suppressive (S) to take-all disease of wheat. This organism may thus be involved with take-all suppressiveness. *Gliocladium* sp. are known to produce gliotoxins (broad-spectrum antibiotic) as well as different classes of fungal cell-wall-hydrolytic enzymes such as chitinases and proteases – all playing an important role in mycoparasitism (Vey et al., 2001). A similar increase in the *Gliocladium* population in adjacent experimental plots incidentally also coincided with suppressiveness to the black pod rot pathogen, *Chalara elegans* Nag Raj and W.B. Kendrick (Van Wyk, P.S., unpublished).

3.3 Functional diversity of soil microbial communities in conducive soils subjected to crop rotation.

A DCA ordination plots illustrating the relationship between microbial communities present in soils conducive to take-all disease of wheat, but which had been subjected to crop rotation using sunflower (Sunf) and soybeans (Soy) as a management practice to induce suppressiveness against take-all, is presented in Figure 6. These crops were cultivated on the upper- and lower section of the experimental plot conducive to take-all during the previous season, before both sections were cultivated with wheat during the time of sampling to evaluate the effect of crop rotation on soil microbial communities, and take-all disease of wheat. The relationship between microbial communities present in the experimental plot with soil conducive (C) to take-all disease which had been subjected to crop rotation, and microbial communities present in the adjacent

experimental plots with soils suppressive (S), and neutral (N) with respect to take-all disease of wheat, is also presented in Figure 6.

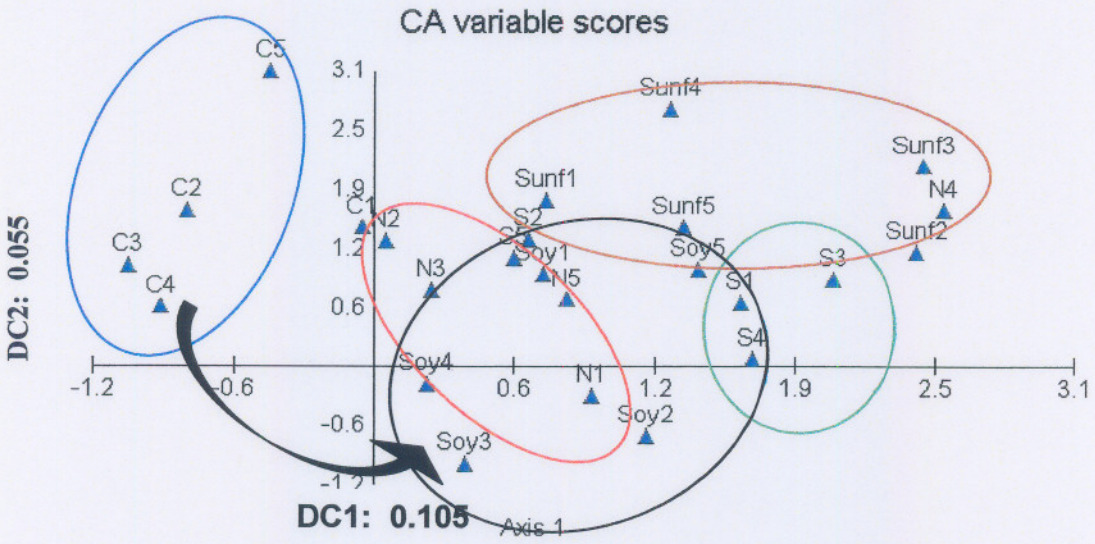


Figure 6. A DCA ordination plots of the community level physiological profiles obtained using GN2 Biolog® microplates for the microbial communities present in an experimental plot with soil conducive (C) to take-all disease of wheat, but which had been subjected to crop rotation with sunflower (Sunfl) and soybeans (Soy). The relationship between microbial communities present in the experimental plot with soil conducive (C) to take-all disease which had been subjected to crop rotation, and microbial communities present in the adjacent experimental plots with soils suppressive (S), and neutral (N) with respect to take-all disease of wheat, is also presented. The eigenvalues for the first two ordination axes of the DCA were 0.105 and 0.055, respectively. These two axes accounted for 24.2% of the total observed variance.

It is evident with that the use of crop rotation with sunflower and soybeans as a management practice to induce suppressiveness against take-all disease, significantly altered the microbial CLPPs (Figure 6). The difference in functional diversity between the two sections of the experimental plot subject to crop rotation, and the functional diversity between soils suppressive (S), and neutral (N) to take-all disease of wheat could possibly be ascribed to the differences in the root exudates by the crops. Since the composition of plant root exudates differ between plant types (Garland, 1996b), leakage of root exudates might attract increasing populations of different rhizosphere microorganisms that are especially well adapted to utilise the specific root exudates very rapidly (Duffy and Weller, 1995). It is evident that the functional diversity of microbial communities in conducive soil subject to crop rotation, was similar to the

functional diversity of microbial communities in soils suppressive to take-all disease of wheat, which might be indicative of the successfulness of crop rotation as a management practice to induce suppressiveness against take-all disease of wheat.

Since the group membership during this study was known *a priori*, multivariate analysis using discriminant analysis (DA) was also applicable. Discriminant analysis selects those variables that yield the best separation according to the given groups. Hypothesis testing and a more rigorous analysis of catabolic profiles can be achieved using discriminant analysis (Buyer and Drinkwater, 1997).

In order to place the various community level physiological profiles (CLPPs), and thus the functional diversity, into perspective, an ordination plot of the first and second canonical function as obtained from the functional diversity of soil microbial communities present in conducive soils where crop rotation had been applied as management practice to induce suppressiveness against take-all disease, is presented in Figure 8.

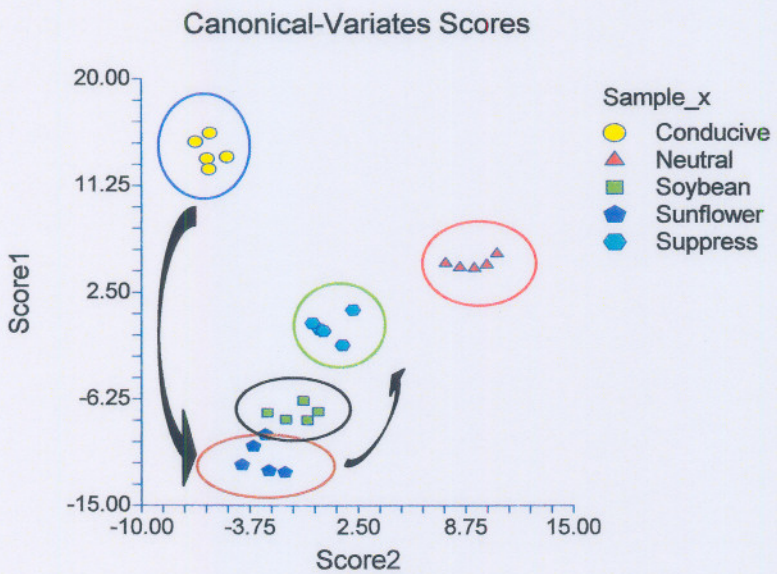


Figure 7. Ordination plot of the first and second canonical function as generated by discriminant analysis of normalised community level substrate utilisation data obtained for the functional diversity of the microbial communities present in an experimental plot with soil conducive to take-all disease of wheat, but which had been subjected to crop rotation with sunflower and soybeans. The relationship between microbial communities present in the experimental plot with soil conducive to take-all disease which had been subjected to crop rotation, and microbial communities present in the adjacent experimental plots with soils suppressive, and neutral with respect to take-all disease of wheat, is also presented. Eigenvalue = 99.16; Wilks' Lambda = 0.000038; F = 12.1; p = 0.0000.

A clear shift in the relationship amongst the functionality of the microbial communities within the two sections due to crop rotation, and soils suppressive, and neutral to take-all disease of wheat is apparent. The first canonical function (CF1) obtained from the discriminant analysis accounted for 73.0% of the total variability observed in the data set. This CF was significant in discriminating between the microbial communities present in the conducive plot subject to crop rotation, and the other various soils. CF1 and CF2 in total, accounted for 97.2% of the total variability observed in the data set.
It is therefore possible to discriminate between the functional diversity of the microbial communities within the two sections in soil conducive to take-all disease of wheat which had been subjected to crop rotation, and soils suppressive, and neutral to take-all disease of wheat (Figure 7). These results might be indicative of the successfulness of crop rotation as a management practice to induce suppressiveness against take-all disease of wheat.

PCA and DCA ordination plots, and a box and whisker plot illustrating the relationship between microbial communities present in soils conducive to take-all disease of wheat, but which had been subjected to crop rotation using sunflower (W) and soybeans (C) as a management practice to induce suppressiveness against take-all, are presented in Figure 8a, 8b, and 8c. These crops were cultivated on the upper- and lower section of the experimental plot conducive to take-all during the previous season, before both sections were cultivated with wheat during the time of sampling to evaluate the effect of crop rotation on soil microbial communities, and take-all disease of wheat.

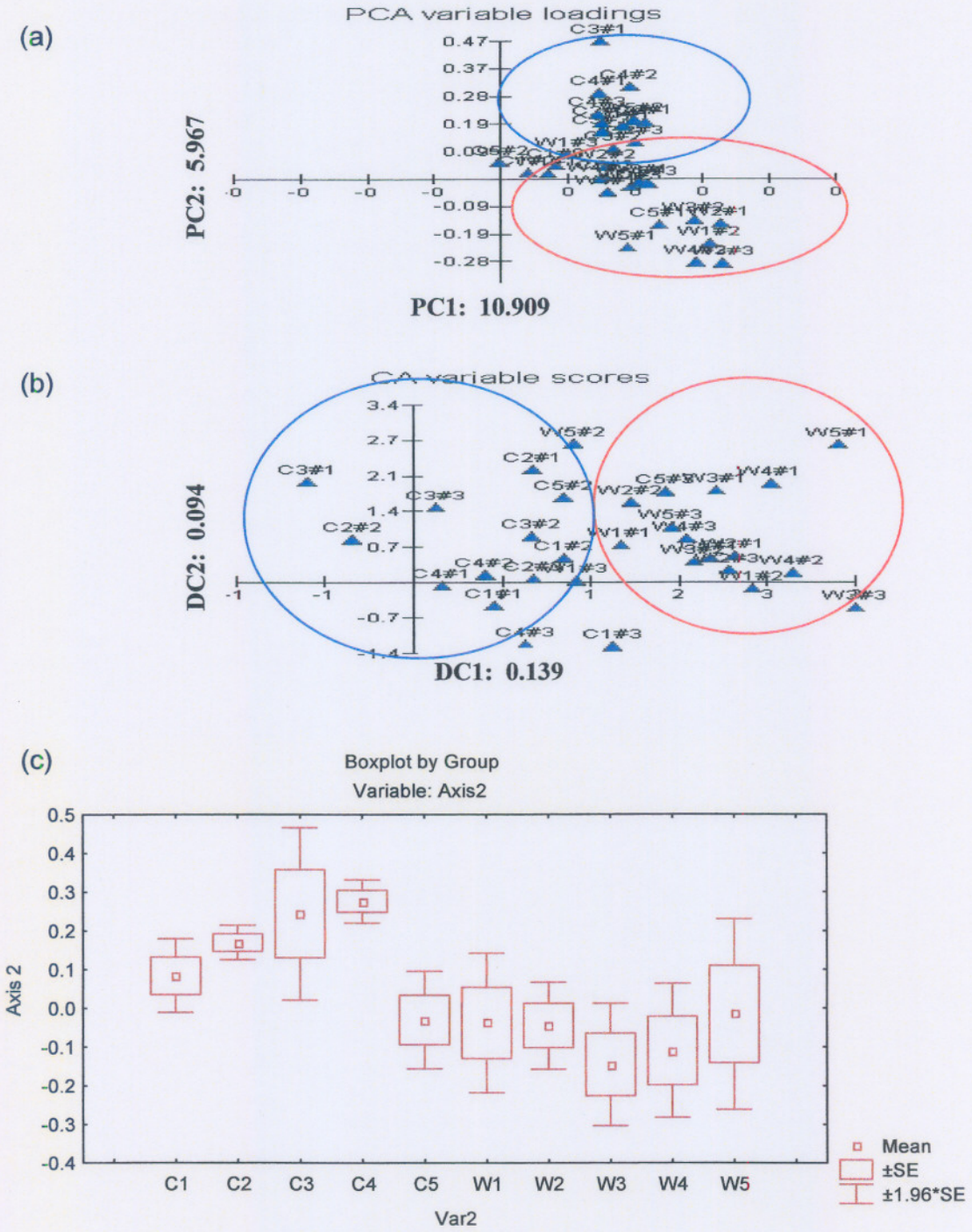


Figure 8. PCA (a), DCA (b) ordination plots and box and whisker plot (c) of the community level physiological profiles obtained using GN2 Biolog® microplates for the microbial communities present in an experimental plot with soil conducive to take-all disease of wheat, but which had been subjected to crop rotation with sunflower (W) and soybeans (C). The eigenvalues for the first two ordination axes of the PCA (a) were 10.909 and 5.967, respectively. These two axes accounted for 28.0% of the total observed variance. The eigenvalues for the first two ordination axes of the DCA (b) were 0.139 and 0.094, respectively. These two axes accounted for 16.5% of the total observed variance.

It is evident with only one exception that the use of crop rotation with sunflower and soybeans as a management practice to induce suppressiveness against take-all disease, significantly altered the microbial CLPPs. The functional diversity of the microbial communities in the two sections of the plot conducive to take-all which had been subject to crop rotation, differed significantly ($p < 0.05$) from each other, as illustrated in the box and whisker plot (Figure 8c). The difference in functional diversity between the two sections of the experimental plot could possibly be ascribed to the differences in the root exudates excreted by sunflowers and soybeans. Since the composition of plant root exudates differ between plant types (Garland, 1996b), leakage of root exudates might attract increasing populations of different rhizosphere microorganisms that are especially well adapted to utilise the specific root exudates very rapidly (Duffy and Weller, 1995).

Garland and Mills (1991) related the separation of samples in PC space to differences in carbon source utilisation by examining the correlation of the original variables to the PCs. Carbon sources which had at least half of their variance explained by PC1 or PC2 (Table 7), were defined as being most important in differentiating among the communities. The degree to which a PC explains the variance in the colour response of an individual carbon source is related to differences in the response in samples with different co-ordinate values for that PC (Garland and Mills, 1991).

Table 7. Correlation of carbon source variables to PCs for analysis of an experimental plot with soil conducive to take-all disease of wheat, where sunflower (W) and soybeans (C) were cultivated on the upper- and lower section of the plot during the previous season, respectively, before both sections were cultivated with wheat during the time of sampling.

PC2	
Carbon source	***r***
Carbohydrates	
D-Mannitol	0.542
ß-Methyl-D-glucoside	0.660
Sucrose	0.594
D-Trehalose	0.756
Phosphorylated chemicals	
Glucose-6-phosphate	0.572
Alcohol	
Glycerol	0.662

Although the soil samples where sunflower and soybeans had been cultivated had similar values for PC1, they differed in their values for PC2. With only one exception, the soybean (C) section possessed higher co-ordinate values. Analysis of PC2 (Figure 8) indicated that the microbial communities in the soybean (C) section utilised four carbohydrates (D-mannitol, ß-methyl-D-glucoside, sucrose, and D-trehalose), glycerol, and glucose-6-phosphate to a relatively lesser degree than the microbial communities in the section where sunflower was cultivated.

A PCA biplot illustrating the relationship between the community level physiological profiles obtained within sections which have been cultivated with sunflower (W) and soybeans (C) in a plot with soil conducive to take-all, is presented in Figure 9.

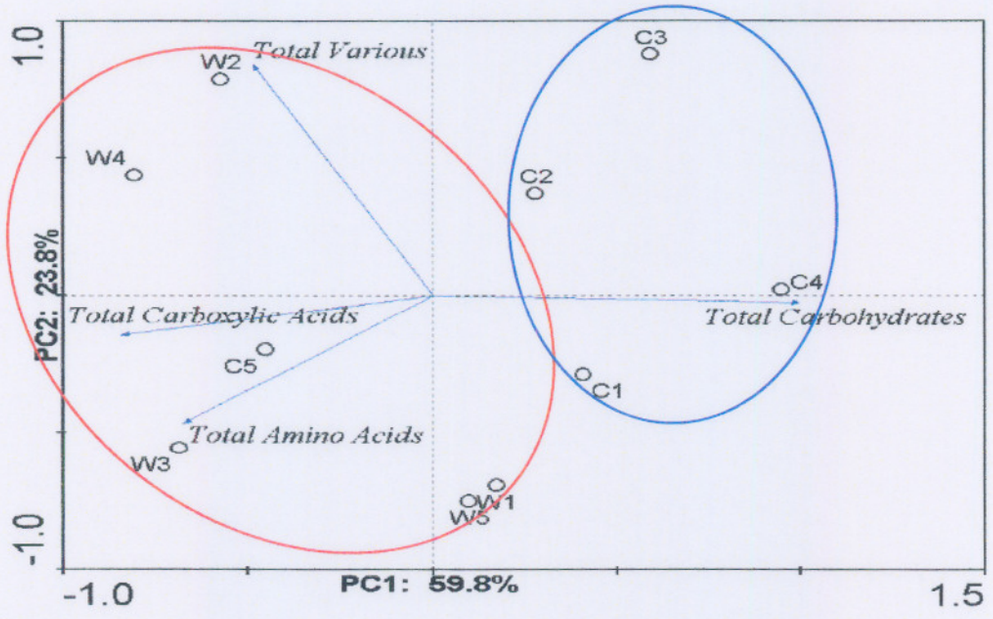


Figure 9. Principle Component Analysis (PCA) biplot of the community level physiological profiles obtained from soil conducive to take-all disease of wheat, but which had been subjected to crop rotation with sunflower and soybean as a management practice to induce suppressiveness against take-all disease of wheat. The eigenvalues for the first two ordination axes were 0.598 and 0.238, respectively. These two axes accounted for 83.6% of the total observed variance.

A clear distinction between the functional diversity of the soil microbial communities during crop rotation was possible. The soils where sunflower crop rotation had been applied grouped loosely together in the bottom- and top-left quadrants of the first ordination axis (Figure 9). Compared to the CLPPs of the soil microbial community

where crop rotation with soybean had been applied, soil microbial communities in the sunflower- (W) section were characterised by the utilisation of a large number of carboxylic acids (Total Carboxylic Acids), amino acids (Total Amino Acids), and various other carbon sources (Total Various), which included esters, alcohols, amides, amines, brominated chemicals, aromatic chemicals, phosphorylated chemicals, and polymers. The soils where soybean (C) crop rotation had been applied grouped loosely together in the bottom- and top-right quadrants of the first ordination axis (Figure 9). Relative to the CLPPs of the soil microbial communities in the sunflower (W) crop rotation section, the CLPPs of the soil microbial communities in the soybean (C) section were characterised by the utilisation of a large number of carbohydrates (Total Carbohydrates).

It is evident that the functional diversity of soil microbial communities present in the sections of the experimental plot with soil conducive to take-all disease of wheat, had undergone significant shifts in functional diversity following the application of crop rotation with sunflower (W) and soybeans (C) with wheat. This resulted in the specific selection of microbial communities with the ability to utilise different carbon sources due to the presence of different root exudates excreted by the specific crops.

Since the group membership during this study was known *a priori*, multivariate analysis using discriminant analysis (DA) was also applicable. Discriminant analysis selects those variables that yield the best separation according to the given groups. Hypothesis testing and a more rigorous analysis of catabolic profiles (CLPPs) can be achieved using discriminant analysis (Buyer and Drinkwater, 1997).

In order to place the various community level physiological profiles (CLPPs), and thus the functional diversity, into perspective, an ordination plot of the first canonical function as obtained from the functional diversity of soil microbial communities present in conducive soils where crop rotation had been applied as management practice to induce suppressiveness against take-all disease, is presented in Figure 10.

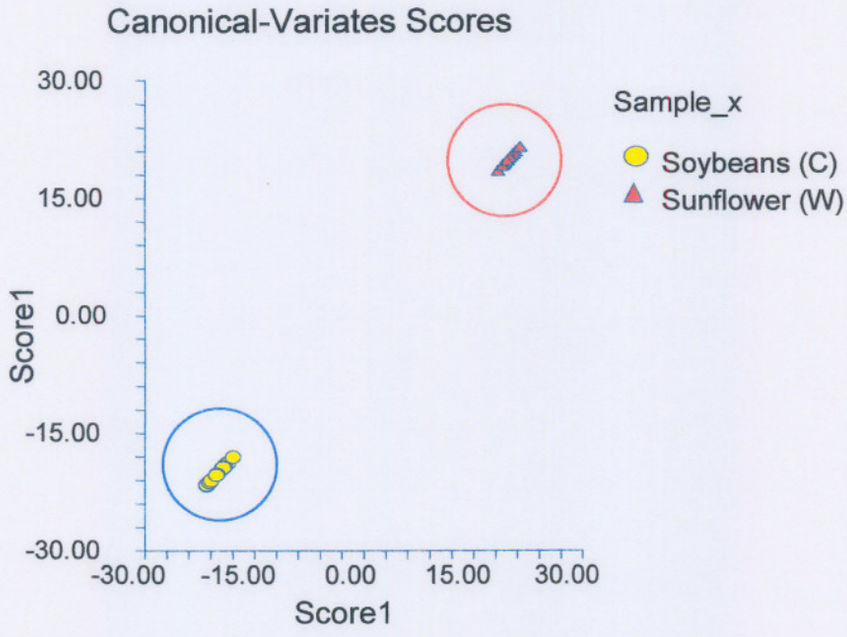


Figure 10. Ordination plot of the first canonical function as generated by discriminant analysis of normalised GN2 community level substrate utilisation data obtained for the functional diversity of the microbial communities present in soil conducive to take-all disease of wheat, using GN2 Biolog® microplates. Crop rotation using sunflower (W) and soybeans (C), had been applied as a management practice prior to the cultivation of wheat. Eigenvalue = 421.66; Wilks' Lambda = 0.0024; F = 342.6; p = 0.0000.

A clear shift in the relationship amongst the functionality of the microbial communities within the two sections due to crop rotation is apparent. The first canonical function (CF1) obtained from the discriminant analysis accounted for 100% of the total variability observed in the data set. This CF was significant in discriminating between the microbial communities present in the conducive plot where crop rotation had been applied.

It is therefore possible to discriminate between the functional diversity of the microbial communities where the sunflower (W) and soybean (C) sections had been rotated with wheat as summer and winter crops, respectively, which differed significantly in the sections of the experimental plot (Figure 10).

The statistically significant difference in community level physiological profiles of soil microbial communities present in the experimental plot which had been subjected to crop rotation, also implied different substrate utilisation capabilities of the soil microbial communities present (Grayston et al., 1998).

The substrate utilisation capabilities (AWCD) of the microbial communities differed significantly ($p < 0.05$) in their ability to utilise 16 of the 95 different carbon sources supplied on the Biolog® GN2 microtiter plate during the date of sampling (Table 8). Some carbon sources may be better for monitoring community changes over time, while others are more suited to examine treatment effects. Staddon et al. (1997) considered carboxylic acids to be an example of the latter.

Table 8. The mean AWCD and standard deviation of the independent variables found to differ significantly in soil conducive to take-all disease but which had been subjected to crop rotation with sunflower and soybeans as a management practice to induce suppressiveness against take-all disease.

Well #	Variables	Sunflower (W)	Soybeans (C)
A02	α-Cyclodextrin	0.40 ± 0.64	0.34 ± 0.69
A05	Tween40	1.41 ± 1.40	2.23 ± 1.60
A06	Tween80	0.76 ± 0.65	2.26 ± 2.40
B07	m-Inositol	1.40 ± 1.67	0.73 ± 0.75
B08	α-D-Lactose	0.51 ± 0.49	0.47 ± 0.67
C09	Turanose	0.31 ± 0.37	0.69 ± 0.63
D06	D-Galacturonic acid	1.46 ± 1.60	1.94 ± 2.09
E01	p-Hydroxyphenylacetic acid	0.52 ± 0.66	0.45 ± 0.68
E03	α-Ketobutyric acid	0.43 ± 0.73	0.17 ± 0.17
E07	Malonic acid	0.19 ± 0.24	1.51 ± 0.92
E08	Propionic acid	0.26 ± 0.25	0.71 ± 0.87
E10	D-Saccharic acid	1.14 ± 1.74	3.55 ± 4.19
F02	Succinamic acid	0.50 ± 0.54	0.63 ± 0.78
G01	L-Histidine	1.88 ± 2.33	0.78 ± 1.39
H05	Phenylethylamine	0.10 ± 0.12	0.22 ± 0.38
H08	2,3-Butanediol	0.20 ± 0.20	0.34 ± 0.66

Differential utilisation of polymers (α-cyclodextrin, tween 40, tween 80), carbohydrates (m-inositol, α-D-lactose, turanose), carboxylic acids (D-galacturonic acid, p-hydroxyphenylacetic acid, α-ketobutyric acid, malonic acid, propionic acid, D-saccharic acid), an amide (succinamic acid), an amino acid (L-histidine), an amine (phenylethylamine), and an alcohol (2,3-butanediol) were responsible for differentiation in community level physiological profiles of soil microbial communities in soils conducive to take-all disease of wheat which have been cultivated with sunflower (W)

and soybeans (C) during the previous season before being cultivated with wheat to demonstrate the effect crop rotation had on soil microbial communities (Table 8).

The effect of crop rotation on the Shannon-Weaver substrate diversity (*H'*) and equitability (*J*) indices, were also evaluated. It was evident that high substrate diversity existed in soils conducive to take-all disease, but which had been subjected to crop rotation (Table 9). According to Magurran (1988), values of the Shannon index fluctuate between 1.5 and 3.5 and rarely increase above 4.5. Values obtained during this study were overall within the higher diversity range (> 3.6), indicative of the achievement of very high substrate diversity values. These values were similar to those obtained for the other experimental plots conducive, suppressive, and neutral to take-all disease of wheat. The high substrate diversity values obtained were established by the fact that a substantial percentage of the carbon sources were utilised, contributing to the very high Shannon-Weaver substrate diversity indices.

The substrate evenness (equitability) (*J*) indices ranged from 0.82 to 0.90 for the soils conducive to take-all disease, but which had been subjected to crop rotation, and did not show significant differences or trends (Table 10). According to Magurran (1988), substrate evenness (equitability) assumes a value between 0 and 1 with 1 being complete evenness, indicating a high diversity. It could therefore be concluded that a high functional diversity existed in both the upper- and lower sections planted with sunflower (W) and soybeans (C), respectively. These values were similar to those obtained for the other experimental plots conducive, suppressive, and neutral to take-all disease of wheat.

Table 9. Shannon-Weaver substrate diversity index values (*H'*) obtained during crop rotation. Soybean (C); sunflower (W).

Sample	*H'* Value
C1	3.69 ± 0.21
C2	3.67 ± 0.01
C3	3.88 ± 0.08
C4	3.73 ± 0.09
C5	3.92 ± 0.09
W1	3.91 ± 0.10
W2	3.79 ± 0.06
W3	3.67 ± 0.07
W4	3.85 ± 0.12
W5	3.72 ± 0.15

Table 10. Substrate equitability indices (*J*) obtained during crop rotation. Soybean (C); sunflower (W).

Sample	*J* Value
C1	0.90 ± 0.02
C2	0.83 ± 0.01
C3	0.86 ± 0.02
C4	0.82 ± 0.02
C5	0.90 ± 0.02
W1	0.87 ± 0.03
W2	0.88 ± 0.02
W3	0.86 ± 0.02
W4	0.86 ± 0.02
W5	0.85 ± 0.01

Whilst the Shannon diversity index emphasises the amount of carbon sources utilised, Sorenson's index emphasises the degree of substrate utilisation. A dendogram constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA), illustrates the similarity in carbon source utilisation of the samples from the previous season's sunflower- (W) and soybean- (C) sections which have been rotated with wheat (Figure 11). The clusters obtained could be distinguished based on their Sorenson's indices. Since the similarity of pairs of sites are studied by taking species abundance into account (Magurran, 1988), a clear distinction could be made between the upper- and lower sections planted with sunflower (W) and soybeans (C), respectively. The clear distinction could be attributed to the selection process occurring in the different plant rhizospheres, resulting in the increase in soil microbial

populations with the ability to utilise the different carbon sources due to the presence of different root exudates (Grayston et al., 1998).

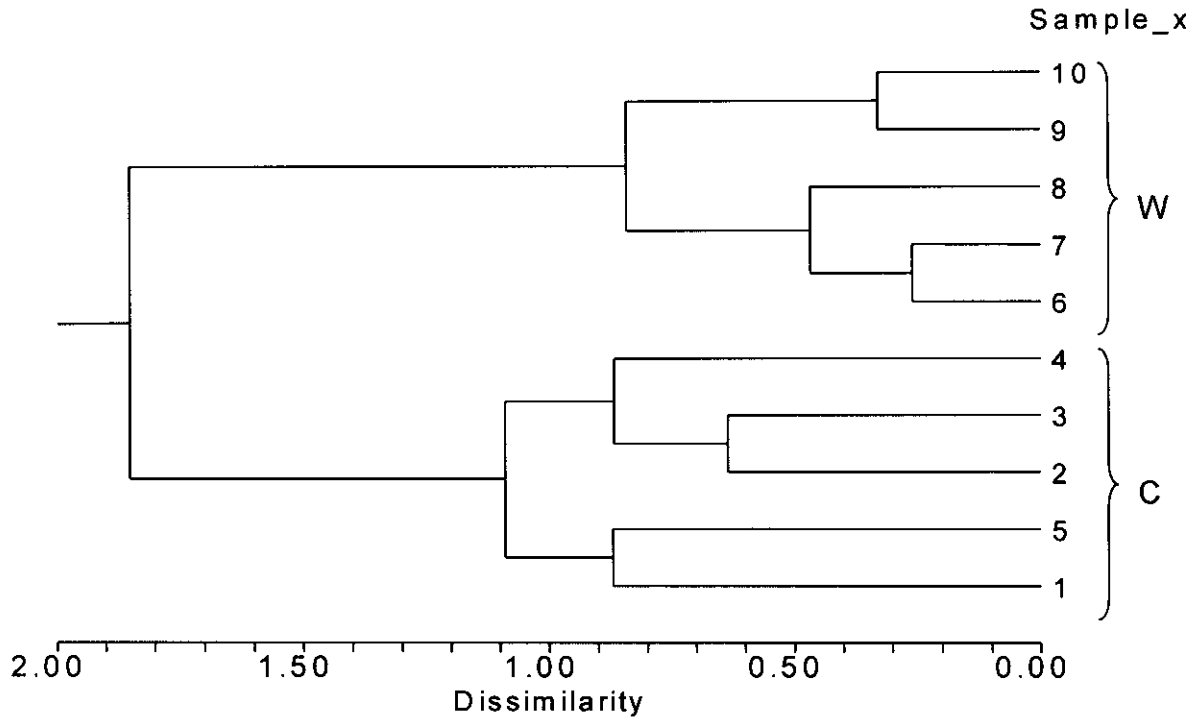


Figure 11. Dendogram constructed from unweighted pair group method with arithmetic mean (UPGMA) illustrating the similarity in carbon source utilisation patterns during crop rotation. Soybean (C); sunflower (W).

4. CONCLUSIONS

Analysis of community level physiological profiles (CLPP) of soil microbial communities in agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat as caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*) was sensitive to enable differentiation of the various samples. Principal component analysis (PCA), detrended correspondence analysis (DCA), and discriminant analysis (DA) of CLPPs illustrated that the microbial community level physiological profiles of soil conducive to take-all disease of wheat, differed significantly from soils suppressive and neutral to take-all disease of wheat. Soils suppressive and neutral to take-all disease were characterised by enhanced utilisation of carboxylic acids, amino acids, and carbohydrates, while conducive soils were characterised by enhanced utilisation of carbohydrates. Shifts in the functional diversity of the associated microbial communities were possibly caused by the presence of *Ggt* and associated antagonistic fungal and bacterial populations in the various soils. During take-all infection, invading hyphae grow through the root cortex of wheat to invade and destroy the root phloem, where after the xylem is invaded. Infected plants' roots have initial black lesions that expand and eventually coalesce, extending existing lesions and / or producing new secondary infections (Sarniguet et al., 1992). It is hypothesised that the development of lesions and the subsequent excessive leakage of root exudates due to take-all infection and possible secondary infections, might attract increasing populations of antagonistic rhizosphere microorganisms that are especially well adapted to utilise these exudates very rapidly (Duffy et al., 1997), a response typical of r-strategists, including Pseudomonads. Although the microbial communities could utilise a wide range of substrates, the communities could only be differentiated by the utilisation of a few substrates, demonstrating the development of different microbial communities with different functionalities in soil conducive, suppressive, or neutral to take-all disease of wheat. When CLPP were statistically analysed in relation to the dominant environmental variables (including physico-chemical characteristics, significant results could be obtained.

Conducive soils were characterised by high nitrate-concentrations, which would result in higher soil pH, and increased take-all severity. The conducive experimental plots were also characterised by low levels of phosphorous and manganese, which might

have resulted in unhealthy plants with low resistance to take-all infection. Duffy et al. (1997) reported that the oxidation of manganese by *Ggt* might decrease the availability of the micronutrients to wheat, which resulted in increased take-all severity. In contrast, experimental plots suppressive to take-all disease were characterised by high ammonium-concentrations. This could contribute to the lowering of the soil pH, which resulted in decreased take-all severity, increased micronutrient availability, and consequently increased antagonistic rhizosphere microbial communities. It is subsequently hypothesised that physico-chemical characteristics of the various soils might have influenced the composition of root exudates, which might also explain the difference in the CLPPs of the associated soil microbial communities.

No significant difference could be observed between the various soil samples using conventional plate counting techniques, possibly due to the restrictive nature of the selective media used, which selected against the unculturable fraction of the microbial community. It is known that less than 1% of soil microbes are culturable. *Fusarium solani* and *F. oxysporum* were found in approximately the same numbers in soils suppressive to take-all disease of wheat, and in conducive soils where crop rotation was practised. *Gliocladium* sp. was mainly isolated from soils suppressive to take-all disease of wheat. *Gliocladium* sp. are known to produce gliotoxins (broad-spectrum antibiotic) as well as different classes of fungal cell-wall-hydrolytic enzymes that play an important role in mycoparasitism (Vey et al., 2001). A similar increase in the *Gliocladium* population in adjacent experimental plots incidentally also coincided with suppressiveness to the black pod rot pathogen, *Chalara elegans* Nag Raj and W.B. Kendrick (Van Wyk, P.S., unpublished).

Distinct differences in the functional diversity of soil microbial community characteristics were also detected in the experimental plots conducive to take-all, but which had been subjected to crop rotation with sunflower and soybean as a management strategy. PCA and discriminant analysis of the CLPPs of the experimental plots conducive to take-all where crop rotation had been applied, enabled differentiation of these plots from the experimental plots conducive, suppressive, and neutral to take-all disease. The CLPPs of the microbial communities associated with the crop rotation were more similar to those of suppressive soil, than to that of the conducive soil, indicating that the application of crop rotation was a suitable management strategy for the suppression of

take-all disease of wheat. It was also evident that the crop used during crop rotation (i.e. sunflower vs. soybean) resulted in a significant difference ($p < 0.05$) in the CLPP of the associated microbial communities. This may possibly be ascribed to differences in the composition of the root exudates, which would select for a specific microbial community. The CLPPs of the soil microbial community associated with the cultivation of sunflower during crop rotation, was characterised by the enhanced utilisation of carboxylic acids, amino acids, and esters, alcohols, amides, amines, brominated chemicals, aromatic chemicals, phosphorylated chemicals, and polymers. In contrast, the CLPP of the soil microbial community associated with the cultivation of soybean during crop rotation was characterised by the enhanced utilisation of mainly carbohydrates.

High Shannon-Weaver substrate diversity (*H'*) and substrate equitability (J) values were obtained for the soil microbial communities in soils conducive, suppressive, and neutral to take-all disease of wheat, as well as the adjacent experimental plot with soil conducive to take-all disease where crop rotation was practised. Hierarchical cluster analysis of the CLPPs obtained, enabled differentiation of the microbial communities associated with the different stadia of take-all disease, and the Sorenson's index values. A clear distinction could be made between the soils.

It is evident that the functional diversity of the soil microbial communities are significantly influenced by the presence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, and that the characterisation of microbial functional diversity by analysis of community level physiological profiles (CLPPs) could successfully be applied as an assessment criterium for the evaluation of agricultural soils conducive, suppressive, and neutral to take-all disease of wheat, as well as for crop rotation systems. These results confirm the report of Schneider et al. (1998) who found phenotypic fingerprinting to be a more sensitive technique than conventional microbiological techniques to detect changes in soil microbial communities. It can thus be concluded that the analysis of the functional diversity of the microbial communities using community level physiological profiles (CLPP) may be of significant value for the evaluation of the effect of management practices on the suppression of the take-all disease of wheat, as well as selection and evaluation of agricultural soils subject to the prevalence of other soilborne diseases.

5. REFERENCES

Aleem, M.I.H., Alexander, M., 1958. Cell-free nitrification by *Nitrobacter*. Journal of Bacteriology 76,510-514

Alef, K., Nannipieri, P. (Eds.) 1995. Methods in Applied Soil Microbiology and Biochemistry. New York: Academic Press. 576p.

Atlas, R.M., Parks, L.C., 1993. Handbook of Microbiological media. CRC Press, Inc., Florida, 1079 pp.

Barnett, S.J., Singleton, I., Ryder, M. 1999. Spatial variation in populations of *Pseudomonas corrugata* 2140 and pseudomonads on take-all diseased and healthy root systems of wheat. Soil Biology and Biochemistry 31, 633-636.

Brennan, R.F. 1993. Effect of ammonium chloride, ammonium sulphate, and sodium nitrate on take-all and grain yield of wheat grown on soils in south-western Australia. Journal of Plant Nutrition 16, 349-358.

Brennan, R.F. 1995. Effect of levels of take-all and phosphorus fertiliser on the dry matter and grain yield of wheat. Journal of Plant Nutrition 18, 1159-1176.

Buyer, J.S., Drinkwater, L.E. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3-11.

Collins, D.J. 1995. Diseases of barley, rye, and triticale in Alabama. [Web:] http://www.aces.edu/department/extcomm/publications/anr/anr-903/anr-903.html [Date of access: May 2001].

Cook, R.J. 1981. The influence of rotation crops on take-all decline phenomenon. Phytopathology 71, 189-192.

Cook, R.J. 1994. Problems and progress in the biological control of wheat take-all. Plant Pathology 43, 429-437.

Cook, R.J., Rovira, A.D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biology and Biochemistry 8, 269-273.

Crawford, D.L., Lynch, J.M., Whipps, J.M., Ousley, M.A. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology 59, 3899-3905.

Duffy, B.K., Weller, D.M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Disease 79, 907-911.

Duffy, B.K., Ownley, B.H., Weller, D.M. 1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopathology 87, 1118-1124.

Gagliardi, J.V., Buyer, J.S., Angle, J.S., Russek-Cohen, E. 2001. Structural and functional analysis of whole-soil microbial communities for risk and efficacy testing following microbial inoculation of wheat roots in diverse soils. Soil Biology and Biochemistry 33, 25-40.

Garland, J.L. 1996a. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology and Biochemistry 28, 213-221.

Garland, J.L. 1996b. Patterns of potential C source utilization by rhizosphere communities. Soil Biology and Biochemistry 28, 223-230.

Garland, J.L., Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351-2359.

Garvin, S., Lindeman, W.C. 1986. Isolation, characterization, and inoculation of N2-fixing bacteria from dicotyledonous plants. Canadian Journal of Microbiology 32, 912-916.

Glandorf, D.C.M., Van Der Sluis, I., Anderson, A.J., Bakker, P.A.H.M., Schippers, B. 1994. Agglutination, adherence, and root colonization by fluorescent pseudomonads. Applied and Environmental Microbiology 60, 1726-1733.

Graham, R.D., Rovira, A.D. 1984. A role for manganese in the resistance of wheat plants to take-all. Plant and Soil 78, 441-444.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Hershman, D., Bachi, P. 1994. Take-all of wheat. [Web:] http://www.ca.uky.edu/agcollege/plantpathology/PPAExten/PPFShtml/ppfagsg1.htm [Date of access: March 2000].

Hintze, J.L. 1997. NCSS 97 – Statistical system for Windows. Number cruncher statistical systems, Kaysville, Utah, 1716 pp.

Huber, D.M., McCay-Buis, T.S. 1993. A multiple component analysis of the take-all disease of cereals. Plant Disease 77, 437-447.

Kersters, I., Van Vooren, L., Verschuere, L., Vauterin, L., Wouters, A., Mergaert, J., Swings, J., Verstraete, W. 1997. Utility of the Biolog system for the characterization of heterotrophic microbial communities. Systematic and Applied Microbiology 20, 439-447.

Lahav, I., Steinberger, Y. 2001. Soil bacterial functional diversity in a potato field. European Journal of Soil Biology 37, 59-67.

Larkin, R.P., Hopkins, D.L., Martin, F.N. 1993. Effects of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83, 1097-1105.

Lupwayi, N.Z., Arshad, M.A., Rice, W.A., Clayton, G.W. 2001. Bacterial diversity in water-stable aggregates of soils under conventional and zero tillage management. Applied Soil Ecology 16, 251-261.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey, pp. 179.

Mazzola, M., Cook, R.J. 1991. Effects of fungal root pathogens on the population dynamics of biocontrol strains of fluorescent pseudomonads in the wheat rhizosphere. Applied and Environmental Microbiology 57, 2171-2178.

McMullen, M.P., Lamey, H.A. 1997. Symptoms and controls of crop diseases. [Web:] http://www.ext.nodak.edu/extpubs/plantsci/crops/pp533w.htm [Date of access: September 2002].

Monsanto Company. 1998. A guide to take-all – a disease in cereal grains. [Web:] http://www.takeall.com/english/default.htm [Date of access: July 2002].

Øvreås, L., Torsvik, V. 1998. Microbial diversity and community structure in two different agricultural soil communities. Microbial Ecology 36, 303-315.

Palojärvi, A., Sharma, S., Rangger, A., Von Lutzow, M., Insam, H. 1997. Comparison of Biolog and Phospholipid Fatty acid patterns to detect changes in microbial community. In: Insam, H., Rangger, A. (Eds.), Microbial Communities - Functional versus Structural Approaches. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 37-48.

Petersen, S.O., Klug, M.J. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Applied and Environmental Microbiology 60, 2421-2430.

Rane, K., Ruhl, G., Sellers, P., Scott, D. 1997. Crop diseases in corn, soybean, and wheat. [Web:] http://www.btny.purdue.edu/Extension/Pathology/CropDiseases/Wheat/Wheat1.html [Date of access: February 2001].

Rengel, Z. 1997. Decreased capacity of take-all fungus to oxidize manganous ions is associated with take-all decline. Journal of Plant Nutrition 20, 455-460.

Rothrock, C.S., Cunfer, B.M. 1991. Influence of small grain rotations on take-all in a subsequent wheat crop. Plant Disease 75, 1050-1052.

Sarniguet, A., Lucas, P. 1992. Evaluation of populations of fluorescent pseudomonads related to decline of take-all patch on turfgrass. Plant and Soil 145, 11-15.

ABSTRACT

Phospholipid fatty acid (PLFA) analyses have been reported to be useful in the characterisation of the structural diversity of soil microbial communities. During this study, the relationship between the structural diversity of soil microbial communities, and physico-chemical characteristics of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker were evaluated. These relationships were investigated using PCA, RDA and discriminant analysis. The structural diversity of the microbial communities in soils suppressive to take-all disease of wheat differed significantly ($p < 0.05$) from soils conducive, and neutral to take-all disease of wheat. A positive association was observed between the microbial phospholipid fatty acid profiles, and dominant physico-chemical variables of the various soils. Soils conducive and neutral to take-all disease of wheat were characterised by high concentrations of manganese, as well as elevated concentrations of monounsaturated fatty acids, terminally branched saturated fatty acids, and polyunsaturated fatty acids which are considered to be indicative of Gram-negative bacteria, Gram-positive bacteria and micro-eukaryotes (primarily fungi), respectively. Compared to suppressive soil, soils conducive and neutral to take-all disease of wheat were characterised by low concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as low soil pH. Soil suppressive to take-all disease of wheat was characterised by the elevated levels of estimated microbial biomass and elevated concentrations of normal saturated fatty acids, considered to be indicative of low diversity. This soil was also characterised by high concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as elevated soil pH. Hierarchical cluster analysis of the major phospholipid fatty acid

groups indicate that the structural diversity differed significantly between soils conducive, suppressive, and neutral to take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*. Results obtained during this study indicate that the analysis of structural diversity of soil microbial communities could be used as a suitable criterion for the evaluation of agricultural soils conducive, suppressive, and neutral to take-all disease of wheat, and could possibly assist in the characterisation of the influence of management practices on the suppression of take-all disease of wheat.

Keywords: Soil microbial community structure; Phospholipid fatty acids (PLFAs); *Gaeumannomyces graminis* var. *tritici*; Take-all disease.

1. INTRODUCTION

Take-all is the name given to the disease caused by a soilborne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker) (*Ggt*). This fungus is responsible for the cause of crown and root rot in wheat, barley, rye and triticale (Rothrock and Cunfer, 1991). *Gaeumannomyces graminis* var. *tritici* is not a seed-borne fungus (Hershman and Bachi, 1994) and survives in crop stubble (Collins, 1995). The disease occurs in early seeded wheat during the growth season and when plants experience nitrogen stress. The most damage is caused during early infections, when both roots and culms are affected (Collins, 1995). When germinating seedlings from newly planted cereal crops come into contact with the take-all pathogen, colonisation of roots continue to occur as the season progresses. Changes in microbial activity and composition can influence plant growth, by increased nutrient turn-over, increasing disease incidence, or even disease suppression (Marschner et al., 2003). Continuous cropping with a susceptible host, or susceptible alternate hosts, can result in the build-up of soil populations of specific plant pathogens, resulting in a decline in crop yield and quality. Take-all is most common in regions where wheat is grown without adequate crop rotation (Hershman and Bachi, 1994).

Soilborne disease suppressiveness is an inherent characteristic of the physical, chemical, and/or biological structure of a particular soil which might be induced by agricultural practices and activities such as planting of crops, or the addition of organisms or nutritional amendments, causing a change in the microfloral environment (Larkin et al., 1993). Suppressive factors can be transferred from soil-to-soil where it could multiply.

Soil is a complex and heterogeneous ecosystem with a character still imposing a momentous challenge. Despite the fact that ecosystems are utterly dependent on microorganisms, microbial community structure, predicting how structure responds to environmental perturbations, or linkages between structure and ecosystem processes have been difficult to determine. The immense diversity of microbial communities, of which only a small portion has been characterised, precludes the use of counting and naming approaches traditionally used in animal and plant community ecology (Vestal and White, 1989). The use of physico-chemical analysis of sand, clay and silt contents,

soil pH, water holding capacity, total nitrogen-, and total carbon contents are also commonly applied, although none of these above-mentioned techniques allows conclusions to be made concerning the biological structures and functions in the soils, due to methodological limitations (Sharma et al., 1998). Although conventional microbiological techniques have been used to quantify and identify culturable soil microbial populations, these results and subsequent conclusions made, are restrictive primarily due to the selective nature of the laboratory media and incubation conditions which exclude most other microorganisms, the unculturability of many microorganisms, and the difficulty in determining microbial community function and structure *in situ.* (Kersters et al., 1997). Although quantitative yields and specific information on biodiversity can be obtained by plate count populations, plating studies are usually incomplete methods for estimating biodiversity (Øvreås and Torsvik, 1998). Recent studies have indicated that less than 1% of all microbes can be cultured, with viable counts of microorganisms from environmental samples accounting for 0.1 - 10% of the total community (Sharma et al., 1998; Vestal and White, 1989). Due to limitations of conventional techniques, numerous new assays and procedures have been developed to study microbial communities *in situ* that show promise for use in complex substrates. Most of these recently developed techniques, which enable the analyses of communities at microbial level, are independent of cell culturability, thus circumventing numerous problems frequently associated with conventional microbiological techniques.

Although the studies in Chapter 3 provide evidence for gross microbial responses to soil management, details concerning the specific responses of the *in situ* microbial community structure, beyond which is known for culturable organisms, are still lacking. With the application of newer biochemical and molecular tools, microbiologists are now better equipped to identify microbial community responses to soil management. In this study, the application of fatty acid methyl esters (FAMEs) to "fingerprint" the structure of the microbial communities in the soils conducive, suppressive, and neutral with respect to take-all disease of wheat, were evaluated (Drijber et al., 2000). Phospholipid fatty acid analysis (PLFA) is a biochemical method that can provide detailed information concerning the structure of the active microbial community and provides a more sensitive measure of changes at the community level than do traditional measures (Vestal and White, 1989; Bossio and Scow, 1998).

The aim of this study was therefore to characterise the soil microbial community structure within agricultural soils conducive, suppressive, and neutral to take-all disease of wheat as caused by the soilborne fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), by analysis of phospholipid fatty acid methyl esters (FAMEs).

The specific objectives of this research were to: (1) characterise the structural diversity of the viable soil microbial community and estimate the viable microbial biomass within agricultural soils conducive, suppressive, and neutral to take-all disease of wheat, and (2) to investigate the relationship between the dominant physico-chemical variables and the microbial community structure in the various soils using multivariate statistical techniques.

2. MATERIALS AND METHODS

2.1 Preparation of sampling sites

Take-all disease increases over years, reaching a maximum 3 – 4 years under wheat monoculture. Following continued monoculture, disease incidence decreases to a level where the crop yield is equal to that of the first year. In a joint venture with the Agricultural Research Council, Small Grain Institute (Bethlehem, South Africa) to breed for resistance against *Gaeumannomyces graminis* var *tritici*, new plots were continuously planted with wheat at the Agricultural Research Council, Grain Crops Institute (Potchefstroom, South Africa) to ensure the availability of diseased plots. Plots where no wheat had been cultivated for at least 30 years prior to sampling, were taken as a natural soil and were considered to be neutral with respect to take-all disease of wheat. Plots where wheat was grown in monoculture for 4 years represented conducive soils while plots under wheat monoculture for 7 years represented suppressive soils.

2.2 Samples

Soil samples were aseptically obtained from adjacent plots of the Agricultural Research Council (Potchefstroom, South Africa) using a randomised plot design. These agricultural plots were conducive, suppressive, and neutral with respect to take-all disease of wheat. Soil samples were collected from each experimental plot in the spring (post-harvest of wheat).

Five sample sites were randomly chosen within each of the experimental plots. Soil samples were taken using a soil auger rinsed with methanol:water (80:20 v/v) between each sampling site to prevent the occurrence of cross contamination. Soil samples consisted of ten sub-samples randomly taken from a 2 m^2 area. Each of the sub-samples were consolidated and mixed thoroughly in order to create a composite sample, which was considered to be representative of the specific sample site within a plot (n = 5). Sampling was only conducted in the topsoil layer (0-15 cm), since it has previously been reported that the number of culturable bacteria and enzymatic activity decline with depth (Riffaldi et al., 2002).

Each soil sample was sealed in plastic bags and immediately frozen in liquid nitrogen on site. Samples were transported in a cooler to the laboratory where they were stored

at -86°C, before being passed through a 2 mm sieve to remove visible organic residues (Riffaldi et al., 2002). Subsamples (± 200 g) of each sample were subsequently lyophilised before PLFA extraction, fractionation and analysis. Physico-chemical characteristion of the various soil samples was done by the Grain Crops Institute, Agricultural Research Council (Potchefstroom, South Africa).

2.3 Determination of the structural diversity

All glassware used for lipid analyses was thoroughly washed with phosphate-free soap (Extran), rinsed ten times with tap water, ten times again with distilled water, air dried, and heated in a muffle furnace at 450°C for a minimum of 4 h to remove any lipid contaminants. All other apparatus underwent the same cleaning procedure, but were also rinsed with methanol and again with chloroform and allowed to dry.

All solvents used were of the highest grade (Burdick & Jackson). Silicic acid and the internal standard, dodecanoic acid (C12:0) were obtained from Sigma Aldrich.

Total lipids were extracted from approximately 5 g lyophilised soil using a modified Bligh and Dyer procedure as described by White and Ringelberg (1998). Total lipids were extracted with a chloroform, methanol, and phosphate buffer mixture (1:2:0.8 v/v). Activated (110°C for at least 1h) silicic acid column chromatography (Palojärvi et al., 1997) was used to fractionate the total lipid extract into neutral lipids, glycolipids and polar lipids. Neutral lipids and glycolipids were eluted from the silicic acid column with 5 ml chloroform and 5 ml acetone, respectively. Polar lipids were then eluted with 10 ml methanol. After the collection of each of the separate fractions, the solvents were removed with nitrogen gas blow-down, and stored under nitrogen at –20°C for further analysis (White and Ringelberg, 1998).

Only the polar lipid fraction (phospholipid fatty acids) was transesterified to the fatty acid methyl esters (FAMEs) by mild alkaline methanolysis (Palojärvi et al., 1997).

The FAMEs were analysed by capillary gas chromatography with flame ionisation detection on a Hewlett-Packard 6890 series II chromatograph fitted with a 30 m HP column (0.250 mm I.D., 0.250 µm film thickness). Definitive identification of peaks was undertaken using gas chromatography-mass spectrometry of selected samples using a Hewlett-Packard 6890 series II chromatograph interphased with a Hewlett-Packard 5973 mass selective detector.

2.4 Fatty acid nomenclature

Fatty acids are designated in terms of A:BωC, where A refers to the total number of carbon atoms, B refers to the number of double bonds, and C refers to the position of the double bonds from the methyl end (aliphatic ('ω') end) of the chain. Cis and trans configurations are indicated by 'c' and 't', respectively. The prefixes 'i', 'a', and '10Me' refer to methyl branching at the iso and anteiso positions and at the 10^{th} carbon from the carboxyl end of the molecule, respectively. Cyclopropane fatty acids have the prefix 'cy'. (Sundh et al., 1997; Bossio et al., 1998.)

2.5 Statistical analyses

All samples were analysed in triplicate. PLFA profiles were analysed with Statistica software (Statsoft, Inc., Tulsa, OK). The arcsine square root transformation was applied to the mole percent PLFA data.

Results of the signature lipid biomarkers analyses were interpreted as described by White and Ringelberg (1998). A three-tiered statistical approach was used where hypothesis testing was first made using analysis of variance (ANOVA) followed by the application of multi-variant analysis such as hierarchical clustering and principal component analysis (PCA) for the determination of sample relatedness. A redundancy analysis (RDA) was subsequently performed with the PLFA group-data as species dependent variables and the most important soil physico-chemical variables as independent environmental factors in explaining variation in PLFA data. The most important soil physico-chemical variables were selected through the forward selection procedure provided in CANOCO, thereby ensuring that only the most important environmental gradients were investigated.

Factor analysis was also used as a data reduction method and in conjunction with hierarchical cluster analyses to assess changes in microbial community structure. An ANOVA was performed on the factor scores and Tukey's Honest Significant Difference test (HSD) was used to identify significant differences between sites, with the within-experiment family-wise error rate was set at $p = 0.05$.

Hierarchical cluster analyses were performed on the means from the transformed mole percent PLFA data using Ward's clustering algorithm. Factor analysis of the estimated biomass and arcsine square root transformed PLFA data were performed using both

iterative and non-iterative extraction techniques. Factor analysis was also used as a data reduction method and in conjunction with hierarchical cluster analyses to assess changes in community structure.

3. RESULTS AND DISCUSSIONS

Estimated viable biomass within each of the samples was calculated on the basis of the amount (pmol g^{-1} dry weight) of C14 – C21 PLFAs recovered in each sample (Balkwill et al., 1988). The estimated biomass for the various samples analysed during this study are shown in Figure 1.

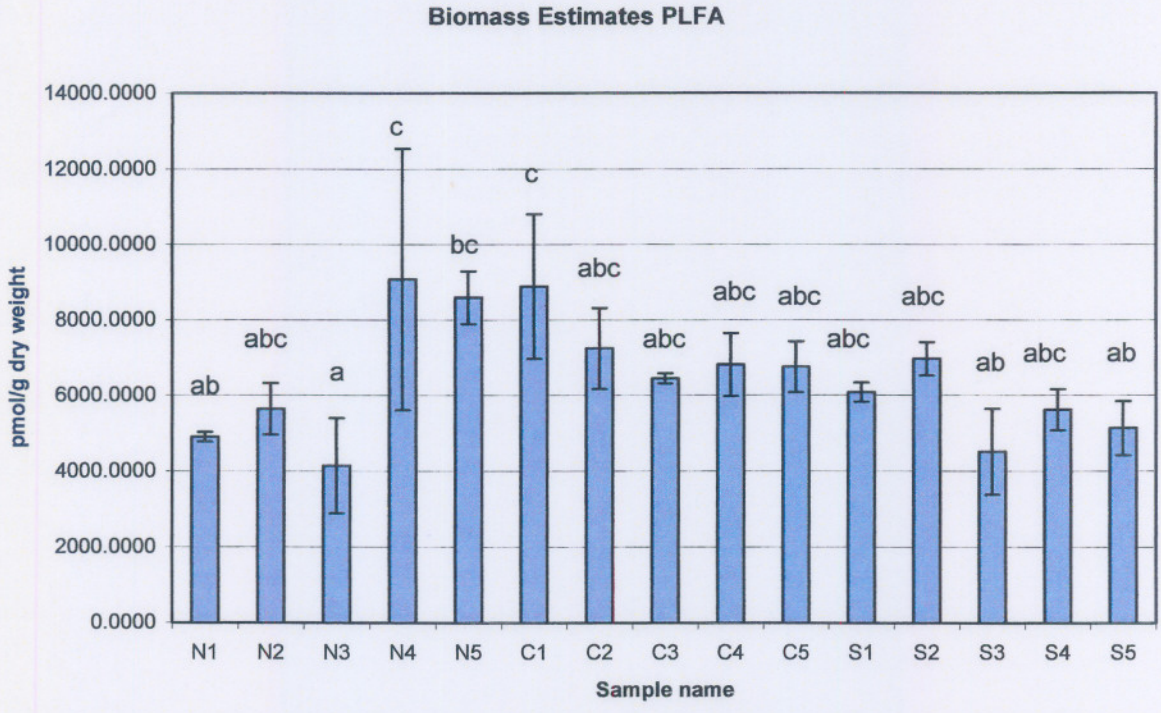


Figure 1. Estimated viable microbial biomass based on PLFA analysis of the various soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat. Samples with the same combination of superscript alphabetical letters indicate no significant differences ($p > 0.05$) among samples for each group. C, conducive; S, suppressive; N, neutral.

Based on the results obtained, it is evident that there is no significant difference ($p > 0.05$) in the estimated biomass of the majority of the soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat (Figure 1).

The estimated number of viable cells was also calculated using the conversion factor of 5.90×10^4 cells / pmol PLFA (Kieft et al., 1994) and 2.50×10^4 cells / pmol PLFA (Summit et al, 2000). The estimated number of cells in the various soil samples as calculated from the PLFAs ranged from 2.44×10^8 to 5.36×10^8 cells / g dry weight (calculated according to Kieft et al., 1994) and 1.04×10^8 to 2.27×10^8 cells / g soil dry weight (calculated according to Summit et al, 2000) (Figure 2). As with any conversion

factor, it is important to realise that the estimated number of cells can vary by up to an order of magnitude (Findlay and Dobbs, 1993).

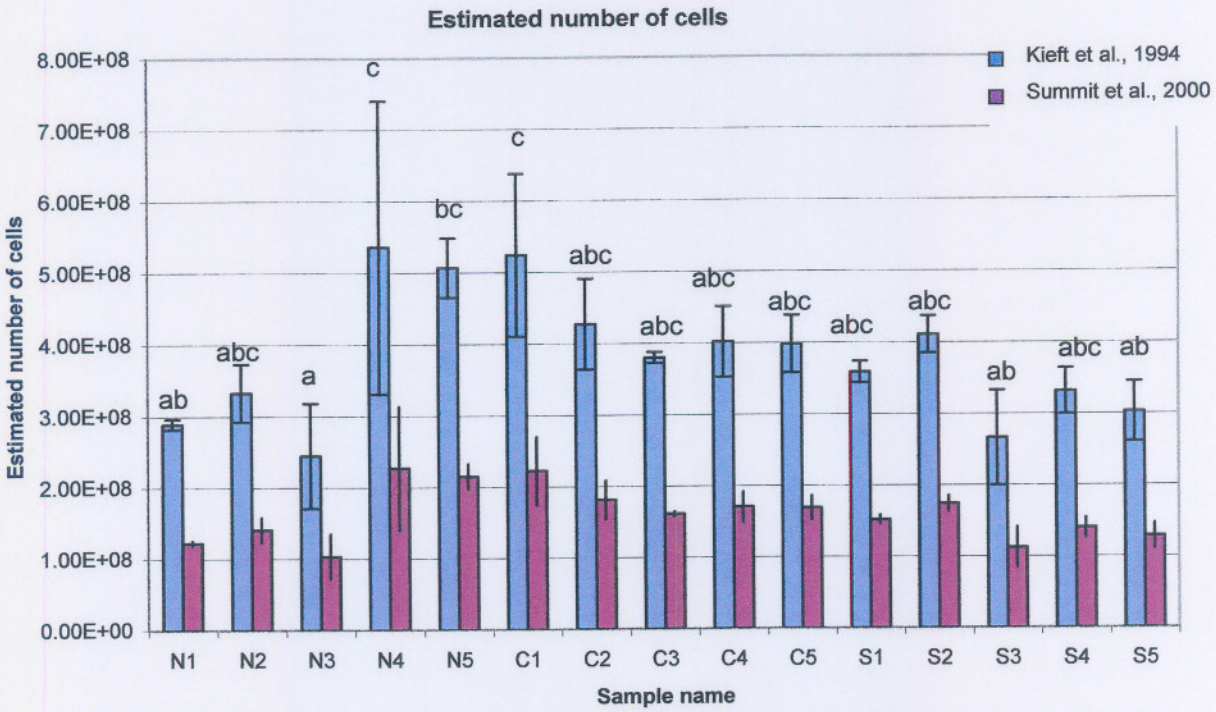


Figure 2. Estimated number of viable cells within the various soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat as calculated using the conversion factors of Kieft et al. (1994) and Summit et al. (2000). Soil samples with the same combination of superscript alphabetical letters indicate no significant difference ($p > 0.05$) among soil samples for each group. C, conducive; S, suppressive; N, neutral.

Obtained results evidently illustrate no significant difference ($p > 0.05$) in the estimated number of viable cells within the majority of the soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat (Figure 2).

Phospholipid fatty acids can be categorised into major groups encompassing normal saturated fatty acids (Nsats), Mid-branched saturated fatty acids (MBsats), Terminally branched saturated fatty acids (TBsats), Branched monounsaturated fatty acids (Bmonos), Monounsaturated fatty acids (Monos), and Polyunsaturated fatty acids (Polys).

The microbial community structure on the basis of the relative mole percentage of the major phospholipid fatty acid groups is presented in Figure 3.

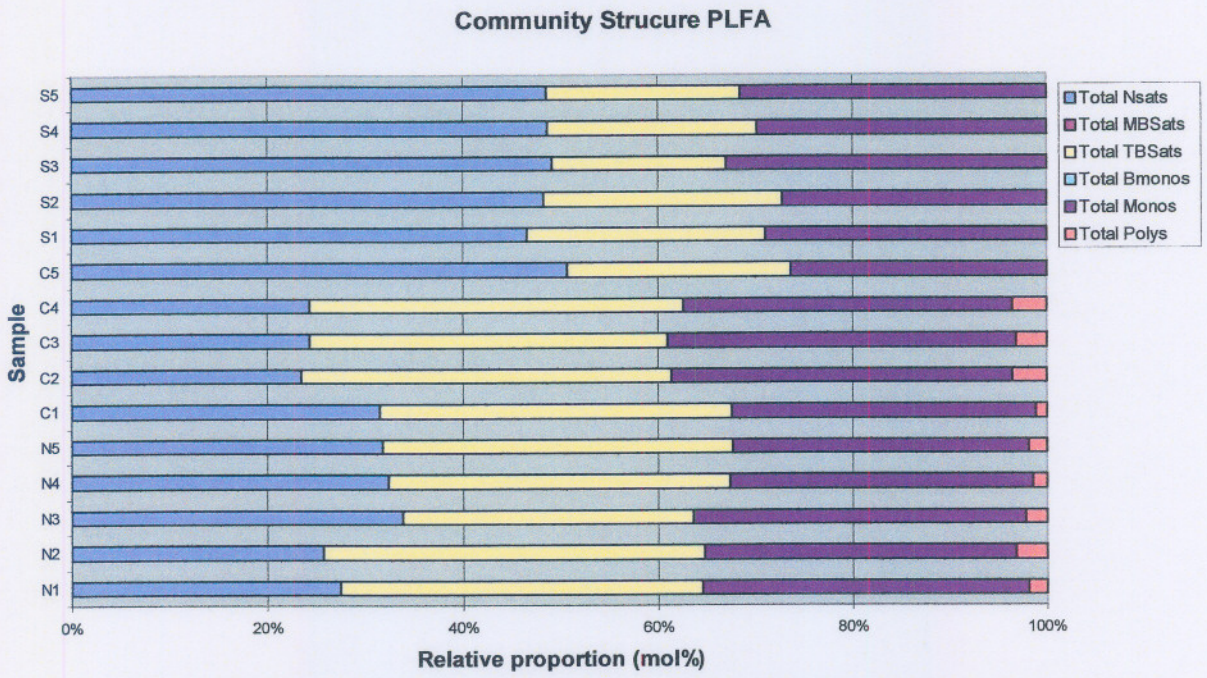


Figure 3. Microbial community structure on the basis of the percentage fraction of the major phospholipid fatty acid groups. Normal saturated fatty acids (Nsats); terminally branched saturated fatty acids (TBsats); monounsaturated fatty acids (Monos); polyunsaturated fatty acids (Polys).

The percentage fraction of the major PLFA groups within each soil sample analysed during this study are summarised in Table 1.

Lack of the majority of the major PLFA groups indicate that the diversity of microbial community in each soil sample was limited. Diversity within a microbial community generally decreases as a result of external disturbances such as plowing, and nutrient deficiencies. On the basis of the results presented in Figure 3 and Table 1, it is evident that a significant shift ($p < 0.05$) had occurred in the structural diversity of the microbial communities in soils suppressive (S) and conducive (C) / neutral (N) to take-all disease of wheat, but no statistical difference ($p > 0.05$) could be observed between soils conducive (C) and neutral (N) to take-all disease.

Normal saturated fatty acids (Nsats) occur in most microorganisms and are therefore considered to be ubiquitous. High levels of Nsats are generally considered to be indicative of low microbial diversity. The percentage fraction of normal saturated fatty acids ranged from 23.518 to 49.162 mole percentage for soils conducive (C) and

Table 1. Mole percentage fractions if the major PLFA groups present in soils conducive (C), suppressive (S), and neutral (N) to take-all disease of wheat.

Sample	Total Nsats	Total TBSats	Total Monos	Total Polys
C1	31.45 ± 2.17 [a]	36.11 ± 1.50 [cd]	31.28 ± 1.49 [ab]	1.16 ± 0.80 [abc]
C2	23.52 ± 2.13 [a]	37.86 ± 3.49 [cd]	35.05 ± 1.281 [ab]	3.57 ± 0.46 [e]
C3	24.36 ± 1.65 [a]	36.57 ± 2.70 [cd]	35.87 ± 3.57 [b]	3.20 ± 0.45 [de]
C4	24.34 ± 1.067 [a]	38.23 ± 2.92 [cd]	33.83 ± 3.00 [ab]	3.60 ± 0.37 [e]
C5	50.71 ± 0.68 [b]	22.87 ± 1.93 [ab]	26.41 ± 2.56 [a]	0 [a]
S1	46.53 ± 2.18 [b]	24.51 ± 0.24 [ab]	28.96 ± 2.39 [ab]	0 [a]
S2	48.36 ± 0.25 [b]	24.43 ± 1.05 [ab]	27.21 ± 0.80 [ab]	0 [a]
S3	49.16 ± 2.47 [b]	17.80 ± 1.28 [a]	33.04 ± 3.75 [ab]	0 [ab]
S4	48.68 ± 3.43 [b]	21.45 ± 1.09 [ab]	29.88 ± 2.59 [ab]	0 [a]
S5	48.57 ± 3.55 [b]	19.87 ± 3.55 [a]	31.56 ± 2.48 [ab]	0 [a]
N1	27.47 ± 1.23 [a]	37.06 ± 0.52 [cd]	33.56 ± 1.39 [ab]	1.91 ± 0.23 [cd]
N2	25.66 ± 1.91 [a]	39.07 ± 4.77 [d]	32.07 ± 2.63 [ab]	3.21 ± 0.31 [de]
N3	33.81 ± 12.03 [a]	29.79 ± 6.33 [bc]	34.15 ± 5.76 [ab]	2.25 ± 0.88 [cd]
N4	32.34 ± 3.25 [a]	35.01 ± 2.98 [cd]	31.24 ± 5.22 [ab]	1.42 ± 0.77 [bc]
N5	31.80 ± 1.98 [a]	35.81 ± 1.82 [cd]	30.42 ± 0.41 [ab]	1.97 ± 0.15 [cd]

‡ Samples with the same combination of superscript alphabetical letters indicate no significant differences ($p > 0.05$) among samples for each group.

suppressive (S) to take-all disease of wheat, respectively. The significant increase ($p < 0.05$) (Table 1) in the percentage fraction of normal saturated fatty acids (Nsats) within soils suppressive (S) to take-all disease of wheat, might be ascribed to possible lower concentrations of root exudates due to less plant tissue damage, resulting in decreased microbial diversity due to healthier plants, because of the specific selection of soil microbial communities antagonistic towards *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in suppressive soils.

Terminally branched saturated fatty acids (TBsats) are generally considered to be indicative of Gram-positive bacteria (Zelles, 1999). The mole percentage of terminally branched saturated fatty acids (TBsats) ranged from 17.803 to 39.070 for soils suppressive (S) and neutral (N) to take-all disease of wheat, respectively. The significant reduction ($p < 0.05$) (Table 1) in the mole percentage fraction of terminally branched saturated fatty acids (TBsats) suggest that the Gram-positive fraction of the soil microbial community within soil suppressive (S) to take-all disease of wheat decreased significantly over time as soil conducive (C) to take-all disease of wheat became suppressive (S) to take-all disease of wheat due to monoculture of wheat. This shift in structural diversity within the suppressive soil corresponded with an increase in the relative percentage of monounsaturated fatty acids, which are generally considered to be indicative of aerobic Gram-negative bacteria (Ratledge and Wilkinson, 1988). The mole percentage of monounsaturated fatty acids (Monos) ranged from 27.209 to 35.867 for soils suppressive (S) and conducive (C) to take-all disease of wheat, respectively. Although the relative mole percentage of monounsaturated fatty acids in soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat did not differ significantly ($p > 0.05$), the increase and decrease in the relative mole percentage of monounsaturated fatty acids in suppressive (S) soil, and conducive- (C) as well as neutral (N) soils, respectively, indicate the increased biocontol activity of the Gram-negative fraction of the microbial communities within the suppressive (S) soil. This might possibly be related to the increased incidence of Pseudomonads.

Polyunsaturated phospholipid fatty acids (Polys) are found almost exclusively in micro-eukaryotes, primarily fungi (White et al., 1996). Although the overall relative mole percentage of polyunsaturated phospholipid fatty acids were very low in soils conducive (C) and neutral (N) to take-all disease of wheat, the relative mole percentage of polyunsaturated phospholipid fatty acids increased significantly ($p < 0.05$) in soils

conducive (C) to take-all disease of wheat. This increase might be attributed to the presence of *Ggt* as well as other fungi in soils conducive (C) to take-all disease of wheat. *Fusarium solani* has been reported to be especially troublesome secondaries in take-all lesions in conducive soil, and tend to pre-empt or overrun the growth of *Ggt* from roots plated on a non-selective medium (Cook, 1981). The suppressed presence of antagonistic fungi such as *Gliocladium* sp. (P.S. van Wyk, personal communication) and *Trichoderma* sp. (Duffy et al., 1997) to *Ggt* in conducive soils should also be considered in the various soils. These results confirm results obtained with the conventional culturing and identification of fungi (Chapter 3). It is of significant importance to note the total absence (below detection level) of polyunsaturated phospholipid fatty acids in the soils suppressive to take-all disease of wheat. These results could possibly be ascribed to the dominance of antagonistic bacteria in these soils.

A PCA biplot illustrating the relationship between the structural diversity of soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat on the basis of the mole percentage fractions of the major phospholipid groups is presented in Figure 4.

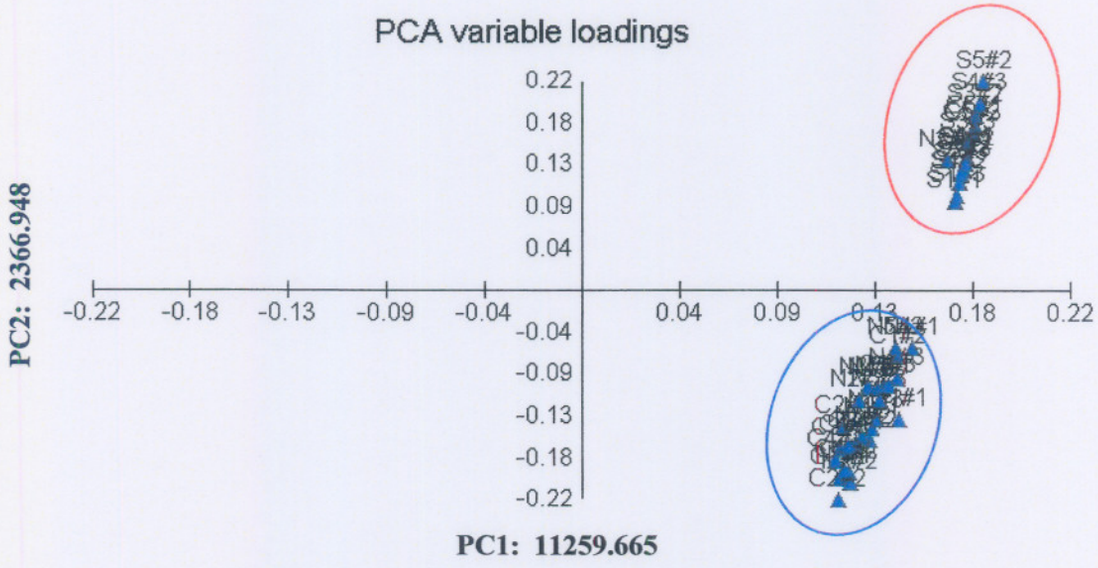


Figure 4. Principal component analysis (PCA) ordination biplot of the mole percentage fractions of the major phospholipid fatty acid groups as present within soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat. The eigenvalues for the first two ordination axes of the PCA are 11259.665 and 2366.948, respectively. These two axes accounted for 98.5% of the total observed variance.

With a single exception, it is clear that soil conducive (C) to take-all disease of wheat, grouped together and could not be differentiated from the soil neutral (N) to take-all disease of wheat due to similar soil microbial structure based on the major PLFA groups (Figure 4).

Since the group membership during this study was known *a priori*, multivariate analysis using discriminant analysis (DA) was also applicable. Discriminant analysis selects those variables that yield the best separation according to the given groups. Hypothesis testing and a more rigorous analysis of catabolic profiles can be achieved using discriminant analysis (Buyer and Drinkwater, 1997).

An ordination plot of the canonical variate scores 1 and 2 as obtained from the discriminant analysis of the percentage fraction of the major PLFA groups within soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat, is shown in Figure 5. This figure illustrates the relationships amongst the structural diversity of microbial communities within the various soils.

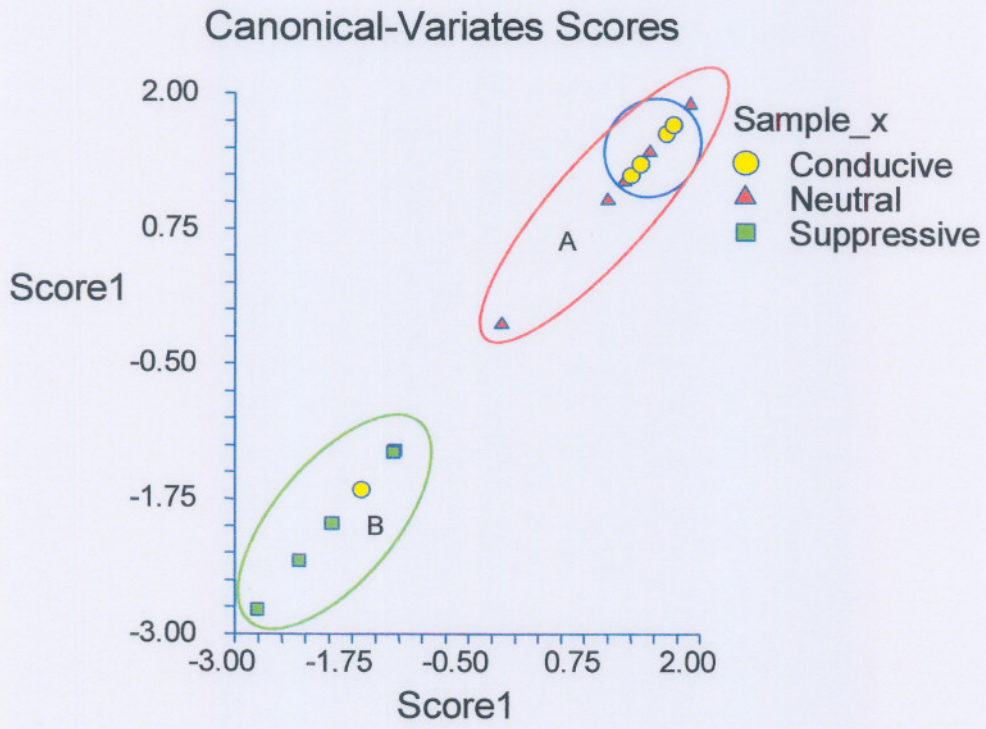


Figure 5. Plot of ordination of canonical variate scores 1 against 2 as generated by discriminant analysis of the mole percentage fraction of the major PLFA groups, showing discrimination between soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat.

The first canonical function (CF1) obtained from the discriminant analysis accounted for 100.0% of the total variability observed in the data set. This CF was significant in discriminating between the soils (Eigenvalue = 2.351; Wilks' Lambda = 0.2984; F = 14.1; p = 0.0007). Since CF1 accounted for 100.0% of the variation, Figure 5 is considered the most representative of the structural diversity relationship between soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat.

With only a single exception, all soils conducive (C) and neutral (N) to take-all disease of wheat grouped together (Group A, Figure 5), thus indicating similarity in the structural diversity of microbial communities present in soils conducive and neutral to take-all disease of wheat. The structural diversity of the microbial communities present in the suppressive soil (Group B, Figure 5) differed significantly ($p < 0.05$) from conducive- and neutral soils. The shift in structural diversity can be clearly seen as conducive (C) soil (Group A, Figure 5) became suppressive (S) to take-all disease of wheat (Group B, Figure 5).

An RDA triplot illustrating the relationship between the dominant physico-chemical characteristics, and mole percentage of the major microbial phospholipid fatty acid groups of the various soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat is presented in Figure 6.

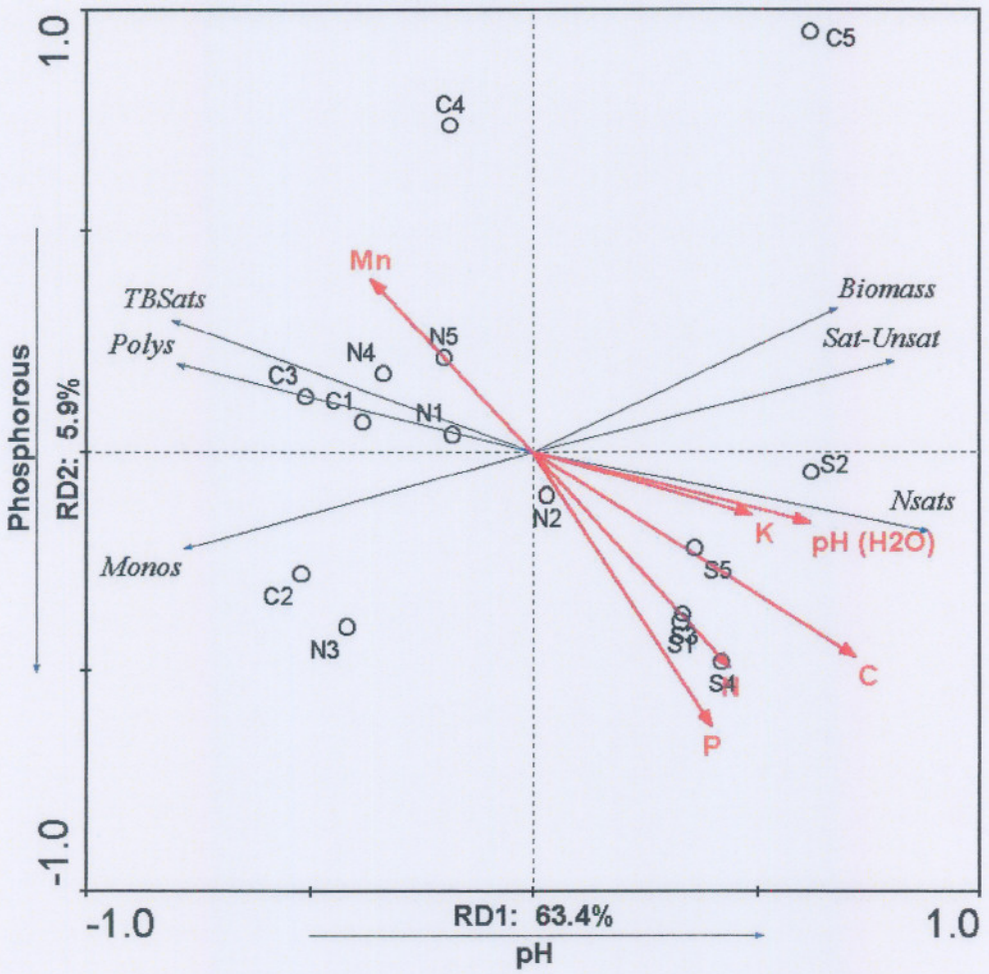


Figure 6. Redundancy Analysis (RDA) triplot illustrating the relationship between the dominant environmental variables, and the mole percentage of the major phospholipid fatty acid groups of soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat. Phospholipid fatty acid groups are represented by blue vectors, and the environmental chemical parameters by red vectors. Eigenvalues for the first three axes are 0.634, 0.059 and 0.041, respectively. Total observed variance of the first two canonical axes is 69.4%. The first canonical axis correlated strongly with pH (r^2 = 0.5739). The second axis correlated with phosphorous (r^2 = -0.392), and the third axis correlated with potassium (r^2 = -0.5126). According to a Monte Carlo Permutation test conducted with 499 permutations, the first canonical axis was statistical significant ($p < 0.002$). The overall effect of the chosen environmental variables on the phospholipid fatty acid groups was also statistical significant ($p < 0.008$).

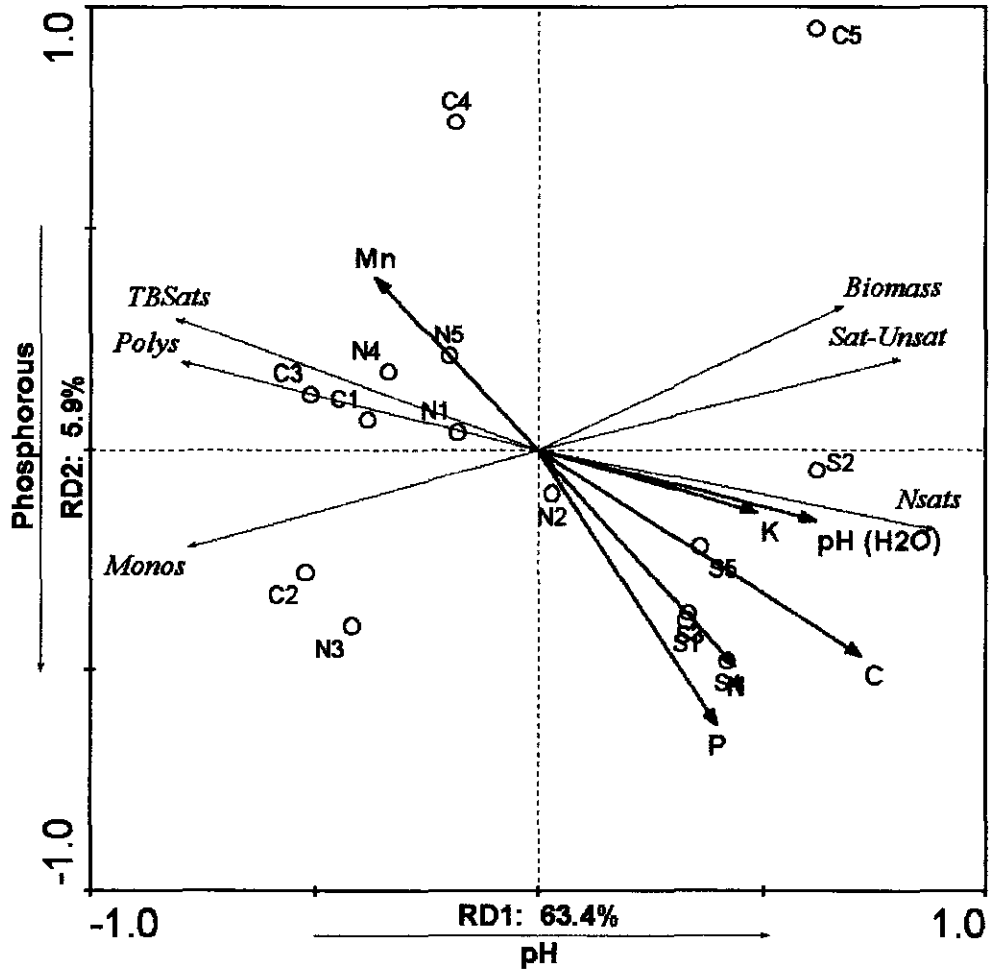


Figure 6. Redundancy Analysis (RDA) triplot illustrating the relationship between the dominant environmental variables, and the mole percentage of the major phospholipid fatty acid groups of soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat. Phospholipid fatty acid groups are represented by blue vectors, and the environmental chemical parameters by red vectors. Eigenvalues for the first three axes are 0.634, 0.059 and 0.041, respectively. Total observed variance of the first two canonical axes is 69.4%. The first canonical axis correlated strongly with pH (r^2 = 0.5739). The second axis correlated with phosphorous (r^2 = -0.392), and the third axis correlated with potassium (r^2 = -0.5126). According to a Monte Carlo Permutation test conducted with 499 permutations, the first canonical axis was statistical significant (p < 0.002). The overall effect of the chosen environmental variables on the phospholipid fatty acid groups was also statistical significant (p < 0.008).

The soil variables pH, phosphorous (P), manganese (Mn), potassium (K), and percentage organic carbon (C) and percentage organic nitrogen (N) were used in the RDA analysis. These chemical variables explained the mole percentage of the major phospholipid fatty acid groups the best during the forward selection procedure with the Monte Carlo Permutation Test using CANOCO. The average values of the environmental variables used in the RDA analysis are summarised in Table 2.

Table 2. Average values of the dominant soil chemical variables used in the RDA from soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat.

	pH	Total Nitrogen (%N)	Total Carbon (%C)	K ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	P ($mg\ kg^{-1}$)
Conducive (C)	7.50 ± 0.07	0.08 ± 0.00	0.72 ± 0.00	87.40 ± 3.50	7.00 ± 1.05	22.74 ± 0.48
Suppressive (S)	7.66 ± 0.08	0.09 ± 0.00	0.81 ± 0.01	96.00 ± 2.21	5.80 ± 0.66	30.19 ± 0.11
Neutral (N)	7.26 ± 0.09	0.07 ± 0.00	0.71 ± 0.00	97.00 ± 1.52	8.20 ± 1.02	29.05 ± 0.05

The dominant chemical characteristics of soils conducive (C), suppressive (S) and neutral (N) to take-all disease of wheat varied significantly between the samples (Table 2).

Soils conducive (C) and neutral (N) to take-all disease of wheat were loosely grouped in the left quadrant of the first ordination axis (Figure 6). These soils were characterised by the highest mole percentage fraction of terminally branched saturated fatty acids (TBsats), polyunsaturated fatty acids (Polys), and monounsaturated fatty acids (Monos) which are generally considered to be indicative of Gram-positive bacteria, micro-eukaryotes, primarily fungi, and Gram-negative bacteria (Ratledge and Wilkinson, 1988), respectively. The conducive (C) and neutral (N) soils were also characterised by the lowest concentration of estimated biomass, and mole percentage fraction normal saturated fatty acids (Nsats) which are considered to be ubiquitous. Based on these results, it could be concluded that soils conducive (C) and neutral (N) to take-all disease of wheat, relative to soils suppressive to take-all disease of wheat, had a high structural diversity due to the decreased presence of normal saturated fatty acids. These soils also had elevated concentrations of Gram-negative bacteria, but the highest concentration of Gram-positive bacteria and micro-eukaryotes (fungi). These soils were also characterised by high concentrations of manganese and the lowest concentrations of phosphorous, potassium, percentage organic carbon, percentage organic nitrogen, and pH (Table 2).

Soils suppressive (S) to take-all disease of wheat were loosely grouped in the right sided quadrants of the first ordination axis (Figure 6). These soils were characterised by high concentrations of phosphorous, potassium, percentage organic carbon, percentage organic nitrogen, pH, as well as the highest elevated levels of estimated microbial biomass, and mole percentage fraction normal saturated fatty acids (Nsats) which are considered to be ubiquitous. The high concentration of normal saturated fatty acids in the suppressive soils are considered indicative of a low structural diversity in these soils. The suppressive soils were also characterised by the lowest concentrations of manganese.

The soils conducive, suppressive, and neutral to take-all disease of wheat could also be grouped by hierarchical clustering analysis of the major phospholipid fatty acid groups (Figure 7). Two major clusters could be identified in the dendogram. With a single exception, all suppressive soil samples clustered together in cluster A, while the conducive and neutral soil samples clustered together in cluster B. Although two sub-clusters were apparent in cluster B, clear differentiation between conducive and neutral soils was not possible. Conducive soils clustered together in sub-cluster B I, while neutral soils clustered together in sub-cluster B II.

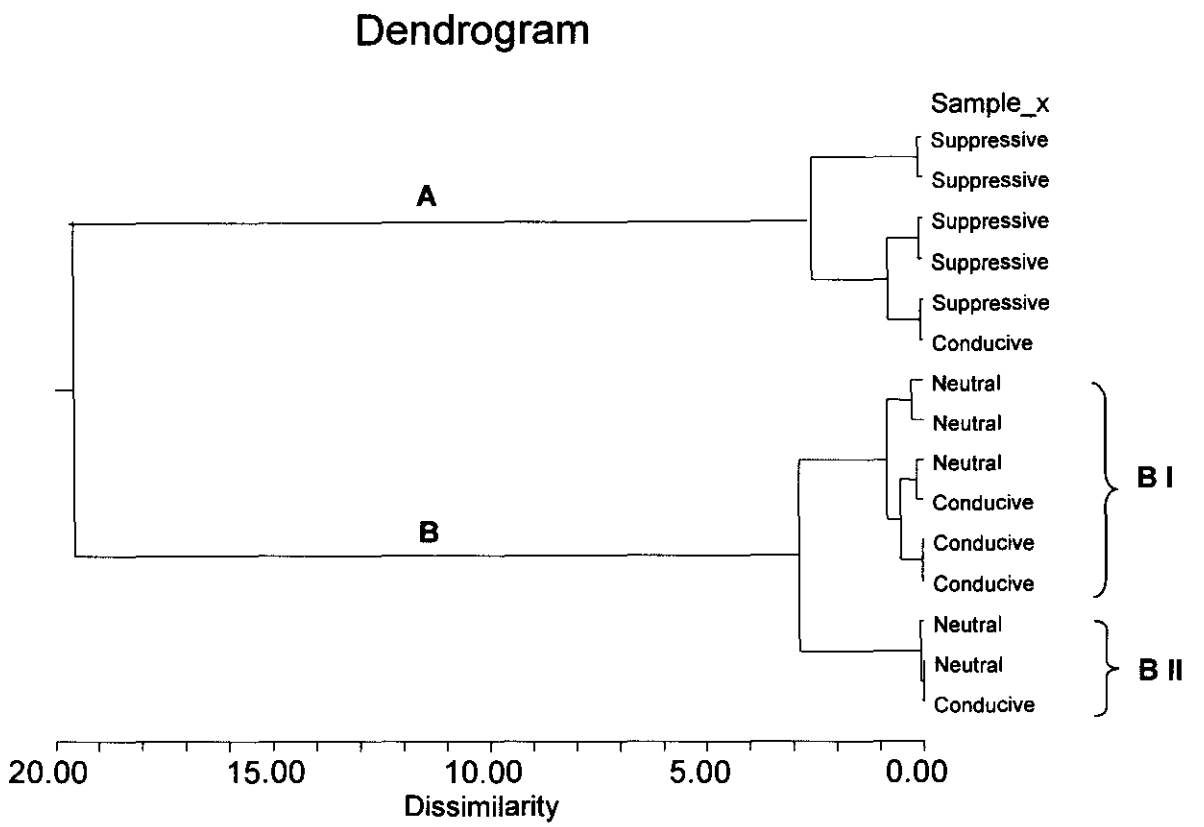


Figure 7. Dendogram illustrating the clustering of soil microbial communities in soils conducive, suppressive, and neutral to take-all disease of wheat based on the mole percentage of the major phospholipid fatty acid groups. Hierarchical cluster analysis was performed using Ward's clustering algorithm.

Results obtained from the hierarchical cluster analysis confirm results obtained during the PCA, DA and RDA analysis of the mole percentage of the major PLFA groups. These results indicate that the structural diversity of soils conducive and suppressive to take-all disease of wheat differ significantly ($p < 0.05$). From the obtained results, it could be hypothesised that soil microbial communities in soils suppressive to take-all disease of wheat (Figure 7; Cluster A) had low microbial diversity with a high level of dominance of specific soil microbial communities. In contrast, soil microbial communities in soils conducive, and neutral to take-all disease of wheat (Figure 7; Cluster B) had high levels of microbial diversity with a low level of dominance of specific soil microbial communities.

4. CONCLUSIONS

During this study, differences in microbial structural diversity were detected with the analysis of phospholipid fatty acid (PLFA) which were found to be adequately sensitive to detect differences in the soil microbial community structure in agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat as caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*). When analysed in relation to environmental variables, including physical and chemical characteristics, significant results could be obtained. Based on the results obtained during this study, it was evident that the structural diversity of the microbial communities associated with soil suppressive to take-all disease of wheat differed significantly from soils conducive and neutral to take-all disease of wheat. Soils conducive and neutral to take-all disease of wheat were characterised by elevated concentrations of terminally branched saturated fatty acids, polyunsaturated fatty acids, and monounsaturated fatty acids which are considered to be indicative of Gram-positive bacteria, micro-eukaryotes (primarily fungi), and Gram-negative bacteria, respectively. Microorganisms have evolved strategies that enable them to successfully survive and maintain themselves within communities and can be classified along an r-K gradient. The r-strategists rely upon high reproductive rates for survival within a community. K-strategists depend upon physiological adaptations to the environmental resources. When resources become limited, r-strategists experience rapid reduction, whereas K-strategists tend to be successful in resource-limited environments. Many Gram-negative microorganisms are considered r strategists. It could thus be concluded that the elevated levels of Gram-negative bacteria in soil conducive to take-all disease of wheat could possible be the response of r-strategists to the presence of excessive nutrients in the form of root exudates. It is, on the other hand, evident that the microbial community within this site would experience a significant reduction in estimated viable biomass once the root exudates composition change due to the natural suppression of take-all disease of wheat, or the death of the plant. These soils were also characterised by the high structural diversity due to the decreased presence of normal saturated fatty acids. Soil suppressive to take-all disease of wheat was characterised by an elevated concentration of biomass and normal saturated fatty acids which are considered to be indicative of most microorganisms. Soils suppressive to take-all disease of wheat, were also characterised by an elevated estimated microbial biomass, as well as elevated concentrations of normal saturated

fatty acids in suppressive soil which are considered to be ubiquitous, and indicative of a low structural diversity. It could thus be concluded that the analysis of phospholipid fatty acids was effective in detecting shifts within soil microbial communities in agricultural soils during different stadia of take-all disease of wheat. This methodology might be of significant value for the evaluation of the effect of management practices on the suppression of the take-all disease of wheat, as well as selection and evaluation of agricultural soils subject to the prevalence of other soilborne diseases.

5. REFERENCES

Balkwill, D.L., Leach, F.R., Wilson, J.T., McNabb, J.F., White, D.C. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. Microbial Ecology 16, 73-84.

Bossio, D.A., Scow, K.M. 1998. Impacts of carbon flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265-278.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid analysis. Microbial Ecology 36, 1-12.

Buyer, J.S., Drinkwater, L.E. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3-11.

Collins, D.J. 1995. Diseases of barley, rye, and triticale in Alabama. [Web:] http://www.aces.edu/department/extcomm/publications/anr/anr-903/anr-903.html [Date of access: May 2001].

Cook, R.J. 1981. The influence of rotation crops on take-all decline phenomenon. Phytopathology 71, 189-192.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology and biochemistry 32, 1419-1430.

Duffy, B.K., Ownley, B.H., Weller, D.M. 1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopathology 87, 1118-1124.

Findlay, R.H., Dobbs, F.C. 1993. Quantitative description of microbial communities using lipid analysis. In: Kemp, P.F., Sherr, B.F. (Eds.), Handbook of methods in aquatic microbial ecology, pp. 271-284.

Hershman, D., Bachi, P. 1994. Take-all of wheat. [Web:] http://www.ca.uky.edu/agcollege/plantpathology/PPAExten/PPFShtml/ppfagsg1.htm [Date of access: March 2000].

Kersters, I., Van Vooren, L., Verschuere, L., Vauterin, L., Wouters, A., Mergaert, J., Swings, J., Verstraete, W. 1997. Utility of the Biolog system for the characterization of heterotrophic microbial communities. Systematic and Applied Microbiology 20, 439-447.

Kieft, T.L., Ringelberg, D.B., White, D.C. 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and environmental Microbiology 60, 3292-3299.

Larkin, R.P., Hopkins, D.L., Martin, F.N. 1993. Effects of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83, 1097-1105.

Marschner, P., Kandeler, E., Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilzer experiment. Soil Biology and Biochemistry 35, 453-461.

Øvreås, L., Torsvik, V. 1998. Microbial diversity and community structure in two different agricultural soil communities. Microbial Ecology 36, 303-315.

Palojärvi, A., Sharma, S., Rangger, A., Von Lutzow, M., Insam, H. 1997. Comparison of Biolog and Phospholipid Fatty acid patterns to detect changes in microbial community. In: Insam, H., Rangger, A. (Eds.), Microbial Communities - Functional versus Structural Approaches. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 37-48.

Ratledge, C., Wilkinson, S.G. 1988. An overview of microbial lipids. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Volume 1. Academic Press, London, pp. 3-22.

Riffaldi, R., Saviozzi, A., Levi-Minzi, R., Cardelli, R. 2002. Biochemical properties of a Mediterranean soil as affected by long-term crop management systems. Soil and tillage research 67, 109-114.

Rothrock, C.S., Cunfer, B.M. 1991. Influence of small grain rotations on take-all in a subsequent wheat crop. Plant Disease 75, 1050-1052.

Sharma, S., Rangger, A., Von Lützow, M., Insam, H. 1998. Functional diversity of soil bacterial communities increases after maize litter amendment. European Journal of Soil Biology 34, 53-60.

Summit, M., Peacock, A., Ringelberg, D., White, D.C., Baross, J.A. 2000. Phospholipid fatty acid-derived microbial biomass and community dynamics in hot, hydrothermally influenced sediments from Middle Valley, Juan De Fuca Ridge. In: Zierenberg, R.A., Fouquet, Y., Miller, D.J., Normark, W.R. (Eds.), Proceedings of the Queen Drilling Program, Scientific Results 169, 1-19.

Sundh, I., Nilsson, M., Borga, P. 1997. Variation in microbial community structure in two Boreal peatlands as determined by analysis of phospholipid fatty acid profiles. Applied and Environmental Microbiology 63, 1476-1482.

Vestal, J.R., White, D.C. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

White, D.C., Ringelberg, D.B. 1998. Signature Lipid Biomarker Analysis. In: Burlage, R.S., Altas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology, University Press, New York, pp. 255-271.

1. BACKGROUND

Take-all is a disease caused by a soil-borne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*) responsible for the cause of crown and root rot in wheat, barley, rye and triticale (Rothrock and Cunfer, 1991). Take-all disease of wheat is most common in regions where wheat is grown without adequate rotation (Hershman and Bachi, 1994), and when plants undergo nitrogen stress (Collins, 1995). Infections of roots result in losses in crop yield and quality because of a lowering in nutrient uptake (Monsanto Company, 1998). Productivity and health of agricultural systems are determined by key roles in functional processes carried out by soil microorganisms and soil microbial communities (Pankhurst et al., 1996). Microorganisms also exhibit direct and indirect beneficial and detrimental interactions with plants (Atlas and Bartha, 1998) since a close relationship exists between plant and microorganism. Reduced capability of plants to survive and maintain their ecological niche, is the result of malfunctioning caused by microbial diseases of plants, resulting in death or a low growth yield of the plant (Cloete, 1999). Several means to control take-all infection have been suggested. Concerns about environmental effects have shifted the focus more towards ecologically sustainable systems that included the use of reduced tillage, inputs of organic materials and nutrient cycling strategies based on crop rotation (Pankhurst et al., 1996). During crop rotation, time is allowed between susceptible crops for the lowering of inoculum potential of soilborne plant pathogens below some economic threshold by resident antagonists (Cook, 1994). Biological control can be achieved with cultural practices enhancing introduced and resident antagonists, but success, in many cases, would involve a combination of introduced and resident antagonists. Soil suppressiveness to plant disease occurs naturally as an inherent characteristic of physico-chemical, and/or biological structure of a particular soil, or it might be induced by some practices and activities such as planting of crops, or the

addition of organisms or nutritional amendments, which cause a change in the microfloral environment (Larkin et al., 1993).

A large obstacle to the evaluation and comparison of soil microbial communities has traditionally been the lack of effective methods to deal with community-level characteristics (Cavigelli et al., 1995). Since only a small percentage of all soil microorganisms are culturable (White et al., 1996), traditional culture-based assays of microbial populations provide very limited information on these complex soil communities. Recently, several useful community level characterisation techniques have been developed that do not rely on culture-based assays, overcoming the limitations of conventional microbiological techniques and substantially contributing to the *in situ* characterisation of soil microbial communities. Microbial ecologists have developed several techniques attempting to reveal more closely the functional role the community plays in nature (Vestal and White, 1989). One approach to translate the information in a microbial ecosystem would be to determine the metabolic diversity (functional diversity) by analyses of community level physiological profiles (CLPPs) based on tetrazolium violet dye reduction as an indicator of sole carbon source utilisation (Garland and Mills, 1991). Commercially available BIOLOG® microtiter plates allow for simultaneous testing of 95 separate carbon sources, and the direct incubation of whole environmental samples (Garland, 1996). The obtained data are especially pliable to multivariate statistical analyses to look at taxonomic diversity in macroorganisms (Zak et al., 1994). The structural diversity of microbial ecosystems can be characterised by the analysis of phospholipid fatty acids as an example of signature biomarker analysis. Phospholipid fatty acid (PLFA) profiles are unique "signature" chemicals that are restricted to specific subsets of a microbial community or bacterial group (Zelles et al., 1992) to act as a fingerprint of the structural diversity of a microbial community (Petersen and Klug, 1994). Measurements of fatty acids in soils have been used to estimate microbial biomass, and to examine community structure (Haack et al., 1994). Although no single approach provides a complete representation of soil microbial characteristics, a slightly different perspective is provided by each of these approaches, with a more complete presentation of soil microbial characteristics achieved with the use of multiple approaches.

2. GENERAL DISCUSSION

During this study, the relationship between functional and structural diversity of soil microbial communities, and physico-chemical characteristics of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*) were evaluated. Conducive soils were characterised by high nitrate-concentrations, which would result in higher soil pH, and increased take-all severity. The conducive experimental plots were also characterised by low levels of phosphorous and manganese, which might have resulted in unhealthy plants with low resistance to take-all infection. Duffy et al. (1997) reported that the oxidation of manganese by *Ggt* might decrease the availability of the micronutrient to wheat, which resulted in increased take-all severity. In contrast, experimental plots suppressive to take-all disease were characterised by high ammonium-concentrations. This could contribute to the lowering of the soil pH, which would result in decreased take-all severity, increased micronutrient availability, and consequently increased antagonistic rhizosphere microbial communities.

It was subsequently hypothesised that the physico-chemical characteristics of the various soils would influence the composition and quantity of the root exudates, which might also explain the difference in the CLPPs of the associated soil microbial communities, as well as the infection of roots by *Ggt* with the subsequent response of antagonistic microbial communities. It is consequently evident that the structural diversity and functional diversity of the soil microbial communities are significantly influenced by the presence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, and that the characterisation of microbial structural diversity and functional diversity by analysis of phospholipid fatty acids and community level physiological profiles (CLPPs), respectively, could successfully be applied as an assessment criterium.

Analysis of community level physiological profiles (CLPPs) of soil microbial communities in agricultural soils conducive, suppressive, and neutral with respect to take-all disease enabled differentiation of the various samples. Profiles of utilisation capacity of carbon substrates were statistically analysed using principal component analysis (PCA), detrended correspondence analysis (DCA) and discriminant analysis (DA). Although no

clear distinction could be made between soils suppressive and neutral to take-all of wheat, it is however, evident that with two exceptions, the functional diversity of the microbial communities present in the soils conducive, and suppressive to take-all disease differed significantly from each other ($p < 0.05$) (Chapter 3; Figure 2c), thus implying different substrate utilisation capabilities. The exceptions could possibly be ascribed to natural variation in biological systems. The difference in functional diversity between the various samples, but especially the large difference between the soils conducive and neutral / suppressive to take-all disease of wheat, could possibly be ascribed to the development of lesions and subsequent excessive leakage of root exudates due to take-all infection and subsequent secondary infections, e.g., *Fusarium*, in soils conducive to take-all disease of wheat. Probable increased leakage of root exudates caused by *Ggt* and other secondary soilborne pathogens, might attract increasing populations of antagonistic rhizosphere microorganisms such as fluorescent *Pseudomonas* spp., that are especially well adapted to utilise root exudates very rapidly (Duffy and Weller, 1995), giving rise to soils suppressive to take-all disease of wheat.

Soils suppressive and neutral to take-all disease were characterised by enhanced utilisation of carboxylic acids, amino acids, and carbohydrates, while conducive soils were characterised by enhanced utilisation of carbohydrates. Shifts in the functional diversity of the associated microbial communities were possibly caused by the presence of *Ggt* and associated antagonistic fungal and bacterial populations in the various soils. During take-all infection, invading hyphae grow through the root cortex of wheat to invade and destroy the root phloem, whereafter the xylem is invaded. Initial black lesions on roots of infected plants expand and eventually coalesce, extending existing lesions and / or producing new secondary infections (Sarniguet et al., 1992). It is hypothesised that the development of lesions and the subsequent excessive leakage of root exudates due to take-all infection and possible secondary infections, might attract increasing populations of antagonistic rhizosphere microorganisms that are especially well adapted to utilise these exudates very rapidly (Duffy et al., 1997), a response typical of r-strategists, including Pseudomonads. Although the microbial communities could utilise a wide range of substrates, the communities could only be differentiated by the utilisation of a few substrates, demonstrating the development of different microbial communities with different functionalities in soil conducive, suppressive, or neutral to take-all disease of wheat. When CLPPs were statistically analysed in relation to the

dominant environmental variables (including physico-chemical characteristics, significant results could be obtained.

Distinct differences in the functional diversity of soil microbial community characteristics were also detected in the experimental plots conducive to take-all, but which had been subjected to crop rotation with sunflower and soybean as a management strategy. PCA and discriminant analysis of the CLPPs of the experimental plots conducive to take-all where crop rotation had been applied, enabled differentiation of these plots from the experimental plots conducive, suppressive, and neutral to take-all disease. The CLPPs of the microbial communities associated with the crop rotation were more similar to those of suppressive soil, than to that of the conducive soil, indicating that the application of crop rotation was a suitable management strategy for the suppression of take-all disease of wheat (Chapter 3; Figure 6,7). It was also evident that the crop used during crop rotation (i.e. sunflower vs. soybean) resulted in a significant difference ($p < 0.05$) in the CLPP of the associated microbial communities (Chapter 3; Figure 8). This could possibly be ascribed to the differences in the composition of the root exudates of sunflower and soybeans. Since the composition of plant root exudates differ between plant types (Garland, 1996), leakage of root exudates might attract increasing populations of different rhizosphere microorganisms that are especially well adapted to utilise the specific root exudates very rapidly (Duffy and Weller, 1995). The statistically significant difference in community level physiological profiles of soil microbial communities present in the experimental plot which had been subjected to crop rotation, also implied different substrate utilisation capabilities of the soil microbial communities present (Grayston et al., 1998). The CLPPs of the soil microbial community associated with the cultivation of sunflower during crop rotation, was characterised by the enhanced utilisation of carboxylic acids, amino acids, and esters, alcohols, amides, amines, brominated chemicals, aromatic chemicals, phosphorylated chemicals, and polymers. In contrast, the CLPP of the soil microbial community associated with the cultivation of soybean during crop rotation was characterised by the enhanced utilisation of mainly carbohydrates.

Shannon-Weaver substrate diversity (H'), equitability (J) and Sorenson's indices were also calculated for the soil microbial communities in soils conducive, suppressive, and neutral to take-all disease of wheat, as well as the adjacent experimental plot with soil conducive to take-all disease where crop rotation was practised. A high Shannon-Weaver substrate diversity (H') index existed in all the various soils conducive,

suppressive, and neutral to take-all disease of wheat. According to Magurran (1988), values of the Shannon index fluctuate between 1,5 and 3,5 and rarely increase above 4,5. Values obtained during this study were overall within the higher diversity range (>3.5), thus indicating the achievement of very high substrate diversity values. The high substrate diversity values obtained were established by the fact that a substantial percentage of the carbon sources were utilised, contributing to the very high Shannon-Weaver substrate diversity indices.

Substrate evenness (equitability) (*J*) indices ranged between 0.83 and 0.90 for all the various soils and did not show significant differences or trends. According to Magurran (1988), substrate evenness (equitability) assumes a value between 0 and 1 with 1 being complete evenness, indicating a high diversity. It could therefore be concluded that a high functional diversity existed in all the various soils.

Whilst the Shannon-Weaver substrate diversity index emphasises the amount of carbon sources utilised, the Sorenson's index emphasises the degree of substrate utilisation. A dendogram constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA), illustrates the similarity in carbon source utilisation of the samples from the various soils (Chapter 3; Figure 4). The clusters obtained could be distinguished based on their Sorenson's indices. Since the similarity of pairs of sites are studied by taking species abundance into account (Magurran, 1988), a clear distinction could be made between the degree of substrate utilisation between soils conducive to take-all disease of wheat, and soils suppressive or neutral to take-all disease of wheat possibly due to the presence of antagonistic soil microbial communities towards the take-all fungus, *Ggt*.Hierarchical cluster analysis of the CLPPs obtained, enabled differentiation of the microbial communities associated with the different stadia of take-all disease, and the Sorenson's index values. A dendogram constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA), illustrates the similarity in carbon source utilisation of the samples from the previous season's sunflower- (W) and soybean- (C) sections which have been rotated with wheat (Chapter 3; Figure 11). A clear distinction could be made between the soils.

No significant difference ($p > 0.05$) could be observed between the various soils based on conventional microbial enumeration techniques using various selective media, possibly due to the restrictive nature of the selective media used, which selected against the unculturable fraction of the microbial community. It is known that less than

1% of soil microbes are culturable. *Fusarium solani* and *F. oxysporum* were found in approximately the same numbers in soils suppressive to take-all disease of wheat, and in conducive soils where crop rotation was practised. *Gliocladium* sp. was mainly isolated from soils suppressive to take-all disease of wheat. *Gliocladium* sp. are known to produce gliotoxins (broad-spectrum antibiotic) as well as different classes of fungal cell-wall-hydrolytic enzymes that play an important role in mycoparasitism (Vey et al., 2001). A similar increase in the *Gliocladium* population in adjacent experimental plots incidentally also coincided with suppressiveness to the black pod rot pathogen, *Chalara elegans* Nag Raj and W.B. Kendrick (Van Wyk, P.S., unpublished).

Structural diversity of soil microbial communities has previously been advantageously characterised by phospholipid fatty acid (PLFA) analyses. During this study, the relationship between the structural diversity of soil microbial communities, and physico-chemical characteristics of agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat were evaluated using principal component analysis (PCA), redundancy analysis (RDA) and discriminant analysis (DA). Soil suppressive to take-all disease of wheat differed significantly ($p < 0.05$) from soils conducive, and neutral to take-all disease of wheat, implying a shift in relationships amongst the structural diversity of microbial communities within the various soils. A positive association was observed between the microbial phospholipid fatty acid profiles, and dominant environmental variables of soils conducive, suppressive, and neutral to take-all disease of wheat. Soils conducive and neutral to take-all disease of wheat were characterised by high concentrations of manganese, as well as elevated concentrations of monounsaturated fatty acids, terminally branched saturated fatty acids, and polyunsaturated fatty acids, which are indicative of Gram-negative bacteria, Gram-positive bacteria and micro-eukaryotes (primarily fungi), respectively. These soils were also characterised by low concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as low soil pH. Soil suppressive to take-all disease of wheat was characterised by the elevated levels of estimated biomass and normal saturated fatty acids, indicative of low diversity. This soil was also characterised by high concentrations of phosphorous, potassium, percentage organic carbon, and percentage organic nitrogen, as well as elevated soil pH.

Hierarchical cluster analysis of the major phospholipid fatty acid groups indicate that the structural diversity differed significantly between soils conducive, suppressive, and neutral to take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*

(Chapter 4; Figure 7). Two major clusters could be identified. Suppressive soils grouped together in a cluster, while conducive and neutral soils grouped together in a different cluster.

3. GENERAL CONCLUSION

Analysis of the functional and structural diversity of soil microbial communities in agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat as caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*), by analyses of CLPPs and PLFAs, enabled differentiation of the various samples. During take-all infection, invading hyphae initially invade and destroy the root phloem, where after the xylem is invaded. Infected plants' roots have initial black lesions that expand and eventually coalesce, extending existing lesions and / or producing new secondary infections (Sarniguet et al., 1992). It is hypothesised that the development of lesions and the subsequent excessive leakage of root exudates due to take-all infection and possible secondary infections, might attract increasing populations of antagonistic rhizosphere microorganisms that are especially well adapted to utilise these exudates very rapidly (Duffy et al., 1997), a response typical of r-strategists, including Pseudomonads. The development of different microbial communities with different functionalities in soil conducive, suppressive, or neutral to take-all disease of wheat, was demonstrated by the utilisation of a wide range of substrates by soil microbial communities. When CLPP were statistically analysed in relation to the dominant environmental variables (including physico-chemical characteristics), significant results could be obtained.

Distinct differences in the functional diversity of soil microbial community characteristics were also detected in the experimental plots conducive to take-all, but which had been subjected to crop rotation with sunflower and soybean as a management strategy. The determination of CLPPs of the microbial communities associated with the crop rotation were more similar to those of suppressive soil, than to that of the conducive soil, indicating that the application of crop rotation was a suitable management strategy for the suppression of take-all disease of wheat. It was also evident that the crop used during crop rotation (i.e. sunflower vs. soybean) resulted in a significant difference ($p < 0.05$) in the CLPP of the associated microbial communities. This may possibly be

ascribed to differences in the composition of the root exudates, which would select for a specific microbial community.

High Shannon-Weaver substrate diversity (*H'*) and substrate equitability (J) values were obtained for the soil microbial communities in soils conducive, suppressive, and neutral to take-all disease of wheat, as well as the adjacent experimental plot with soil conducive to take-all disease where crop rotation was practised. Hierarchical cluster analysis of the CLPPs obtained, enabled differentiation of the microbial communities associated with the different stadia of take-all disease, and the Sorenson's index values. A clear distinction could be made between the microbial communities in the various soils.

Differences in microbial structural diversity were also detected with the analysis of phospholipid fatty acid (PLFA) which were found to be adequately sensitive to detect differences in the soil microbial community structure in agricultural soils conducive, suppressive, and neutral with respect to take-all disease of wheat as caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*). Based on the results obtained during this study, it was evident that the structural diversity of the microbial communities associated with soil suppressive to take-all disease of wheat differed significantly from soils conducive and neutral to take-all disease of wheat. Based on these results, it could be concluded that conducive and neutral soils, relative to soils suppressive to take-all disease of wheat, had a high structural diversity due to the decreased presence of normal saturated fatty acids. These soils also had elevated concentrations of Gram-negative bacteria, but the highest concentration of Gram-positive bacteria and micro-eukaryotes. Microorganisms have evolved strategies that enable them to successfully survive and maintain themselves within communities and can be classified along an r-K gradient. The r-strategists (zymogenous) are opportunistic soil microorganisms with high levels of activity and rapid growth on easily utilisable substrates (i.e., root exudates). These microorganisms rely upon high reproductive rates for survival within a community, while K-strategists depend upon physiological adaptations to the environmental resources. When resources become limited, r-strategists experience rapid reduction, whereas K-strategists tend to be successful in resource-limited environments. Many Gram-negative microorganisms are considered r-strategists. It could thus be concluded that the elevated levels of Gram-negative bacteria in soil conducive to take-all disease of wheat could possibly be the response of opportunistic r-strategists with high levels of activity, due to the presence of excessive easily utilisable

substrates in the form of root exudates. It is, on the other hand, evident that the microbial community within this site would experience a significant reduction in estimated viable biomass once the root exudates composition change due to the natural suppression of take-all disease of wheat, or the death of the plant.

From the obtained results, it could be hypothesised that soil microbial communities in soils suppressive to take-all disease of wheat (Chapter 4; Figure 7; Cluster A) consisted of low microbial diversity with a high level of dominance of specific soil microbial communities. This might be the result of the presence of specific root exudates excreted by plant roots determining the diversity level due to the specific selection of soil microbial communities. Therefore, soil microbial communities in soils conducive, and neutral to take-all disease of wheat (Chapter 4; Figure 7; Cluster B) consisted of high microbial diversity with a low level of dominance of specific soil microbial communities.

It is consequently evident that the structural diversity and functional diversity of the soil microbial communities are significantly influenced by the presence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, and that the characterisation of microbial structural diversity and functional diversity by analysis of phospholipid fatty acids and community level physiological profiles (CLPPs), respectively, could successfully be applied as an assessment criterium. These results confirm the report of Schneider et al. (1998) who found phenotypic fingerprinting to be a more sensitive technique than conventional microbiological techniques to detect changes in soil microbial communities. It can thus be concluded that the analysis of the structural- and functional diversity of the microbial communities using phospholipid fatty acid- and community level physiological profiles (CLPP), respectively, might be of significant value for the evaluation of the effect of management practices on the suppression of the take-all disease of wheat, as well as selection and evaluation of agricultural soils subject to the prevalence of other soilborne diseases.

4. RECOMMENDATIONS AND FUTURE RESEARCH

The observed difference in the structural diversity suggests that the analysis of PLFAs was considerably more sensitive than the technique used to characterise functional diversity (community level physiological profiles). The Biolog® method is making data interpretation problematic, since it is still suffering from the same problems encountered

with culture plating methods, and the danger also exists that direct measurements of soil microbial community functional diversity might provide information more relevant to the functioning of soils than species diversity (Zak et al., 1994).

According to Smalla et al. (1998), the following should be considered concerning the community level physiological profiles within Biolog® microtiter plates: 1) Carbon source utilisation in the Biolog® system represents the amount of species present, since each species contributes in terms of its carbon source utilisation; 2) dominance would be indicated by the same number of carbon sources utilised upon dilution of the sample; 3) if the inoculum density and incubation time were sufficient, the community level physiological profile would be stable, while the number of organisms would change (Winding and Hendriksen, 1997); 4) the same number of carbon sources utilised upon dilution of the sample, if organisms were diluted at the same ratio, would indicate equitability. It is important to remember that inoculum density and incubation time should be sufficient; 5) an unequal distribution of species present in a sample would be indicated by a linear decline of carbon sources utilised, since some of the species would be diluted out – disappearing their contribution to carbon source utilisation.

Future work with ecologically meaningful substrates, might make the method more appropriate for use with soil microbial communities. Despite the fact that the determination of CLPPs with the use of Biolog® microtiter plates is not ideal for studies of the composition of natural microbial communities when used alone, it still provides useful means of understanding the growth habitat, development, and potential function of microorganisms from soil habitats. The importance of studying the functional diversity of soil microbial communities in combination with structural diversity is therefore evident. It is thus recommended that the determination of community level physiological profiles be used in combination with other culture-independent approaches to reveal more complete information regarding the composition of soil microbial communities (Haack et al., 1995; Hill et al., 2000).

Current knowledge regarding genetic diversity is deficient, and even less is known regarding the manner in which genetic and taxonomic diversities affect functional diversity and ecosystem properties (Øvreås and Torsvik, 1998). Understanding is restricted because of methodological limitations and the lack of basic taxonomic information of soil microbial communities (Øvreås and Torsvik, 1998). Comparing phenotypic and DNA-based analyses of bacterial diversity, e.g. denaturing gradient gel electrophoresis (DGGE) analysis, may contribute to a better understanding of microbial

populations in soil (Garland, 1996). These techniques have also been frequently applied in numerous other studies for the assessment of microbial diversity.

An in-depth study of the compounds present in the root exudates of sunflower and soybeans might reveal the nature of the observations made in this study. It is thus recommended that the spatial and temporal variation in the functional and structural diversity be characterised, especially post-planting, and pre-cultivation when wheat plants have matured and / or while the soybeans and sunflower are still on the experimental plots.

Since the conservation of the microbial diversity in agricultural soils is essential to preserve the soil fertility, all the various factors affecting the diversity and the conservation thereof is also an important field of study. With the determination of the functional and structural diversity of microbial communities, the capacity to quickly characterise potential pathogens, access suppressive strategies, and evaluate subsurface communities and their recovery during and after perturbation, will only enhance agricultural technologies.

5. REFERENCES

Atlas, R.M., Bartha, R. 1998. Microbial ecology. 4th Ed. Benjamin/Cummings Science Publishing, Menlo Park, California, 694pp.

Cavigelli, M.A., Robertson, G.P., Klug, M.J. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99-113.

Cloete, T.E. 1999. Microbial Ecology. In: Principles in Microbiology '99. [CD-ROM.]

Collins, D.J. 1995. Diseases of barley, rye, and triticale in Alabama. [Web:] http://www.aces.edu/department/extcomm/publications/anr/anr-903/anr-903.html [Date of access: May 2001].

Cook, R.J. 1994. Problems and progress in the biological control of wheat take-all. Plant Pathology 43, 429-437.

Duffy, B.K., Weller, D.M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Disease 79, 907-911.

Duffy, B.K., Ownley, B.H., Weller, D.M. 1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopathology 87, 1118-1124.

Garland, J.L., Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351-2359.

Garland, J.L. 1996. Patterns of potential C source utilization by rhizosphere communities. Soil Biology and Biochemistry 28, 223-230.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Haack, S.K., Garchow, H., Klug, M.J., Forney, L.J. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Applied and Environmental Microbiology 61, 1458-1468.

Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.J. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60, 2483-2493.

Hershman, D., Bachi, P. 1994. Take-all of wheat. [Web:] http://www.ca.uky.edu/agcollege/plantpathology/PPAExten/PPFShtml/ppfagsg1.htm [Date of access: March 2000].

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15, 25-36.

Larkin, R.P., Hopkins, D.L., Martin, F.N. 1993. Effects of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83, 1097-1105.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey, pp. 179.

Monsanto Company, 1998. A guide to take-all – a disease in cereal grains. [Web:] http://www.takeall.com/english/default.htm [Date of access: July 2002].

Øvreås, L., Torsvik, V. 1998. Microbial diversity and community structure in two different agricultural soil communities. Microbial Ecology 36, 303-315.

Pankhurst, C.E., Ophel-Keller, K., Doube, B.M., Gupta, V.V.S.R. 1996. Biodiversity of soil microbial communities in agricultural systems. Biodiversity and Conservation 5, 197-209.

Petersen, S.O., Klug, M.J. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Applied and Environmental Microbiology 60, 2421-2430.

Rothrock, C.S., Cunfer, B.M. 1991. Influence of small grain rotations on take-all in a subsequent wheat crop. Plant Disease 75, 1050-1052.

Sarniguet, A., Lucas, P., Lucas, M., Samson, R. 1992. Soil conduciveness to take-all of wheat: Influence of the nitrogen fertilisers on the structure of populations of fluorescent pseudomonads. Plant and Soil 145, 29-36.

Schneider, C., Liss, S., Allen, G. 1998. Phenotypic fingerprinting as a management tool for wastewater treatment systems. [Web:] http://www.chem-eng.utoronto.ca [Date of access: August 2003].

Smalla, K., Wachtendorf, U., Heuer, H., Liu, W-T., Forney, L. 1998. Analysis of BIOLOG® GN substrate utilization patterns by microbial communities. Applied and Environmental Microbiology 64, 1220-1225.

Vestal, J.R., White, D.C. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

Vey, A., Hoagland, R.E., Butt, T.M. 2001. Toxic metabolites of fungal biocontrol agents. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Fungi as biocontrol agents. CAB International, pp. 311-346.

White, D.C., Ringelberg, D.B., Macnaughton, S.J. 1996. Review of PHA and signature lipid biomarker analysis for quantitative assessment of *in situ* environmental

microbial ecology. 1996 International Symposium on Bacterial Polyhydroxyalkanoates 161-170.

Winding, A, Hendriksen, N.B. 1997. BIOLOG® substrate utilisation assay for metabolic fingerprints of soil bacteria: incubation effects. In: Insam, I., Rangger, A. (Eds.), Microbial Communities: Functional versus Structural Approaches. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 195-205.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology and Biochemistry 26, 1101-1108.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology and Biochemistry 24, 317-323.