CRANFIELD UNIVERSITY

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SCHOOL OF APPLIED SCIENCES

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ABSTRACT

The effects of xenobiotics application on the composition and function of soil microbial community were investigated in mountain grassland (Slovakia) and agricultural (UK) soils. Slovak soil was Cambisol, sampled from the mountain grassland regions of Greater Fatra, Lesser Fatra, Lower Tatras and Slovak Ore Moutains. UK soil was sampled from the Cottenham, Faulkborne and Denchworth series located in an agriculture farm at Silsoe. Soils thereby differed in climate conditions, soil type, soil texture and land use. Initially soils were characterized by texture, moisture, pH, total carbon, oxidizable carbon, total nitrogen, microbial biomass, hydrolytic enzyme activity, soil respiration and PLFA composition. Results distinctly showed that the microbial community structure, especially abundance of G^+, G^- bacteria and fungi, varied between different soil types.

An experiment was established using the UK soils. The effects of the xenobiotics polyvinylalcohol, a fungicide (Fundazol) and a herbicide (Gesagard) on soil microbial community and activity were investigated one day and forty-two days following xenobiotic application. The functional stability in the terms of resistance and resilience using the method described by Orwin and Wardle (2004) was calculated from the soil respiration rate data. The experimental treatments caused a significant difference in the PCA profile of PLFA data. Soil type and textural classification affected the altered microbial profile. Treatments also altered microbial activity and microbial biomass. The arable soils were more resistant to xenobiotic perturbation than grassland soils. The reduction of functional stability was associated with the altered soil microbial community composition. Thus, soil type had a greater role than treatment type in determining microbial community composition whereas the treatment type was more determining factor of catabolic profile.

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LIST OF ABBREVIATIONS

4-MUF	4-methylumbelliferone
a	an used amount of 0.1M KOH
A.S.L	Above Sea Level
ANOVA	analysis of variance
APVMA	The Australian Pesticides and Veterinary Medicines Authority
ARDRA	amplified rDNA restriction analysis
b	an used amount of 0.1M HCl by titration of soil sample
B+D	Bligh and Dyer solvent
BBU	1-(2-benzimidazolyl)-3- n-butylurea
BS	British standard
C (All chapters)	carbon
C (Material and Methodology)	the mass of pipetted clay sample
c	an used amount of 0.1M HCl by titration of control sample
C_0	control soil
Ca	calcium
CLPP	Community-level physiological profile
C_{mic}	microbial carbon
C_0	Cottenham
CO_2	carbon dioxide
C_{ox}	oxidisable carbon
Cu	copper
D (Literature Review)	Simpson index
D (Material and Methodology)	Dispersant factor
De	Denchworth
d.s.	dry soil
D_0	difference between C_0 and D_0
DGGE	Denaturing gradient gel electrophoresis

DNA	deoxyrinonucleic acid
Do	Lower Tatras
Du	Slovak Ore Mountain
dw	dry weight
Dx	difference between C_x and D_x
E	evenness
eDNA	environmental DNA
F (Material and Methodlogy)	Factor
f	a factor of solutions
F (Results)	Fundazol
Fa	Faulkborne
FAME	fatty acid methyl esters
Fe	iron
g	gram
G^-	gram negative
G (Results)	Gesagard
G^+	gram positive
GC	Gas chromatography
Glu	Glucose
H	hydrogen
H'	Shannon index
HCl	hydrochloric acid
hr	hour
ISO	International Organization for Standardization
K	potassium
kg	kilograms
KOH	potassium hydroxide
LSD	Least Significant Differences
m_0	the weight of an empty drying tin
m_1	the weight of the drying tin plus fresh soil

m_2	the weight of the drying tin plus oven-dried soil
MF	Lesser Fatra
Mg	magnesium
mg	milligram
ml	millilitre
Mn	manganese
N	the total number of individuals (in equation)
N	nitrogen
ni	the number of individuals
N_t	total carbon
P (Literature Review)	phosphorus
P (Material and Methodology - equation)	production of CO_2
P (Results - charts)	polyvinylalcohol
P (Results)	probability
P_0	disturbed soil
PCA	Principal component analysis
PCR	Polymerase chain reaction
pi	the proportion of individuals
PLFAs	phospholipid fatty acids
PTFE	polytetrafluoroethylene
PVA	polyvinylalcohol
PVAL	polyvinylalcohol
qCO2	metabolic quotient
RABIT	Rapid Automated Bacterial Impedance Technique
rDNA	ribosomal DNA
RL	index for resilience
RNA	ribonucleic acid
rRNA	ribosomal RNA

RS	index for resistance
S	species richness
S (Literature Review)	sulphur
S (Material and Methodology)	the total mass of sand
SAIA	Slovak Academic Information Agency
SIR	Substrate-induced respiration
Sk	Experiment involved Slovakian soil
SPE	Silic acid column chromatography
SR	soil respiration rate
SSCP	single-stranded conformal polymorphism
STB	3-butyl-1,3,5-triazino[1,2a]-benzimidazol-2,4(1H,3H)dione
TGGE	Temperature gradient gel electrophoresis
TPF	2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium formazan
T-RFLP	terminal restriction fragment polymorphism
UK	Experiment involved soils from UK
USDA	U.S. Department of Agriculture
VF	Greater Fatra
W	Water
WHC	water holding capacity
WHO	World Health Organisation
X	the amount of KOH bound by CO_2 in the soil sample
Y	an amount of KOH bound by CO_2 in the control sample
Z	the mass of pipetted silt plus clay sample
Zn	zinc

1 LITERATURE REVIEW

1.1 Introduction

Analysing the relationships between biodiversity and ecosystem functioning in soils has been a major issue in soil ecology for the last decade (Degens *et al*., 2001; Griffiths *et al.*, 2001; Nannipieri *et al*., 2003; Griffiths *et al*., 2004; Crawford *et al*., 2005). Microorganisms are fundamentally important because they mediate 80 – 90 % of the soil processes (Nannipieri *et al*., 2003) such as decomposition of organic matter, nutrient mineralization, plant productivity and carbon cycling. Despite this knowledge, the influence of perturbations on soil microbial dynamics is still largely unknown. More about the theory of the soil microbial community and ecosystem functioning can be found in this chapter.

1.2 Soil as a habitat

Soil plays an essential role for the life of animals, plants and humans on the Earth. It is the part of the Earth's terrestrial surface which forms the principal environment for living organisms such as plants, microorganisms, and soil macrofauna. Also, soil is a dynamic system in which numerous processes can be found. These processes and their products are the basic for the functioning of other ecosystems on the Earth and thus affecting sustainability of the life on the planet. Moreover, the soil is a closely integrated ecosystem, in which living organisms are integrated with particular components of soil, such as inorganic minerals, decayed organic matter, water and soil atmosphere.

There are many definitions of soil. U.S. Department of Agriculture (USDA) defines soil loosely as "the natural medium for the growth of land plants" (Gardiner and Miller, 2008). According to Voroney (2007), soil environment is "the totality of living organisms which occupy soil, including plants, animals and microorganisms and their abiotic environment".

Soil formation is a complex and long-term process that involves continuous transformation of parent material (rocks, small mineral particles) through primary (fedspars, micas) and secondary minerals (silicate clays) to forming the particular

horizons. Many processes, such as physical and biochemical weathering, geological, and biological are essential for the transformation of parent material. Similarly, anthropogenic processes are essential for soil formation but in the highly industrialized world they play a crucial role in bringing pressure on soil systems (e.g. agricultural, pollution, climate change). Soil organisms and plants represent another basic component of the formation of soil. Moreover, the parent material, topography and time scale are the remaining factors responsive to the formation of soil.

The architecture of soil habitat provides the living space for soil biota (Young and Ritz, 2005; Kibblewhite *et al*., 2008). Mineral particles, together with organic matter, microorganisms, and inorganic cements, form aggregates which are linked by pore networks. The walls of soil particles provide a surface for colonisations by bacteria, fungi and the macrofauna. Additionally, this structure supports the resistance of soil to chemical breakdown. The larger pore spaces are important for the flow of the underground water, nutrients, the growth of plant roots, communication between living organisms and interactions between soil biota and habitat.

The soils consist of different proportion of clay-sized, silt-sized and sand-sized particles (Gardiner and Miller, 2008). The architecture of these particles influences the availability of soil surface to microorganisms. Soil aggregates, comprised of silt-sized particles, minerals (aluminium, silicon, iron, magnesium oxide and hydroxide, aluminium and iron silicate) and humus and non-humus organic matter, provide the optimal conditions for growth of microorganisms. The size and diversity of the soil microbial community are influenced by chemical and physical properties of soil particles, which are colonized by microorganisms.

1.2.1 Soil health

Soil, which takes along time to regenerate, is one of the main resources on the Earth and it plays a crucial role in ecosystem. However, a large proportion of soil surface has been degraded due to human intervention including intensive agriculture, mineral extraction, dexilification and landfill sites (Doran and Safley, 1997). It is calculated that the degraded area reaches 40% of the world's arable soil (Oldeman, 1994) and 6 to 10 % of arable soil is believed to be severely degraded. Therefore, there has been a big concern

with long-term sustainability of soil and maintaining the soil quality in the last two decades (Doran and Parkin, 1996; Lal, 1998; Bandick and Dick, 1999; Bloem *et al*., 2006).

The terms 'soil quality' and 'soil health' are often used as equivalents in scientific papers. Numerous definitions of soil health have been proposed in the literature (Doran and Parkin, 1996; Acton and Gregorich, 1995; Karlen *et al*., 1997; Pascual *et al.*, 2000). A most widely accepted one defines soil health as "the capability of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health for an indefinite period of time" (Bloem *et al*., 2006).

Measurement of soil health is problematic because of some difficulties which are linked to the properties and structure of the soil ecosystem (Kibblewhite *et al*., 2008). Firstly, soil is a complex system in which numerous interactions can be found. Secondly, soil is a multifunctional system with a variety of soil processes. Thirdly, the soil ecosystem is an open system which is affected by environmental factors. Lastly, the changes in soil ecosystem are usually evident only after significant time period. For this reason, a set of indicators should be used for measurement of the soil health, not only one of these indicators alone. However, the final set of indicators which would reflect land agriculture and long- term sustainability of soil productivity have not yet been identified.

Physical (parent material, topography, structure, and texture), chemical (especially pH, nutrient content, organic matter content) and biological indicators may be used for measurement of soil health. However, physical and chemical properties are relatively stable and they can change very slowly (Pascual *et al.*, 2000) after disturbance. Biological indicators are more sensitive to any changes which occur in the soil environment, consequently they are more appropriate for measuring the impact of environmental changes, changes in soil utilization as well as contamination (Doran and Parkin, 1996; Nannipieri *et al*., 2003; Nannipieri and Badalucco, 2002; Gil-Sotrés *et al*., 2005). Moreover, the soil organisms are important for soil processes, especially

decomposition of organic matter, forming soil aggregates and nutrient cycles. For this reason, the biological indicators, such as microbial biomass, enzyme activities, soil respiration, and presence of some specific groups of microorganisms, are often used for assessment of soil health.

The impact of different changes in soil can be determined at different levels of microbial community diversity. Winding *et al*. (2005) distinguished several basic profiles of microbial community which include an enzymatic profile (see Material and Methodology), a functional profile – a community-level physiological profile and a catabolic profile, further a phenotypic profile, and a genotypic profile of microbial community.

1.2.2 Community-level physiological profile (CLPP) of soil microbial community

CLPP is a method which allows measuring the amount of carbon dioxide released by soil microorganisms via a broad range of carbon substrates. CLPP applies ninety-six wells microtitre BIOLOG® plates containing nutrients and tetrazolium salt solutions for detecting the growth and it involves from 31 to 95 different carbon substrates. The growth of microorganisms and utilization of specific substrates is measured 24 and 48 hours after adding soil inoculated solution into the plates (Garland and Mills, 1991). The wells of plates are different coloured which depends on the ability of microorganisms to utilize selected carbon substrates. This method is based on microorganisms which affect the process of proliferation, either by growing or by influence on the growth of another species (Winding *et al*., 2005).

CLPP has been successfully used for measurements of changes in soil caused by soil management and soil contamination (Campbell *et al*., 1997; Johnson *et al*., 1998; Yao *et al*., 2000), such as soil contaminated by oil or for assessing the microbial rhizosphere community.

The application of this method is simple and can be automated. This method itself is sensitive, and provides number of information about functional diversity of microbial community. However, its usage is limited by various factors such as sampling,

concentration of carbon source and physiological conditions of microbes. The main disadvantages of BIOLOG application in soil microbiology is the growth of microbial cells in inoculated solution of soil. Moreover, this growth is similar to growth of microbial cells on agar plates. Consequently, the diversity of microorganisms present on the plates is probably much lower than total amount of species in soil microbial community (Torsvik *et al*., 2002).

1.2.3 Catabolic profile of soil microbial community

The catabolic profile of soil microbial community is based on different abilities of microorganisms to respire specific organic substrates and can be easily detected by application of different substrates (Campbell *et al*., 2003). This provides information about microbial biomass in a particular soil sample. Moreover, the application of different organic substrates related to various aspects of cellular metabolism allows the scientists to better understand the function of microorganisms in the decomposition process.

Substrate-induced respiration (SIR), which measure the amount of CO_2 released before and after an addition of a substrate, is an alternative approach to CLPP. Many approaches, which use SIR for measurement of functional diversity of soil microbial community, have been proposed (Garland and Mills, 1991; Zak *et al*., 1994). The response of SIR of microorganisms is measured first six hours after addition of carbon substrates because at that time only the original microbial community is still present and other competitive microorganisms, related to added substrates, have not started to grow yet (Degens and Harris, 1997).

Degens and Harris (1997) developed a whole-soil SIR method which assesses the whole soil microbial population. In this method, the soil microorganisms are not cultivated and the whole microbial community should cooperate on the utilization of carbon substrates.

Campbell *et al.* (2003) developed an easy and reproducible approach which can be used for measurement of functional diversity of whole microbial community. It is based on the application of the MicroResp™ system which consists of a deep-well microtiter

plate for soil, an interconnecting gasket and a reverse top plate with a detection gel and substrates.

1.2.4 Phenotypic profile of soil microbial community

Because of some limitations of traditional cultivation as well as chemical methods, the phospholipid fatty acid analysis (PLFAs) is frequently used for measurements of the microbial biomass, the structure of soil microbial community and the abundance of fungi and bacteria. Additionally, PLFAs may be applied for assessing the changes in a soil microbial community in consequence to the soil management and disturbance (Pennanen, 2001).

Phospholipid fatty acids are basic components of living cells. Individual PLFAs are specific for specific subgroups of microorganisms such as gram positive and gram negative bacteria, metanotrophic bacteria, sulfate-reducing bacteria, actinomycetes, arbuscular-mycorhizae fungi and ectomycorrhiza fungi (Zelles, 1999). These specific PLFAs are relatively conservative in concentration within these groups. A measurement of concentration of different PLFAs extracted from soil may provide biological fingerprinting of soil microbial community. Profiles of PLFAs mostly refer to the dominant groups of community structure. However, PLFAs do not provide any information about quantity of species in community.

The total amount of PLFAs can be applied for measurement of microbial biomass in environmental samples (PLFAs). The bacterial biomass can be evaluated by summing up the abundances of abundances of bacterial PLFAs (i15:0, ai15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, ai17:0, 17:0, cyc-17:0, 18:1ω7 a cyc-19:0) (Tunlid *et a*l., 1989; Frostegård *et al*., 1993). Ratio of biomass of fungi to bacterial biomass is determined by PLFAs ratio of fungi (18:2ω6c) to bacteria.

1.2.5 Genotypic profiles of soil microbial community

The application of molecular methods plays an important role in assessing of soil microbial community in soil microbiology. The technique of nucleic acids may be used to determine the total structure of soil microbial community, the dynamics of particular populations, as well as the genes of community (Sayler and Layton, 1990). These

methods overcome limitations of cultivation methods and have contributed to discovering new species in soil in last decades (van Elsas *et al*., 2000).

Genetic diversity is usually characterised by diversity of DNA genes coding for 16S rRNA. The 16S rRNA genes are used for bacteria and archaea domains, whereas 18S rRNA genes are present in species of fungi (Alef and Nannipieri, 1995; Head *et al*., 1998; Liesack *et al*., 1997).

Up until now, many manufacturer's kits and other methods, which extract the total environmental DNA (eDNA) of the soil microbial community from soil samples, have been developed. There are several methods known which can be used to assess the diversity of rRNA gene sequences coded by eDNA. In soil ecology, the most widely used molecular techniques include denaturing gradient gel electrophoresis (PCR-DGGE), temperature gradient gel electrophoresis (PCR-TGGE), terminal restriction fragment polymorphism (T-RFLP), amplified rDNA restriction analysis (ARDRA) and single-stranded conformal polymorphism (SSCP) (Lee *et al*., 1996; Smit *et al*., 1997; Heuer and Smalla, 1997; Felske *et al*., 1998; Dunbar *et al*., 1999).

1.3 Biodiversity and ecosystem functioning

1.3.1 Biological diversity

Over the last few decades there has been an increasing interest in biodiversity research. Groups such as vascular plants, molluscs, birds and mammals, have been investigated to a much greater extent than others such as prokaryotes and fungi (Storch *et al*., 2007). The development of biochemical and molecular analytical methods has improved the understanding of microbial species and led to the discovery of novel species thereby improving knowledge of the Earth's biodiversity.

Biological diversity encompasses all living organisms. Various definitions of biodiversity have been suggested. The definition stated in the Convention on Biological Diversity (Glowka *et al.*, 1994) is probably the most often used. Biological diversity was defined as "the variability among living organisms from all sources, including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of

which they are part; this includes diversity within species, between species and of ecosystems" (Heywood and Bates, 1995).

Swift *et al*. (2004) defined diversity based on four values:

1. *Intrinsic* – own value of biodiversity which is unusable in direct relationships with humans.
2. *Utilitarian* - primary or usable value of biodiversity (genes, species) to society.
3. *Serependic* or bequest value, which comprises undiscovered benefits of biodiversity and their application in the future.
4. *Functional value* or indirect usage of biodiversity which link biodiversity with ecosystem function, structure and integrity.

Biodiversity can be defined in three groups: genetic diversity, species or organismal diversity and ecosystem diversity. These groups can be considered separately in order to explain their main features. However, they are dependent on each other and they form a hierarchy (Gaston and Spicer, 1998). For example ecosystems involve organisms which belong to different kingdoms, phyla, families, genera, species, populations and individuals. Each organism is formed by set of chromosomes which consists of genes and nucleotides.

1.3.2 Genetic diversity

Genetic diversity comprises the variation of genetic components (nucleotides, genes, chromosomes, and genomes) within structured organisms. Genetic diversity within populations as well as between populations of organisms is studied (Øvreås and Torsvik, 1998; Griffiths *et al*., 1997; Dunbar *et al*., 1999). Genetic diversity is represented by different levels of hierarchy of genetic information, which is essential for coding of biological information and is therefore, a critical component of biodiversity.

The basic level of genetic diversity involves variation in sequences of nucleotides (adenine, cytosine, guanine and thymine) within DNA. Hereditary sections of DNA which occupy specific places of chromosomes are called genes. There may be present different amount of alleles (copies of genes) within any organism. Organisms may

contain different amount of chromosomes. Most contain two sets of chromosomes (diploid), however organisms with one (monoploid), three (triploid) and four (tetraploid) sets of chromosomes have been discovered.

Multiple variation in organisms may be caused either by mutation of genetic components or as a results of sexual reproduction by recombination and natural selection (Harrison *et al*., 2004a). Variation of genetic information can be determined directly or indirectly. Direct variation refers to in nucleotides, genes and chromosomes. Genetic diversity may be also be measured indirectly by monitoring the variation in phenotypic features, such as biochemical, physiological and anatomical characteristics.

1.3.3 Species diversity

The term *species* is defined as "a group of interbreeding natural populations unable to successfully reproduce with other such groups, and which occupies a specific niche in Nature" (Bisby and Coddington, 1995).

Diversity of species encompasses variety of species and involves two primary factors – species richness (number of species) and evenness (relative abundance). Magurran (1988) aggregates them into single index that can be used as indicator of the wellbeing of the ecological system. Species richness was defined as "the number of different species in a particular area or number of individuals or biomass" (Magurran, 1988). Additionally Harrison *et al*., (2004b) described species evenness or species equitability as relative abundance of species in a defined area.

Species diversity can be described using different indices. Whittaker (1972) distinguished three types of indices - alpha, beta and gamma diversity. These indices can also be used for measuring ecosystem diversity. Alpha diversity was defined as the number of species present in an ecosystem, beta diversity the total number of unique species within the compared ecosystems and gamma diversity was defined as the overall diversity of different ecosystems within a region or geographic-scale species diversity (Hunter *et al*., 2002).

Magguran (1988) classified the species diversity indices into three general categories. These categories are species richness indices, species abundance models and indices based on the proportional abundances of species.

Species richness indices provide a useful measure of diversity, particularly because they can be measured in practice and existing patterns in species richness have already been published (Gaston and Spicer, 1998). The application of indices of species richness is commonly used in botanic and aquatic studies. However, there are many limitations associated with using these indices, especially in soil ecosystem. It is unlikely that all the species present would be counted in species rich community such as soil microbial systems. Also, indices of species richness do not take into account different linkages between species and ecosystem function such as decomposition or primary production, especially in soil (Bengtsson, 1998).

The indices based on proportional abundances of species overcome these limitations associated with the species richness indices. They group species richness and evenness into one single figure. Two indices – the Simpson (Simpson, 1949) and the Shannon index (Shannon, 1948) are commonly used to determine biodiversity in soil microbial community (Staddon *et al*., 1997; Mäder *et al*., 2002; Fierer and Jacskon, 2006; Xu and Jiang, 2005).

The Shannon index is also called Shannon-Wiener function (Krebs, 1989) after being independently derived by Claude Shannon and Norbert Wiener or Shannon-Weaver index after Shannon's co-author. Shannon index supposes randomly sampling of individuals from an "indefinitely large" population and a presence of all species in the sample. The last assumption is theoretical and difficult to reach in soil microbiology research because of undetected species. The Shannon index is used for comparison between two different habitats and for evaluation of single habitat over time. The Shannon index is defined by equation:

$$H' = -\Sigma\ (pi)\ (\log_e pi)$$

The quantity *pi* is the proportion of individuals belonging to *i*th species and is defined as

$$pi = ni/N$$

where *ni* is the number of individuals of the *i*th species and N is the total number of individuals.

The Shannon index often uses $\log_2$ in the equation, but any log base may be adopted provided there is a consistency in the choice of log base. The values of the Shannon index for real community usually fall between 1.5 and 3.5 and can rarely reach 4.5 (Margalef, 1972). The Shannon index is used for species richness (S) and evenness (E). The evenness is calculated from modified equation:

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

The values of evenness are always between 0 and 1. All species are equally abundant when the equation is E=1.

The Simpson index was defined as "the probability of any two individuals randomly drawn from infinitely large community belonging to different species"(Simpson, 1949) and is calculated from the following equation:

$$D = \Sigma(pi)^2$$

where *pi* is the proportion of individuals belong to *i*th species.
The diversity decreases with increasing value of the Simpson index. Therefore, the Simpson index is usually expressed as 1-D or 1/D.

Both the Simpson and Shannon indices are easy to calculate and interpret. However, there are differences in application between these two indices. The Shannon index is weighted towards species richness, whereas the Simpson index is weighted towards species evenness. The Simpson index is focused on common species and the Shannon index favours rare species. Broadly speaking, application of these two indices provides two different approaches to measuring the species richness and abundance which allows ecologists to choose more appropriate for their research.

1.3.4 Ecosystem diversity

The term *ecosystem* is used for describing the natural and dynamic functional unit, which involves community, their physical environment and interactions between the

biotic and abiotic components. Odum (1975) defines three essential features of ecosystems:

1. permanent state of energy flow,
2. capability of self-development,
3. counteracts the effects of entropy.

Ecosystem diversity encompasses ecological differences of community, habitats, niches and biomes and variation of ecological processes within the biosphere. Three basic factors, the physical characteristics of the environment, diversity of species present and the interactions between species themselves and with the environment, affect ecosystem diversity (Harrison *et al*., 2004c). The physical characteristics comprise, for example, temperature, topography of ecosystem and flow energy. For example, it is generally known that the warm tropical ecosystems are species richer than cold temperate ecosystems.

1.3.5 Microbial diversity in soil

Microbial diversity encompasses variation at different level of biological organisation. Microbial diversity includes: variation of genetic information within microbial species (genetic diversity), the distribution of individuals within different species (Atlas and Bartha, 1998), species richness, and species diversity. Microbial diversity can also be expressed at the ecosystem level and includes processes, interactions, the number of trophic levels and the number of functional groups (Torsvik and Øvreås, 2002; Nannipieri *et al*., 2003).

Soil microbial community comprises species of bacteria, actinomycetes, algae, protozoa and nematodes. Soil microbial community are probably the most diverse (Torsvik and Øvreås, 2002) and are the most abundant (Whitman *et al*., 1998) of all organisms on the Earth, but their total number is still not known, because some of microbial species have not yet been discovered. In terms of soil ecosystem, microbial community is the species richest in comparison to other terrestrial ecosystems (Giller *et al*., 1997). There are at least 10^4 bacterial species and up to 1.5×10^6 species of fungi per gram soil (Torsvik *et al*., 2002; Hawksworth, 2001)

There are a number of different diversity indices and mathematical models which have been proposed for measurement of diversity in soil. The most used mathematical models involve the Log normal distribution (Preston, 1962) the Geometric series (May, 1975), the Logarithmic series (Fisher *et al*., 1943), the Broken stick model (MacArthur, 1960) and model calculated with relative abundance of r- and K- selected species (Hughes, 1984). The most known indices encompass the theory of alpha, beta and gamma diversity (Whittaker, 1972), the Shannon index, the Simpson index (for all three indices see Chapter Species Diversity), the Brillouin index (Pielou, 1975) and the McIntosh index (McIntosh, 1967).

The study of species richness, functional groups and keystone species are often used to describe microbial diversity in soil (Bengtsson, 1998; Suzuki *et al*., 2005). In the past, plate-counting methods were frequently applied for assessing of species richness in soil. However, it was detected, that only 0.001-0.3 % of total microbes in soil can be cultivated (Benedetti and Dilly, 2006). Therefore, new methods such as nucleic acid approach (Griffiths *et al*., 1997; Felske and Akkermans, 1998; Øvreås and Torsvik, 1998) and phospholipid fatty acid analysis (Frostegård and Bååth, 1996; Pankhurst *et al.*, 2001) have been developed. Application of these methods has greatly enabled soil ecologist to identify novel species of microorganisms in soil and to increase the number of detected species in soil (Coleman and Whitman, 2005).

Functional groups are groups of organisms with similar function in respect to chemical transformations such as soil respiration and enzyme activities. De Ruiter (2003) argues that ecosystem complexity is defined by diversity of functional groups as well as amount of interactions between functional groups. According to Bengtsson (1998), diversity of functional groups associates measurements of species diversity with functional diversity. However, Anderson (2003) claims that the linkage between taxonomic, genetic and functional diversity remains unsolved because the functional groups are aggregated units.

For this reason, Anderson (2003) prefers application of keystone species to the measuring the species diversity. “Keystone species” or “microbial species which control

keystone processes" are limited number of organisms and groups of organisms that seem to control critical processes necessary for ecosystem functioning (Folke *et al*., 1996). These keystone species may change over time and space. The loss or decline of these species would result in dramatic change in the ecosystem in which they live, which could lead to loss of other species especially keystone dependent species (Walker, 1992). This theory is frequently used in ecology (Carpenter and Kitchell, 1993; Power and Mills, 1995; Moore and de Ruiter, 2000).

1.3.6 Ecosystem function

Ecosystem function includes ecosystem processes and ecosystem stability (Bengtsson, 1998). Ecosystem functions are provided by various component of ecosystem, not only individual species (Bengtsson, 1998; Kibblewhite, 2008). Millennium Ecosystem Assessment (2003) distinguishes several groups of ecosystem services. These include groups which participate in productivity (e.g. fibre, water), support of life on this planet (e.g soil formation, nutrient cycling), regulation of ecosystem processes (e.g. disease control). The last group involves non-material cultural services.

It is the soil microbial community which plays the key role in basic ecosystem services in soil. According to Barrios (2007) and Kibblewhite *et al.* (2008), four main ecosystem functions can be identified in soil. They are transformation of carbon, nutrient cycling, soil structure modification and biological regulation of soil populations.

1. Transformation of carbon in soil encompasses decomposition and synthesis of soil organic matter. Decomposition or mineralization of organic material into simple molecules is essential part of biochemical cycles, particularly the global carbon cycle. Additionally, decomposition provides essential nutrients for synthetic processes in soil and contributes to removal of waste from soil and to regulation of greenhouses gases such as methane and carbon dioxide. These processes are carried out by bacteria, fungi and intervertebrates and their enzymatic activities.
2. The cycling of nutrient include cycles of primary macronutrients (N,P,K), secondary macronutrients (Ca, Mg, S) and micronutrients (e.g. Fe, Mn, Zn, Cu). In particular nitrogen, phosphorus, sulphur and micronutrients are released by

decomposition of soil organic matter. Nitrogen cycle, especially presence of microorganisms such as nitrifiers and denitrifiers, contributes to regulation of another greenhouse gas - nitrogen dioxide.

3. Soil structure is defined as formation of aggregates of different sizes from clay, sand, silk particles and soil organic matter, by organic and organic factors (Barrios, 2007). Formation of soil structure involves formation and stabilization of migroaggregates, macroaggregates, biostructures and pore networks. These products of formation of soil structure provide living environment suitable for the microorganisms, soil macrofauna as well as root growth in soil. These processes are mostly carried out by bacteria, fungi, ants, termites and earthworms.
4. Biological regulation of soil populations includes regulation of pest and disease of plants and animals by diverse food web of healthy soil community (Susilo *et al*., 2004).

1.3.7 Linkage between biodiversity and ecosystem functioning

Determining the relationship between different levels of biodiversity and ecosystem functioning has been an important research topic for many years (Woodwell and Smith, 1969; Schulze and Mooney, 1994; Vitousek and Hooper, 1993; Swift *et al*., 2004). A numbers of different theories have contributed to the understanding the influence of biodiversity on ecosystem functioning in terrestrial (Hooper and Vitousek, 1997; Coleman and Whitman, 2005) and marine ecosystems (Duffy *et al*., 2001; Emmerson et *al*., 2001; Stachowicz *et al*., 2002).

The influence of biodiversity on ecosystem functioning in soil ecosystem is still relatively unclear because of poor knowledge of microbial diversity. Many theories assume that microorganisms play essential roles in functioning of the processes in soil (Finlay *et al*., 1997; Andrén *et al*., 1999). However Bengtsson (1998) argued that the diversity influences ecosystem functioning indirectly, through presence and activities of functional groups and keystone species.

Four models of relationship (Figure 1.1) between changes in species richness and ecosystem processes exist (Lawton, 1994; Johnson, 1996):

1. *Null hypothesis* argues that the addition or deletion of species does not affect ecosystem services.
2. *Rivet hypothesis* or *rivet-popping* (Lawton, 1994) suggests that species in ecosystem are like rivets in aeroplane. A loss of one species may not have pronounced effect on ecosystem functioning. However, the ecosystem will fail if several species are lost which is in analogy with the loss of too many rivets on an airplane wing.
3. *Redundant species hypothesis* or *functional compensation* (Walker, 1992; Schulze and Mooney, 1994) is species richness irrelevant. This hypothesis is based on presence of two types of species. First group involves "redundant" species, which are equivalent, with little significance in basic ecosystem services. The second group comprises certain species such as primary producers, decomposers, consumers which are essential part of basic ecosystem processes. The ecosystem will function well even in presence of few species if the biomass of certain species is maintained.
4. *Idiosyncrasy hypothesis* assumes that species present in ecosystem have various and complex roles. For this reason the influence of species richness on ecosystem processes is unpredictable.

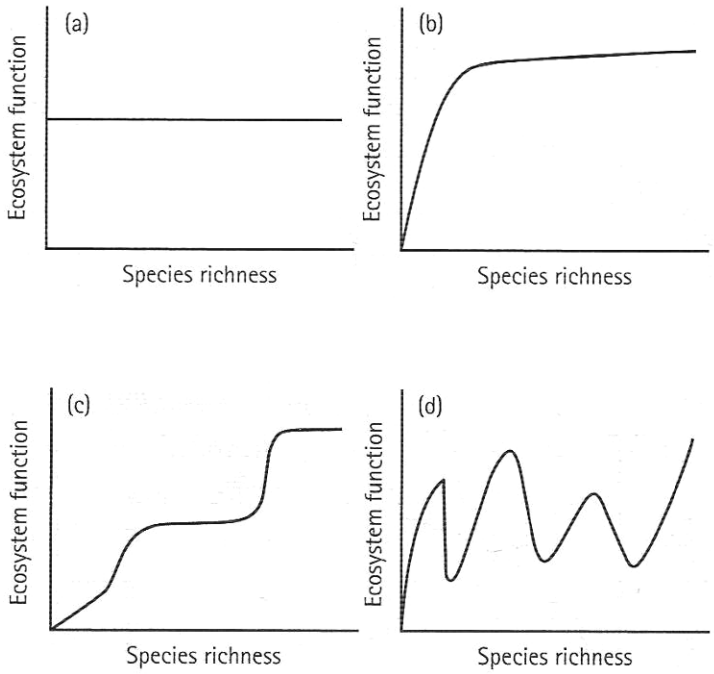


Figure 1.1 Models of relationships between species richness and ecosystem functions (Naeem *et al*., 1994).

Various authors have experimentally assessed models of the relationship between biodiversity and ecosystem functioning (Naeem *et al*., 1994; Tilman *et al*., 1996; Schwartz *et al*., 2000). Investigation of these models has resulted in the hypothesis that a diverse or species rich population is ecologically important for stability and functioning of ecosystem. In a species rich community, there is a greater probability of presence of large number of species with strong impact on ecosystem processes. Furthermore, there is a higher chance that the remaining species have similar functional roles as lost species and can compensate their function. In species poor community, there is a greater probability that a loss of species will be apparent.

1.3.8 Ecosystem stability

Ecosystem stability of soil microbial community is a part of a broad concept of soil quality or soil health. Many definitions of ecosystem stability have been proposed. Ecosystem stability describes the ability of microorganisms to resist or recover from a perturbation (Pimm, 1984; Orwin and Wardle., 2004). Margalef (1968) defined ecosystem stability as "the ability to return to a state reasonably close to its original state in the presence of perturbation." The faster recovery and the less fluctuation an ecosystem has, the more stable it is (Holling, 1973). The ecosystem stability comprises

two components: resistance and resilience. Resistance is the inherent capacity of the ecosystem to withstand a disturbance event and resilience is the ability to recover after perturbation (Pimm, 1984; McNaughton, 1994; Seybold *et al*., 1999).

The relationship between biodiversity and ecosystem stability has been studied by numerous authors (McNaughton, 1977; van der Heijden *et al*., 1998; Kuan *et al*., 2006; Orwin *et al*., 2006; Wertz *et al*., 2007). The relationship between biodiversity and ecosystem function has been studied in terrestrial ecosystems, mainly in plant microbial community (McNaughton *et al*., 1977; Wardle *et al*., 2000) as well as in aquatic microbial community (Steinman *et al*., 1990; McGrady-Sreed *et al*., 1997; Petchey *et al*., 2002). Recently, the study of relationship between biodiversity and ecosystem stability has focused mainly on soil microbial community (Griffiths *et al*., 2001; Müller *et al*., 2002; Orwin *et al*., 2006; Wertz *et al*., 2007) because of their importance in soil processes which play crucial roles in functioning of all ecosystems on the Earth. However, the response of soil microbial community to disturbance and factors which influence this response remain largely unknown.

The concept of ecosystem stability and its two components, resistance and resilience, can be used in relation to two parts of soil ecosystem, soil microbial community structure and functional diversity. Generally, the more diverse an ecosystem is the more stable it becomes. Specifically, the ecosystem is more stable if it is occupied by a large number of species or new organisms entering the ecosystem (MacArthur, 1955). Another theory, which focuses on differences between fast-growing and slow-growing species, can be applied to soil microbial community. According to this theory, slow-growing species tend to be resistant, but not resilient. Fast-growing ones are resilient but not resistant (Grime, 2001). Therefore, the stable ecosystem should contain both types of species.

It was experimentally confirmed (Briones and Raskin, 2003; Botton *et al*., 2006) that microbial community with greater stability of species diversity tend to have less of functional diversity. The main reason is probably explained by the theory that dominant

microorganisms commonly present in soil community are not essentially those adapted to perturbation (Fernandez *et al*., 2000).

The concept of ecosystem stability is well defined but it still remains difficult to choose the right indicators for the measurement of resistance and resilience and for evaluating the obtained data. As I mentioned above, the ecosystem stability is a component of concept of soil health or soil quality. For this reason, the same indicators for measurement of soil quality can be applied for assessing the ecosystem stability. Similarly, it is necessary to apply a set of physical, chemical and microbial indicators, not only one alone. Several indices of quantifying of resistance and resilience have been proposed in the literature (Herbert *et al*., 1999; Wardle *et al*., 2000; Griffiths *et al*., 2001). Orwin and Wardle (2004) suggest indices which should accurately express the response of soil microbial community to disturbances and are standardised by a control value (undisturbed soil).

The index for resistance is defined as:

$$RS(t_0) = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

where D_0 is the difference between the control (C_0) and the disturbed soil (P_0) at the end of disturbance

The index for resilience is defined as:

$$RL(t_x) = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1$$

where D_x is the difference between the control (C_x) and the disturbed soil (P_x). These indices are located between -1 and +1. The value of + 1 shows the maximal resistance and the maximal resilience respectively, or no effect of disturbance and maximal recovery. Lower values indicate less resistance and less resilience of particular soil samples.

1.4 Xenobiotics and contaminants in soil

Sustainability of soil quality is essential for maintaining the biodiversity and the functioning of terrestrial ecosystems. The main factor which declines the quality of soil and damages the soil structure and community of living organisms is contamination,

particularly by the more toxic and persistent chemicals. Recently, numerous xenobiotics, which are present in the soil due to the anthropogenic impact and natural emissions, have been identified. The impact of xenobiotics has been investigated more in the aquatic ecosystems than in soil, probably due to the greater complexity of soil components. However, a better understanding of transformation of xenobiotics in soil could help to protect soil against degradation.

A xenobiotic is defined as "a chemical (or chemical mix) which is foreign for an organism and it is not normally produced or expected to be present in it, or it is chemical found in much higher concentration than usual" (Richardson, 1996). Chemicals which are present in elevated concentrations in soil may be considered contaminants (Peijnenburg, 2000). A broad range of xenobiotics are present in soil. Soil can be contaminated through direct application, atmospheric fall-out, industrial chemicals and urban waste (Edwards, 1992). These chemicals can either be of low toxicity, degradable by microorganisms, or high toxicity, capable to accumulate in soil over long-time period, or they can only be toxic to particular taxa or trophic groups. Peijnenburg (2004) distinguishes several basic structural groups of xenobiotics. These are nutrients, organic chemicals, pesticides, and heavy metals.

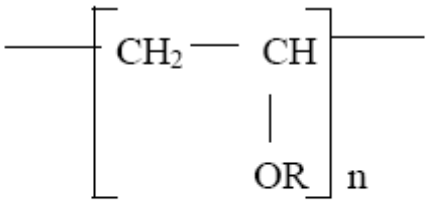
Currently, the international research is focused on impact of pesticides on soil ecosystem (Edwards, 1992; Chen *et al*., 2001a; Burrows and Edwards, 2004; Bellinaso *et al.*, 2003). Various types of pesticides have been used in land management for many years, aiming to maintain the crop productivity and to obtain a higher crop yield. When the pesticides began to be used, there was no research regarding the impact of the application of these pesticides on soil ecosystem. Since discovering that some of the used pesticides were toxic to soil microbial community, a great effort has been made to determine which pesticides are non-toxic to a soil ecosystem. However, the knowledge about transformation of pesticides, especially fungicides, in soil is still largely unknown because of numerous different interactions between abiotic and biotic components in soil.

The ability of microorganisms to degrade xenobiotics is considered as a key driver for the sustainability of soil quality and soil fertility. The biological transformation of xenobiotics involves various metabolic pathways of microorganisms, particularly the catabolic activity of microorganisms. The process of biodegradation of xenobiotics involves mineralization of xenobiotic molecule into carbon dioxide and other inorganic components which microorganisms can utilize for growth and as a source of carbon (Bollag and Liu, 1990). However, the processes, which control the ability of microorganisms to degrade the xenobiotics in soil, have not yet been fully explained. Nevertheless, some xenobiotics are resistant to biodegradation and are accumulated in the soil in the long term. These non-degradable xenobiotics may affect structure of soil aggregates and inhibit some groups of soil microoorganisms which result in soil quality and soil fertility decline.

1.4.1 Polyvinylalcohol

Polyvinylalcohol is usually termed as PVA or PVAL. It is a water-soluble chemical with many properties which make it useful for application in many industrial fields. These are good film-forming, emulsifying, adhesiveness, odourless, high flexibility, and resistance to oil, grease and solvent.

PVA exists only as a synthetic polymer; the monomer structure has not yet been discovered. For this reason, the manufacturing preparation is not based on polymerisation of monomers, however the PVA is prepared by partial or complete hydrolysis which comprises replacing the acetate groups with hydroxyl groups. The chemical structure of polyvinyl alcohol (partially hydrolyzed) is following (Saxena, 2004):



where R = H or $COCH_3$

PVA is usually sold as a clean granular material in variety of molecular weights or as a plastic wrap.

There were few studies (Öztas *et al*., 2004; Özbek, 2004) focused on linkage between application of polymers and soil structure in last decades. It was found out that addition of polymers, especially polyacrylamide, polyvinylalcohol and polysaccharide, as soil conditioners improves soil structure. The main reason is that they function as glue and cements and they stabilise soil aggregates and develop soil strength. A linkage was found between soil texture and application of PVA. Öztas *et al*. (2004) reported different effects of application of PVA on soils with different soil textures. The PVA increased aggregate stability in sandy and sandy clay loam soil up to 95 %, but only 72 % in clay soil. Öztas *et al.* (2004) and Özbek (2004) found out that the bulk density declines and porosity increases with increasing amount of added PVA in sandy soil.

1.4.2 Herbicide Gesagard (prometryne)

Gesagard is a product which contains prometryne as an active component. Other synonyms for Gesagard are Caparol, Mercasin, Promet, Prometrex and Primatol Q. Prometryne was first commercially produced in 1965 by Ciba – Geigy Corporation (US company). The chemical names of prometryne are N^2, N^4 – di-isopropyl-6methyl-1,3,5-triazine-2,4-diamine and N, N´-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine.

Prometryne is white, crystalline solid which is unstable under strongly acidic conditions. It is soluble in organic solvents such as ethanol, methanol, acetone, dichloromethane and toluene. It is stable under normal temperatures and pressures and starts to decompose at temperatures between 118 – 120 ^{o}C. In thermal decomposition of prometryne, the products including toxic oxides of carbon, nitrogen and sulphur may be released into the atmosphere (Ciba-Geigy, 1987).

Gesagard is classified as a general use pesticide. It is pre- and post- emergence herbicide, which is applied to inhibit growth of annual grasses and broadleaf weed in variety of crops including cotton, celery, carrots, parsley and leeks. It affects their growth via inhibition of photosynthesis of particular grasses and weed. It is applied as a liquid or wettable powder.

The application of Gesagard was banned in Slovak Republic in 2006. The main reasons were probably potential toxicity as well as resistance of the weed which it should act against. This herbicide had been permanently used in agriculture for more than 40 years. Therefore, there is a high probability that the particular grasses and weed, photosynthesis of which Gesagard should inhibit, might have become resistant to its application. In the rest of Europe, the Gesagard and their equivalents are still applied in agriculture.

There has been only little research focus on toxicity of Gesagard. It is classified as possible human carcinogen and endocrine disruptor by the U.S. Environmental Agency and World Health Organisation and belongs to toxicity class II or III. It is slightly to moderately toxic and it may cause skin irritation, skin sensitization and eye irritation.

Gesagard binds to the most soils, especially to soils with high clay and organic matter content. It can remain in soil for up to 18 months (Ciba-Geigy Corporation, 1987). Soil microorganisms can decompose it and some of them can utilize this decomposed herbicide as a source of energy, nitrogen and sulphur.

1.4.3 Fungicide Fundazol (benomyl)

Fundazol is a product which contains benomyl as an active component. Benomyl was firstly introduced as fungicide in 1968 by US Company Du Pont (Tomlin, 1994). Other commercial names for products containing benomyl were Agrodit, Bener, Benlate, Benosan, Fungicide 1991, and Tersan. The chemical names of benomyl are carbamic acid or [1-(butylamino) carbonyl–1H- benzimidazol-2-yl] carbamate.

Fundazol (benomyl) is a systematic pre- and post- harvest benzimidazole fungicide. It was the first widely used systematic fungicide. It is toxic to microorganisms, especially to saprophytic and parasitic fungi, and to a certain extent earthworms. It is used for treatment of many fungal diseases, particularly on crops, fruits, mushrooms, nuts, and some kinds of vegetables.

The main action of Fundazol as an antifungal agent is its binding to cell microtubules, which results in inhibition of vital function of cells, such as cell division, and extra cellular transportation in which the microtubules play essential roles. The application of Fundazol as fungicide is selective because the fungal microtubules are more sensitive than mammalian microtubules. The different sensitivity of different groups of fungi is explained by different affinity of fundazol for fungal tubulin (WHO, 1993).

The benomyl is tan, crystalline solid which decomposes above melting point of 140 ^{o}C. It has vapour pressure < 5.0×10^{-6} Pa at 25 ^{o}C and density of 0.38 g cm^{-3}. Benomyl does not dissolve in water.

Recently, the application of benomyl has been considered problematic. Firstly, benomyl has been used more than 40 years in agriculture. For this reason, many strains of parasitic fungi, which should be killed by benomyl, have become resistant to it. Secondly, there are many questions and studies about toxicity and toxic effect of benomyl, and particularly its main metabolite carbendazim to humans, animals, plants and particular ecosystems. Therefore, original manufacturer of benomyl fungicides, Du Pont company, stopped to produce benomyl fungicides in 2001. Hovewer, because of Du Pont's patent expiration, other manufacturer still produce it.

Benomyl is classified as possible human carcinogen by the US Environmental Protection Agency. It is because of study on mice, which shows occurrences of liver tumour after the application of benomyl (WHO, 1993). Moreover, its application can causes skin irritation, moderate eye irritation, skin sensitivity (Extoxnet, 1994), occasionally headaches, diarrhoea, and sexual dysfunction when working with it for longer. Thomas and Gartwhaite (1993) published a study which showed possible health threat of benomyl to pregnant women and their children being born with damage to the optic system (anophthalmia, blindness).

For the mentioned reasons, the application of Fundazol was banned in Slovak Republic in 2007. Similarly, it is prohibited to supply or use products containing benomyl after 6 December 2006 in Australia (APVMA Gazette, 2005).

In soil, solutions and plants, benomyl is degraded to main metabolite carbendazim (methyl-1H-benzimidazol-2-carbamate), and to 2-AB, STB (3-butyl-1,3,5-triazino[1,2a]-benzimidazol-2,4(1H,3H)dione) and BBU (1-(2-benzimidazolyl)-3- n-butylurea). Benomyl and their soil degradation metabolites are strongly adsorbed to soil organic matter, particularly in surface soil layer. They can remain in soil for up to 3 years (WHO, 1993).

1.5 Aims and objectives

The aims of the research are:

1. to determine effect of treatments of xenobiotics on functional characteristics (soil respiration, enzymatic activity), total amount (microbial biomass) and structure (phenotypic profile) of soil microbial community in two different soil types, arable soil and grassland soil,
2. to determine the impact of xenobiotic application on ecosystem stability of soil microbial community, in terms of resistance and resilience.

1.6 Hypotheses

1. The microbial community of soils with different soil types will have differing abilities to resist xenobiotic (polyvinyl alcohol, Gesagard and a fungicide Fundazol) perturbation as indicated by phenotypic and catabolic characteristics
2. Application of xenobiotics will alter ecosystem stability, in terms of resistance and resilience, as described by Orwin and Wardle (2004).

2 MATERIAL AND METHODOLOGY

2.1 Experimental sites and sampling

The soils were sampled from two countries. The Slovakian soils were used in an experiment hereafter referred to as Experiment 1 or 'Sk". The UK soils were used for further experiment hereafter referred to as Experiment 2 to or "UK". The Slovakian sampling sites are described according to Chlpik (2007).

The Slovakian soils were Cambisols sampled from four grassland valleys of the Greater Fatra, Lesser Fatra, Low Tatras and Slovak Ore Mountain regions. A spade-made probe, which allowed distinguishing particular horizons of soil, was used for sampling. The top layer (0 horizon) with undecomposed plant residues was removed. The samples were taken randomly in three replicates from the A horizon to a depth 10 cm.

The mountains Greater Fatra is a part of the Outer Western Carpathians and was declared a National Park on 1 April 2002. It lies predominantly in the Zilina region but also goes into the Banska Bystrica region. The sampling site is situated on a meadow located in the Lubochna valley, under the Western slopes of Ploska, near the Cottage under Borisov. It is located approximately 1200 m A.S.L (Above Sea Level). Terrain has North-eastern aspect with an inclination angle of 10^{o}.

The National Park of the mountains Lesser Fatra is located in the Northwestern part of Slovakia and it spreads across the regions of Zilina, Martin, Dolny Kubin and Prievidza. The sampling site is situated in the valley Strungovy Grun between villages Parnica and Zazriva and it is 1100 m A.S.L. The terrain has an eastern aspect with an inclination angle of 20^{o}.

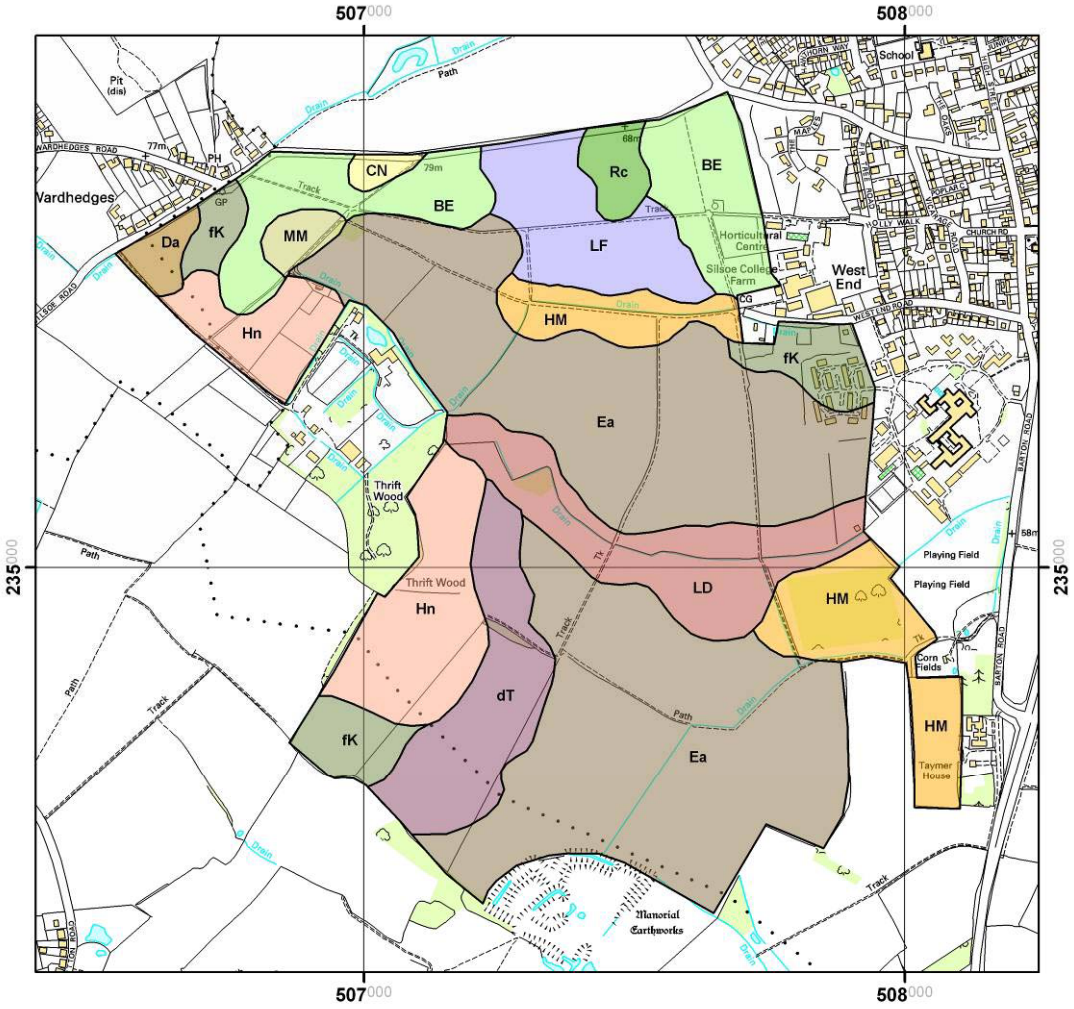
The National Park of the mountains Low Tatras is lies in central Slovakia south of High Tatras. The areas of Low Tatras and High Tatras are separated by valleys of the rivers Vah and Hron. The sampling site is situated in location under hill Kecka, with altitude approximately 1000 m A.S.L. The terrain has an Eastern aspect with an inclination angle about $8 – 10^{o}$.

The mountains Slovak Ore Mountains are situated in the Spis Region in the South-western part of Slovakia. It belongs to Inner Western Carpathians. Sampling site is situated on the borderline of the Veporsky and Stolicky Mountains, above the location Dubakovo. It is located approximately 920 m A.S.L. and it has a southern aspect with an inclination angle of 15 – 20^{o}.

Soils in the UK were taken from three locations within the farm at Cranfield University (Figure 2.1). The locations were selected on the basis of differing textural classifications and soil types. The Cranfield farm is located in Silsoe in the South-east of U.K., with an altitude of about 60 m A.S.L and an average annual rainfall of about 584 mm. The three soil types were arable soils belonging to the Cottenham (loamy sand), Faulkborne (sandy clay) and Denchworth (clay loam) series.

Cottenham soil is deep, permeable, iron-rich sand which is situated at the top of a moderate slope. The Faulkborne soil is deep, permeable, flinty loam which is located in the middle of a gentle slope. The Denchworth soil series is slowly permeable, mottled, calcareous clays situated at the bottom of a gently slope (Verma and Bradley, 1988).

Soils were sampled by Dutch auger at nine points 5 m apart along a “W” shape transect to a depth 15 cm. After sampling, the samples were homogenised separately and sieved through 2 mm mesh.



	Series symbol	Series name	Soil subgroup name	Description
	Da	Denchworth	Pelostagnogleysol	Clayey passing to clay or soft mudstone
	LD	Lawford	Pelostagnogleysol	Swelling clay drift passing to clay or soft mudstone
	Ea	Evesham	Typical calcareous pelosol	Clayey passing to clay or soft mudstone
	dT	Drayton	Typical calcareous pelosol	Swelling clay drift passing to clay or soft mudstone
	Hn	Hanslope	Typical calcareous pelosol	Clayey chalky drift
	HM	Holdenby	Typical argillic pelosol	Clayey drift passing to clay or soft mudstone
	fK	Faulkbourne	Typical argillic pelosol	Clayey chalky drift
	BE	Bearsted	Typical Brown Earth	Coarse loamy passing to sandstone
	Rc	Rivington	Typical Brown Earth	Coarse loamy over lithoskeletal sandstone
	MM	Maplestead	Typical Argillic Brown Earth	Coarse loamy drift with siliceous stones
	LF	Ludford	Typical Argillic Brown Earth	Fine loamy drift with siliceous stones
	CN	Cottenham	Typical Brown Sand	Sandy ferruginous

Figure 2.1 Cranfield farm map (scale 1:8,125), Silsoe (Verma and Bradley, 1988)

2.2 Disturbance treatments and conditions of experiments

Experiment 1 (Sk) and 2 (UK) differ in respect to the sampling location and the experimental variables. In both experiments, the same four treatments (glucose, PVAL, herbicide and fungicide) were added to samples (50 g).

The applied treatments were:

1. Control-Untreated
2. Glucose (2 g kg^{-1} d.s.)
3. Polyvinylalcohol (PVAL - 2 g kg^{-1} d.s.)
4. Herbicide – Gesagard 80WP (0.6 g kg^{-1} d.s.)
5. Fungicide – Fundazol 50WP (0.24 g kg^{-1} d.s.)

The control, untreated sample and the samples treated by glucose were used for comparison of the effect of xenobiotic perturbation on soil microbial community. Glucose is a commonly used substrate for measurement of soil respiration because it provides an easily utilised carbon source for the soil microbial community. The effect of glucose on soil respiration is well-known and has been observed by many researchers (Zak, 1994; Degens *et al.*, 2001; Griffiths *et al.*, 2001). The treatments (polyvinylalcohol, Gesagard and Fundazol) were chosen to determine their effect on soil microbial community.

In Experiment 1 (Sk) pH, moisture content, oxidizable carbon and N_t, were measured from fresh soil. The biological characteristics including microbial biomass, enzyme activities and soil respiration were evaluated after eight week pre-incubation at 4±1 °C. Afterwards, the treatments were added to the soil samples. The samples with added treatments were incubated at 28 °C for three weeks. At the end of the experiment, soil respiration was measured and the PLFA profiles were evaluated. The characteristics were measured in Slovakia exception PLFA analysis which was done at Cranfield University

In Experiment 2 (UK) moisture content, pH, water holding capacity, soil texture, soil respiration, and total carbon and nitrogen were measured from fresh soil samples.

Subsequently, the soil samples were incubated at 25 ºC for one week. After incubation, the measurement of soil respiration, enzyme activity, microbial biomass and PLFA peaks were made. Afterwards, the treatments were added to triplicate soil samples to reach 40 % water holding capacity (WHC). The soil samples were incubated with the added treatments at 25 ºC for six weeks. At the end of the experiment, the same characteristics were measured as at the beginning of experiment.

2.3 Physical and chemical methods

2.3.1 Soil moisture content

The amount of water present in soil is a very important characteristic of a soil ecosystem. It affects the rate and functioning of various microbial, chemical and physical processes in the soil.

The gravimetric method is commonly used for the measurement of soil moisture. Soils were dried in an oven at 105 ± 5 ºC for a minimum of 24 hours. Moisture content was calculated by comparison of the soil weight before and after drying using the following equations (ISO 11465:1993):

$$\text{Moisture content (\%)} = \frac{m_1 - m_2}{m_2 - m_0} \times 100$$

$$\text{Dry mass content (\%)} = \frac{m_2 - m_0}{m_1 - m_0} \times 100$$

where m_0 is the weight of an empty drying tin (g)

m_1 is the weight of the drying tin plus fresh soil (g)

m_2 is the weight of the drying tin plus oven-dried soil (g)

2.3.2 Soil pH measurement

The term pH was firstly used by Sörensen in 1909. The determination of soil pH is based on the measurement of concentration of hydrogen ions in soil solution and is defined as

$$pH = \log_{10}(1/[H^+]) = - \log_{10}[H^+]$$

Soil pH affects many soil processes and properties such as nutrient availability, solubility of heavy metals, clay mineral formation, and microbial activity (Pawlett, 2002).

The three solvents – deionised water, 1M potassium chloride and 0.01M calcium chloride are commonly used for measurement of pH. Deionised water (50 ml) was added to soil sample (10 ml) and shaken with it for one hour at room temperature. Afterwards, the samples were allowed to settle for few minutes. Finally, the pH of soil samples was measured after three-point calibration (pH 4, pH 7, and pH 10) of pH meter.

2.3.3 Water-holding capacity (WHC)

Water holding capacity is the storage capacity of soil expressed in the amount of water held by a unit mass of soil under normal conditions. It is primarily affected by soil structure and the amount of soil organic matter. Soils with small particles (e.g. clay soil) have larger surface and therefore less spaces than soil with larger particles (e.g. sandy soil). For this reason clay soils have higher water-holding capacity. Also, water holding capacity is higher in soils with higher amount of organic matter. It is because of affinity of water to organic matter. The determination of water-holding capacity is necessary for incubation period of experiment. It is very important to set up the same WHC for each sample and then keep it during the whole experiment.

The apparatus for measuring the water holding capacity consisted of a glass funnel with glass wool at the bottom, a stoppered drain attached to the outlet of the funnel, and a measuring cylinder. The sieved fresh soil sample (25 g) was placed in the funnel. Subsequently, deionised water (50 ml) was added to the funnel and allowed to drench the sample for 30 minutes, after which time surplus water was drained into the measuring cylinder. The volume of blank was determined by the same method, but without the soil. The water-holding capacity can be calculated using the following equation:

100 % WHC = soil moisture (ml g^{-1}) + volume of retained water (ml g^{-1})

2.3.4 Soil carbon and nitrogen content

Total carbon content in soil encompasses organic and inorganic carbon in soil. Organic carbon is an essential part of all organic compounds and constitutes between 48 and 58% of soil organic matter.

The sum of inorganic nitrogen and organic nitrogen is the total nitrogen in soil. The inorganic forms of nitrogen, especially nitrate or ammonium, can be absorbed and used by plants. However, the organic nitrogen represents the larger part, between 95 and 97 %, of the total nitrogen present in soil. This form of nitrogen is bound to organic matter and is not available to plants.

The carbon and nitrogen contents in soil were measured using the Perkin-Elmer CHN elemental analyzer (ISO 10694:1995). The principle of this method is based on oxidation of carbon and nitrogen to the gases CO_2 and N_2 by heating the soil in a pure oxygen environment to at least 900 ºC. The released gases (CO_2, N_2) were separated by frontal chromatography and were quantified by thermal conductivity detector.

The soil samples which had been dried in the oven at 105 ºC for at least 2 hours were used for measurement of soil carbon and nitrogen contents. The soil samples used for measurement of total nitrogen were ground to a fine powder before packaging. Small size samples (0.001 mg) were packed into small aluminium-foil capsule and put into the carousel of the automatic sample feeder.

2.3.5 Soil texture

Soil texture describes the relative proportion of soil inorganic particles. The soil particles, which are larger than 2 mm in diameters, such as gravels or rocks, are excluded from the definition of soil. Soil particles are grouped into different sizes fractions. The United States Department of Agriculture (Soil Survey Staff, 1951) distinguishes three major groups of soil separates. Sand-sized particles, larger than 0.05 in diameter, are the largest ones. The silt-sized particles have diameters between 0.002 and 0.05 mm. The smallest particles are the clay-sized particles which have diameter smaller than 0.002 mm. The fractions of the separates present in soil refer to soil texture

classifications such as clay, silt, sand, loam, silty clay, sandy clay, clay loam, silty clay loam, sandy loam, loamy sand, and silt loam.

In this study, the size of fractions of soil separates was determined by using the British standard which applies slightly different sizes of soil particles. Sand fraction has diameters between 0.063 and 2 mm, medium silt fraction has diameters between 0.002 and 0.63, and clay fraction is less than 0.002 mm in diameter. The soil texture triangle (Figure 2.2) consists of all texture classifications which can be obtained by the end of calculation which will be described shortly.

The determination of particle size distribution was based on several points (ISO 11277:1998). Firstly, hydrogen peroxide solution was added to air-dry sieved soil samples. Addition of 30 % hydrogen peroxide solution resulted in decomposition of soil organic matter. Afterwards, the deionised water was added to fill each bottle to 200g in weight. Then, the 5 % buffered sodium hexametaphosphate solution was added to each bottle to disperse the liquid product. Subsequently, the sizes of soil separates were determined by combination of weighing, sieving, sedimentation and the pipette method.

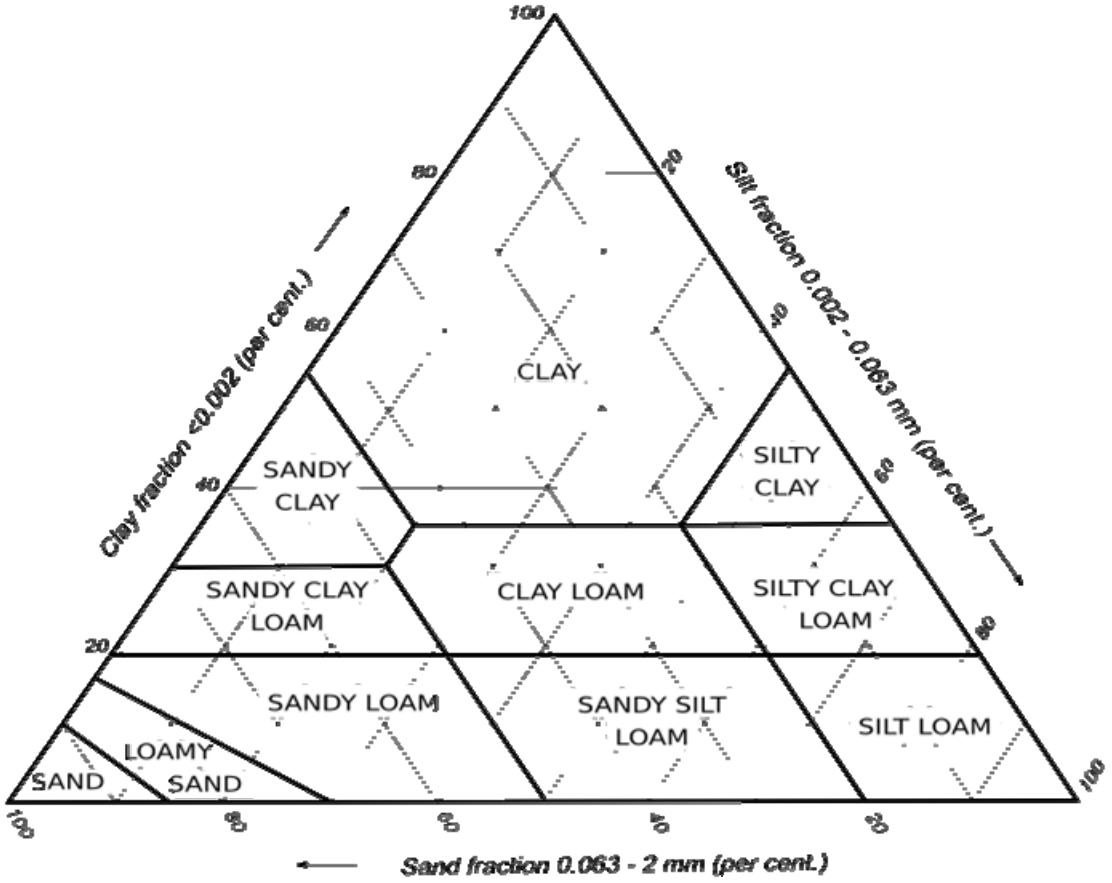


Figure 2.2 Soil texture triangle according to BS 7755 (1998)

The percentage of soil mineral fractions and separates can be calculated by following equations:

Dispersant factor (D) = d/20

Factor (F) = S + ((Z-D) × 20)

$$\% \text{ Sand} = \frac{\text{Mass of particular sand fraction}}{F} \times 100$$

$$\% \text{ Silt} = \frac{(Z - C) \times 20}{F} \times 100$$

$$\% \text{ Clay} = \frac{(C - D) \times 20}{F} \times 100$$

where d is oven-dry mass of sodium hexametaphosphate solution (g)

Z is the mass of pipetted silt plus clay sample

C is the mass of pipetted clay sample

S is the total mass of sand

2.4 Microbial methods

2.4.1 Microbial biomass

Principle

Microbial biomass is a critical part of the soil ecosystem due to its responsibility for energy and nutrient cycling and regulation of soil processes, such as decomposition or mineralization of soil organic matter (Turco *et al*., 1994). It is defined as the total amount of living soil micro biota (Jenkinson and Ladd, 1981), usually expressed in carbon units. Microbial biomass involves all the species of bacteria, actinomycetes, fungi, micromycetes, algae, protozoa and nematodes which are smaller than 5 μm. The microbial biomass is often used as an "indicator" of soil health and the ability of soil to recover after perturbation.

There are many techniques which may be applied for measurement of microbial biomass including fumigation-extraction method (Vance *et al*., 1987), fumigation-incubation (Jenkinson, 1988), substrate-induced respiration (Anderson and Domsch, 1978), arginine ammonification (Alef *et al*., 1988), and measurement of microbial adenosine tri-phosphate (Jenkinson, 1988). Each method has advantages and disadvantages, but all the methods are able to detect differences among soils. The fumigation-extraction method is the one most commonly used because of its simplicity and applicability to wide range of soils (Turgay and Haraguchi, 2000).

Method

The fumigation-extraction method (Vance *et al.*, 1987) is based on the fumigation of soil samples by chloroform thereby resulting in the death of microbial cells and subsequently the lysis of microbial membranes and release of protoplasm. The released carbon and nitrogen can then be extracted by 0.5M potassium sulphate.

Prior to fumigation, sieved soils were adjusted to 40 % WHC. Each soil was partitioned into two portions. One portion (10 g) was fumigated with chloroform, soda lime and damp tissue in desiccator for 24 hours. The second portion (10 g) was used as a non-fumigated control sample. Then the 0.5 M sodium sulphate (40 ml) was added to the fumigated as well as the non-fumigated samples. There was also one extra sample

containing only potassium sulphate without soil (blank sample). The microbial carbon present in the soil samples was measured using the Segmented Flow analyser SFA2000 (Burkard Scientific). The principle of this method is based on oxidation of soil organic carbon to carbon dioxide in the presence of potassium persulphate. The released gas was measured by infra-red or ultraviolet spectrometric detection.

2.4.2 Hydrolytic enzyme activities

Principle

Soil enzymes represent an essential part of the soil ecosystem and processes in soil. Soil enzymes are the mediators and catalysts of all transformations present in soil. For this reason, the measurement of enzymatic activities in soil is a sensitive indicator of soil health, soil degradation, effect of pollutants, and recovery of soil (Dick, 1997). Enzymatic activity in soil is provided mostly by bacteria, fungi and plant roots and it is responsible for circulation of carbon, nitrogen and other elements in biogeochemical cycles (Shaw and Burns, 1996). Oxidoreductases, transferases and hydrolases are the most studied groups of soil enzymes because their roles in decomposition of various organic compounds, presence in organic cycling and soil organic matter formation.

There are two major groups of enzymes present in soil. The first group involves those which are present inside the microbial cells (intracellular enzymes), either bound to microbial cell membrane or releasing from damaged cells. The second one comprises enzymes that catalyse reactions on the surface of cells or outside of cells (extracellular enzymes).

The activity of soil enzymes is affected by various physical, chemical and biological factors such as moisture content, soil temperature, aeration, structure, soil pH, nutrient content, soil organic matter content and presence of activators and inhibitors.

Method

The method described by Marx *et al*. (2001) was used. This method is simply to perform and allows direct measurement of functional diversity in soil without extraction or purification of the product. Similarly, this method allows processing of large number of different samples in a relatively short time as well as by low concentration of

substrates. The main reason for the mentioned advantages is usage of ninety-six-well microplates and flourometrically-labelled substrates which contain highly fluorescent compound 4-methylumbelliferone (4-MUF). The addition of 2-N-(morpholino) ethanesulfonic acid buffer is important to achieve the optimal range of pH which for many enzymes is around 6.1.

The defrosted sieved soil samples (0.5 g) were dispersed in deionised water (50 ml). Then the samples were shaked for 30 min. After shaking, the samples were transferred to beaker. The magnetic stirrer was placed into each sample and the samples were stirring to ensure an homogenous mixture. Then the soil suspension was taken from this mixture and was dispensed to the microplate. The ninety-six-well microplates were used for the measurement of enzyme activities in soil samples. Two soil samples in triplicates and one blank sample in duplicate were analysed within one microplate. At the same time of putting the soil suspension at the bottom of the microplate, the control sample was prepared by substituting the sample with sterile deionised water (50 µl). Secondly, the appropriate amount of buffer was added to each well. Subsequently, 10 mM substrates solutions including 4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-β-D-glucoside, 4-MUF-phosphate, 4-MUF-N-acetyl-β-D-galactosaminide, 4-MUF-β-D-xyloside, 4-MUF-β-D-galactopyranoside, and 4-methylumbelliferyl sulphate, were added to the first eight columns separately. Then, the appropriate amount of 100 µM 4-MUF standard solution was added to last four columns to obtain final amounts of 0, 0.5, 1.5 and 2.5 nm of 4-MUF per reaction. Afterwards, the plates were incubated at 25 °C for three hours. Finally, the plates were read with an excitation wavelength of 360 nm and emission wavelength of 460 nm on Molecular Devices UV reader. The standard curve for each sample was prepared using the data of four last columns. The remaining data was used for the calculation of rate of hydrolytic enzyme activities in soil (nmol 4-MUF g^{-1} dw soil hr^{-1}).

2.4.3 Soil respiration

Principle

Soil respiration is an important process, which provides the output of carbon from the soil to the atmosphere and the input of oxygen from the atmosphere to the soil. Water

content in soil, concentration of oxygen and availability of carbon are the major factors that affect the soil respiration process. This process is provided mostly by the soil microbial community and soil fauna. Generally, the microorganisms are responsible for 90 % of released carbon dioxide whereas soil fauna only for remaining 10 % (Javoreková *et al*., 2008).

According to Šantrůčková *et al.* (1993), two types of soil respiration may be measured. The first type includes measurement of activity of soil which is not influenced by any substrate addition (basal respiration) and the second group involves substrate-induced respiration, thus measurement of potential respiration of soil after addition of organic chemicals or nutrients (Anderson and Domsch, 1978).

Numerous studies (Insam, 2001; Plaza *et al*., 2003) have used a ratio of soil respiration to microbial biomass instead of evaluation of these parameters alone. This ratio is usually termed as metabolic quotient (qCO_2) or specific respiration rate and is usually expressed as CO_2-C $h^{-1}mg^{-1}$ per unit of microbial biomass C. The metabolic quotient may be used as a sensitive indicator of soil quality, effect of perturbation or effect of pollutants (Anderson and Domsch, 1990).

Method

In Experiment 1 (Sk), soil respiration was measured by the absorption-titration method (Weaver *et al*., 1994). The principle of this method is based on the binding of carbon dioxide released by microorganisms in a presence of 0.1M potassium hydroxide (KOH). The amount of released carbon dioxide was determined by back titration using 0.1M chloride acid (HCl). Atmospheric CO_2 was used as the control. The basal respiration and the substrate-induced respiration were measured. Subsequently, the production of CO_2 (P) was calculated via following equation:

$$P= (x-y) \times 2.2 \times 20$$

$$x= a - b$$

$$y= a - c$$

where x is an amount of KOH bound by CO_2 in the soil sample

y is an amount of KOH bound by CO_2 in the control sample

a is an used amount of 0.1M KOH (ml f^{-1})

b is an used amount of 0.1M HCl by titration of soil sample (ml f^{-1})

c is an used amount of 0.1M HCl by titration of control sample (ml f^{-1})

f is a factor of solutions

In Experiment 2 (UK), a method of Ritz *et al.* (2006) was applied. This method uses the Rapid Automated Bacterial Impedance Technique (RABIT) equipment developed by Don Whitley Scientific Ltd. Firstly, RABIT cells with a mixture of agar and potassium hydroxide at the base were prepared. Then, the RABIT cells were placed into incubation modules to stabilise. Afterwards, the soil samples with added substrate in triplicate were placed in glass boats inside the cells. One control (deionised water) and seven substrates were used in this experiment. They were D-glucose, L-arginine, Citric acid, Malonic acid, α-ketoglutaric acid, amino butyric acid and acetyl glucosamine. Subsequently, electrical changes of alkaline agar due to ionization of CO_2 to carbonate were monitored every six minutes for 16 hours at 25 °C. Finally, the obtained RABIT data was converted to soil to μg C-CO2 g^{-1} soil using a constant of 0.0298.

2.4.4 Phospholipid fatty acid (PLFA) analysis

Principle

Phospholipid fatty acids are one of the three basic types of lipids which are present in cell membrane of each living cell. They are metabolised relatively quickly after cell's death, what ensures the analysis of the living cells only (White *et al*., 1979), and they are widely used to determine a "phenotypic profile" (Zelles, 1999).

Method

PLFA profiles were analysed by the method described by Frostegård *et al.* (1991). The method of several stages including extraction of phospholipids, fractionation of lipid extracts, mild alkaline methanolysis, clean-up procedure and gas chromatography.

Extraction of phospholipids is an important part of PLFA analysis which allows separation of lipids from the protein-lipid complex and the cell membrane lysis. The most commonly applied method uses 15 – 20 ml of Bligh and Dyer (B+D) extraction solvent (Bligh and Dyer, 1959) which is added to freeze-dried soil samples (5 – 10 g). This solvent consists of 0.15M citrate buffer (0.15M citric acid dehydrate and 0.15M trisodium citrate), chloroform and methanol at a ratio of 0.8:1:2 (v/v/v), respectively.

Butylated hydroxyl toluene (0.005%) was added as anti-oxidant to the extraction solvent. The PTFE tape was placed at the top of bottles to avoid of plasticide contamination. After 30 min. sonication and 30 min. shaking, the solution was split into an organic layer and a water layer. After another addition of chloroform (4 ml) and citrate buffer (4 ml), the organic (upper) layer which contained extracted lipids was separated into two layers. The nitrogen-dried organic (lower) layer was then used for fractionation of phospholipids.

Fractionation of lipids is a process of separation of a lipid extract into classes of neutral lipids, glycol-lipids and polar lipids. It was done using commercially prepared Solid phase extraction (SPE, also known as Silic acid column chromatography) columns. These columns, which contain silica, were added to SPE manifold attached by tubes to a vacuum pump. A small amount of sodium sulphate was placed into each cartridge to remove moisture from the soil samples. The silica was washed by 2ml of chloroform. Then the solvent was dried. Afterwards, 2 ml of chloroform were added. From this stage onwards small part of solvents was left in SPE cartridges to avoid drying of silica. Fractionation of the particular lipid classes was based on their polarity to silic acids and selectively washes by chloroform, acetone and methanol. Polar lipid fractions were collected into new bottles and dried under stream of nitrogen at 37 °C.

The phospholipid fractions were methylated by mild alkaline methanolysis. This stage allowed the transformation of fatty acids to their methyl esters. This transformation decreased the polarity of fatty acids and made the subsequent gas chromatography analysis possible. During this stage, it was important to keep all the used solvents moisture-free as this attack the double bonds and compete with methanol for the fatty acids in order to obtain free fatty acids rather then methyl esters.

In the stage of base wash, the samples were cleaned and any underrivated fatty acids and other contaminants were removed using 0.3 M sodium hydroxide and mixture of hexane and chloroform at a ratio of 4:1, respectively.

The last stage was the gas chromatography. The soil samples were dissolved in hexane and transfer to the G.C. vials with G.C inserts. Then, the samples were placed into the carousel of the automatic sample feeder of Agilent Technologies 6890N G.C. The Agilent G2070 ChemStation for G.C. systems were used for detection of total area of peaks and their retention time. The G.C. was fitted with a splitless injector and a HP-5 capillary column (30m length, 0.32 mm ID and 0.25 μm film) which was 5 % phenylmethyl siloxane. Helium was used as the carrier gas (1 ml per min). The FAMEs were separated using temperature program, starting at 50 ^{o}C for 1 min (splitless hold time), increasing at 25 ^{o}C per min to 160 ^{o}C followed by 2 ^{o}C per min to 240 ^{o}C and 25 ^{o}C per min to 310 ^{o}C. Samples were injected (1 μl) using an autosampler. The G.C. equipment identifies FAMEs (retention time) of each sample and detects relative concentration (total area) of individual PLFAs. The retention time of each identified fatty acid is described in Table 2.1. The peak values obtained by GC were normalised by dividing the amount of each PLFA by the total amount of PLFA in that particular sample. The fatty acids identified in the sample were used for calculation of tentative abundance of specific microorganisms (G^{+} bacteria, G^{-} bacteria and actinomycetes) as well as total bacteria and fungi in the soil samples (Table 2.2).

Table 2.1 Fatty acids identified in this research and their retention time

Fatty acid elution order	Fatty acid	Retention time (min)	Fatty acid elution order	Fatty acid	Retention time (min)
1	14:0	17.106	20	Me i17:0	27.462
2	c14:1	17.851	21	i17:0c	27.842
3	c14:1	18.242	22	i17:0	28.085
4	c14:1	18.793	23	ai17:0	28.451
5	c14:1	19.228	24	i17:0	28.663
6	c14:1	19.686	25	17:0c	29.049
7	c14:1	19.970	26	17:1	29.345
8	i15:0	20.424	27	17:0	29.590
9	ai15:0	20.712	28	i17:0	29.777
10	i16:1	23.311	29	18:0	31.296
11	3OH 14:0	23.491	30	18:2w6,9	32.500
12	i16:0	24.090	31	18:1w9c	32.840
13	ai16:0	24.539	32	18:1w9t	33.135
14	16:1w7c	24.804	33	18:1w7t	33.301
15	16:1w7t	24.878	34	i18:1	33.426
16	16:1co5	25.144	35	18:0	33.839
17	16:0	25.624	36	19:2	35.486
18	Me i17:0	27.156	37	19:0c	37.574
19	Me i17:0	27.319			

Table 2.2 PLFAs used for calculation of relative abundance of selected taxonomic microbial groups (Piotrowska-Seget and Mrozik, 2003)

Microbial identification group	Fatty acid group	Fatty acid
Bacteria	Various fatty acids	*i*15:0, *ai*15:0, 15:0, *i*16:0, 16:1ω9, 16:1ω7t, *i*17:0, *ai*17:0, 17:0, *cyc*-17:0, 18:1ω7, *cyc*-19:0
Gram-negative	Cyclopropyl and Mono-unsaturated	16:1ω9c, 16:1ω7c, 16:1ω7t, 16:1ω5c, 18:1ω9c, 18:1ω7c 18:1ω7t, 17:0c
Gram-positive	Terminally branched	*i*15:0, *ai*15:0, i16:0, *i*17:0, *a*17:0
Actinomycetes	Methyl branching on the 10^{th} carbon	10:Me16:0, 10Me17:0, 10Me18:0
Eucaryotes, particularly fungi	Polyunsaturated, straight chain	18:2ω6,9

2.5 Data analysis by statistical methods and indices

Data obtained by all the techniques mentioned above was statistically evaluated using software STATISTICA 8.0. Significant differences between different treatments and sites were determined by one-way analysis of variance (ANOVA). Repeated measures ANOVA was used for observation of the development of soil respiration over time. Principal component analysis was a useful approach to a statistical analysis of the PLFA profiles, soil respiration and enzyme activities data. This method is based on a reduction of independent variables such as fatty acid G.C. peaks, and data of soil respiration and enzyme activities after an addition of eight substrates to a smaller set of uncorrelated variables that contain most of the information of the original variables.

The soil respiration data were applied for calculation of the resistance and the resilience of soil against a disturbance (addition of xenobiotics) according to Orwin and Wardle's indices (2004).

3 RESULTS

3.1 Experiment 1 (Sk)

3.1.1 Soil chemical properties

Soil chemical properties including pH, soil moisture, C_{ox}, N_t, C:N ratio, and soil texture, were analysed to characterise the sampling sites . All soils were grassland soils. Table 3.1 shows slight variation in the soil texture and the soil moisture. Soil textural classifications included sandy loam soil in the Greater Fatra, Lesser Fatra and Low Tatras and loamy sand soil in the Slovak Ore Mountains. Soil moisture content varied between sites. The loamy sand soil had the lowest moisture content. Soil pH ranged from 4.86 to 5.33 (Table 3.1). The most acidic soil was located at Lower Tatras.

Table 3.1 Mean values of the analysed characteristics for each field. Standard errors are in brackets.

Parameter	Field			
	Greater Fatra (VF)	Lesser Fatra (MF)	Lower Tatras (Do)	Slovak Ore Mountains (Du)
pH	5.16 (0.01)	5.32 (0.07)	4.90 (0.02)	5.08 (0.01)
Moisture (%)	23.48	27.64	19.36	12.02
C_{ox}	6.75 (0.04)	3.98 (0.09)	5.10 (0.11)	2.49 (0.11)
C:N	13.04 (0.08)	10.79 (0.24)	13.01 (0.29)	13.70 (0.58)
Soil texture	sandy loam	sandy loam	sandy loam	loamy sand

Legend: C_{ox} – oxidizable carbon, C – carbon. N – nitrogen

C_{ox} (oxidizable carbon) values ranged from 2.49 to 6.75. There were significantly different ($P<0.05$) Cox values between all the fields (Table 3.1). The site with the highest C_{ox} was Greater Fatra (VF). However, as can be seen from the Table 3.1, there was no statistically significant difference ($P>0.05$) in the carbon to nitrogen ratio between the locations Greater Fatra, Lower Tatras (Do) and Slovak Ore Mountains. This ratio was significantly lower ($P\leq0.05$) in the location Lesser Fatra.

3.1.2 Microbial biomass data

The results of the microbial biomass measurement are shown in the Figure 3.1.

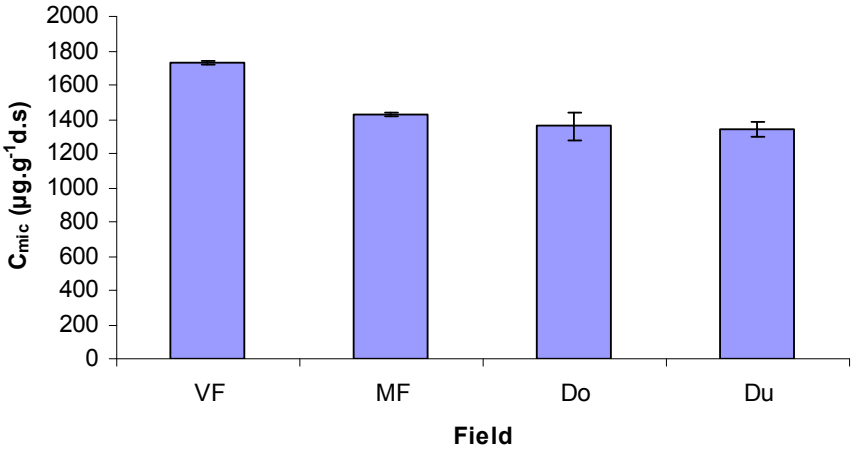


Figure 3.1 Microbial biomass data for each site of sampling. The bars show standard error (n = 3).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains

There was a significantly greater ($P<0.05$) microbial biomass at Greater Fatra, compared to the other sampling sites (Figure 3.1). There was no significant ($P>0.05$) difference between the sites MF, Do or Du.. The mean values of microbial biomass ranged from 1070.41 to 1728.38 µg $C.g^{-1}$ d.s.

The ratio of microbial biomass to the amount of total or oxidizable carbon (Figure 3.2) is an important indicator of the ecosystem's sensitivity to different changes in the ecosystem (Insam and Domsch, 1988; Anderson, 2003; Růžek *et al.*, 2004). In the sites of sampling, the ratio ranged from 2.56 to 5.42. The lowest and similar values of the ratio were found in the areas Greater Fatra (2.56) and Lower Tatras (2.65). These two locations were significantly different ($P<0.05$) from the more sensitive areas, Lesser Fatra and Slovak Ore Mountains. The area Slovak Ore Mountain had not only the highest value of the ratio C_{mic}/C_{ox} of all the locations but also the greatest variation among the areas of sampling.

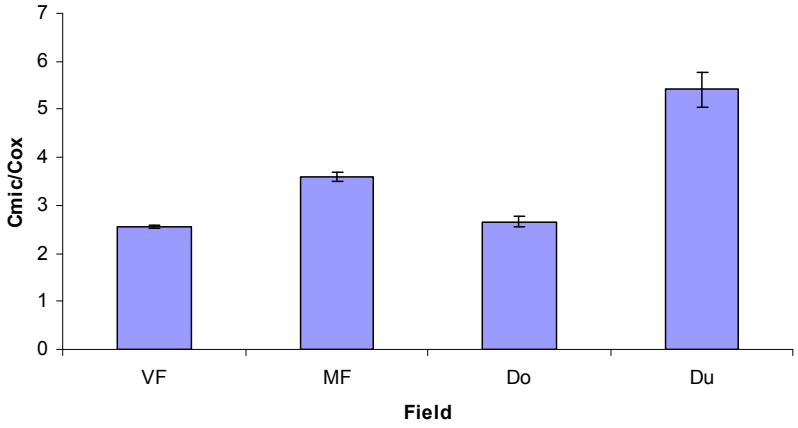


Figure 3.2 Mean values of ratio Cmic/Cox. The bars show standard error (n = 3).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains

3.1.3 Microbial activities data

Results of the hydrolytic enzyme activity and soil respiration were analysed using Analysis of Variance (ANOVA). Dehydrogenase activity (DHA) was analysed after eight week of pre-incubation (4 ^{o}C). The enzyme activity of the areas ranged from 13.65 to 22.92 μg TPF.g^{-1}.d.s.h^{-1}. Statistically significant differences ($P<0.05$) were observed between the sites Lesser Fatra and Donovaly, which had similar values, and the sites of Greater Fatra and Slovak Ore Mountains. The greatest hydrolytic activity was present in Slovak Ore Mountains (Figure 3.3).

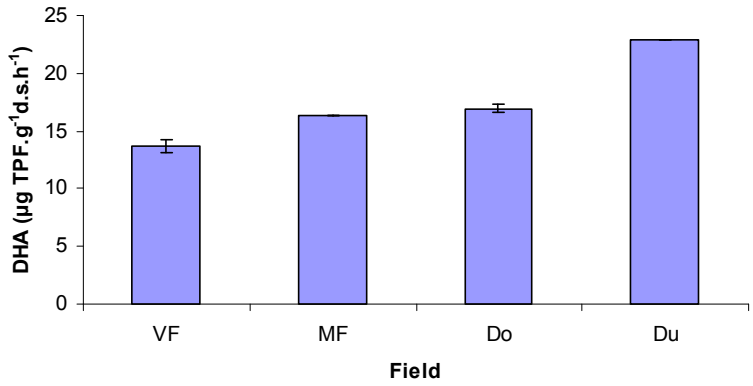


Figure 3.3 Dehydrogenase activities values for each field. The bars show standard errors (n = 3).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains, TPF – triphenylformazan, DHA – dehydrogenase activity, d.s. – dry soil

The respiration of soil treated with polyvinylalcohol, Fundazol, Gesagard, and glucose were analysed at the beginning of the experiment (24 hours after addition of xenobiotics) and at the end of experiment (3 weeks incubation with treatments). Results were compared to an untreated control.

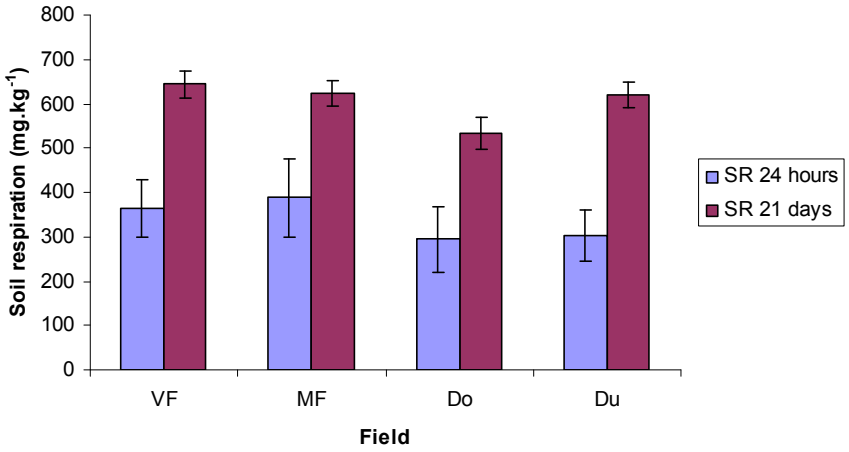


Figure 3.4 Soil respiration (SR) of each field measured at the beginning (24 hours) and at the end (21 days) of experiment. The bars show standard errors (n = 4).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains

Figure 3.4 shows a statistically significant increase ($P<0.05$) in the soil respiration after 21-day incubation of the soil samples. There were no statistically significant differences ($P>0.05$) between the sites at the beginning of the experiment. However, a statistically significant decrease ($P<0.05$) was found in the area Lower Tatras after 21 days of incubation of soil samples with the treatments. The main reason for this decrease was a significantly lower rate of the soil respiration within all treatments compared to the other sites.

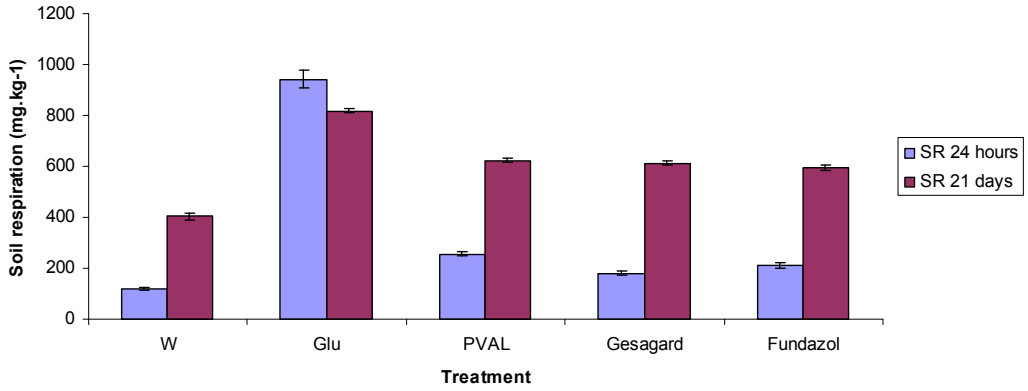


Figure 3.5 Soil respiration (SR) of each treatment measured at the beginning (24 hours) and at the end (21 days) of experiment. The bars show standard errors (n = 4).

Legend: W – control sample, Glu – glucose, PVAL - polyvinylalcohol

With the exception of glucose, soil respiration was higher for all treatments after 21 days of incubation compared to the 24h incubation results (Figure 3.5). Where soil was treated with glucose, respiration declined over the 21 days of incubation. All the analysed treatments (Fundazol, Gesagard and PVAL) influenced the process of the soil respiration significantly ($P<0.05$) n comparison to the control sample. The highest increase of the soil respiration could be found in the soil samples with an addition of glucose which were significantly different ($P<0.05$) from the other analysed treatments.

The ratio of basal respiration to microbial biomass data (metabolic quotient) is another indicator of microbial stress associated with changes in environmental conditions. The values of the respiratory ratio (Figure 3.6) reached similar low values for all the fields. The greatest variation was in the soil sampled from the location Lesser Fatra.

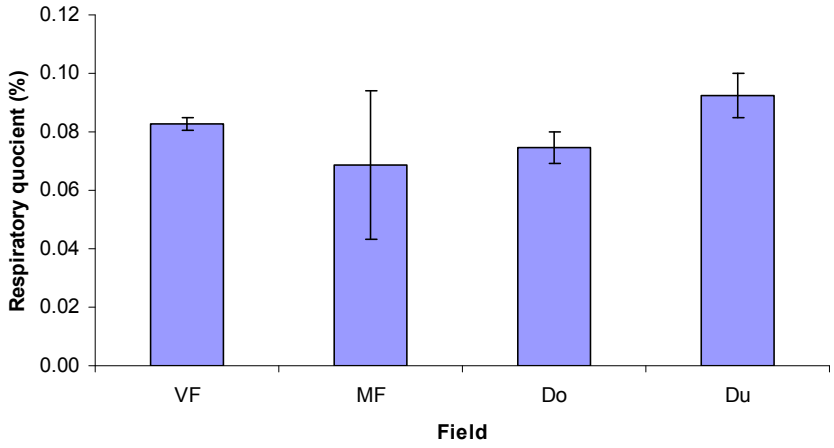


Figure 3.6 The metabolic quotient (%) for each field. The bars show standard errors (n=3).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains

Table 3.2 The resistance index for each treatment in total and within fields

Field	Treatment	Resistance
Greater Fatra	PVAL	0.37
	Gesagard	0.43
	Fundazol	0.40
Lesser Fatra	PVAL	0.26
	Gesagard	0.24
	Fundazol	0.30
Lower Tatras	PVAL	0.13
	Gesagard	0.14
	Fundazol	0.19
Slovak Ore Mountains	PVAL	0.41
	Gesagard	0.46
	Fundazol	0.58
Total	PVAL	0.30
	Gesagard	0.32
	Fundazol	0.37

The indices of resistance and resilience were calculated according to Orwin and Wardle (2004). The mean values of resistance for each treatment within all fields are given in Table 3.2. Generally, there could be seen high resistance of soil samples against the treatments. The resistance index reached relatively similar values for samples treated by PVAL, Gesagard and Fundazol. The soil sampled from location Lower Tatras seemed to be less resistant against all three applicated treatments than other locations. The highest resistance was present in the area Slovak Ore Mountains.

3.1.4 Microbial community structure

Eighteen PLFAs including saturated, unsaturated, methyl-branched and cyclopropane (14:0, c14:1, 3OH 14:0, i15:0, ai15:0, i16:0, 16:1w5, 17:0, Me i17:0, i17:0, ai17:0, 17:1, 18:0, 18:2w6,9, 18:1w9c, 18:1w7t, 19:1, 19:0c) , were used to compare the sites and the effects of pesticide treatment.

Figure 3.7 shows complete comparison of PLFAs of all fields and treatments. According to the both main principal components, the grouping of soil sampled from locations Greater Fatra and Lesser Fatra could be seen. The exception was soil sampled from Lesser Fatra and treated by Gesagard (MF G) which was separated from others samples of Lesser Fatra according to PCA 2. Some of samples of Lower Tatras, specifically samples treated by Fundazol and Gesagard, were separated from the areas Lesser Fatra and Greater Fatra according to PCA 1. According to the PCA 1, the samples of area Slovak Ore Mountain was completely separated from the locations Lesser Fatra and Greater Fatra. PCA 2 grouped almost all samples of all fields into one group, exception the soil sampled from location Lower Tatras and treated by Fundazol, polyvinylalcohol as well as the control sample from the same area. In location Slovak Ore Mountain the separation of soil treated by Fundazol and polyvinylalcohol from the control sample was found. The samples from Greater Fatra and Lesser Fatra showed separation only soil sampled from Lesser Fatra and treated by Gesagard. In the area Lower Tatras, separation of all observed treatments from the control sample could be seen. These results are in more details described separately for fields and treatments in the Figure 3.8 and the Figure 3.10, respectively.

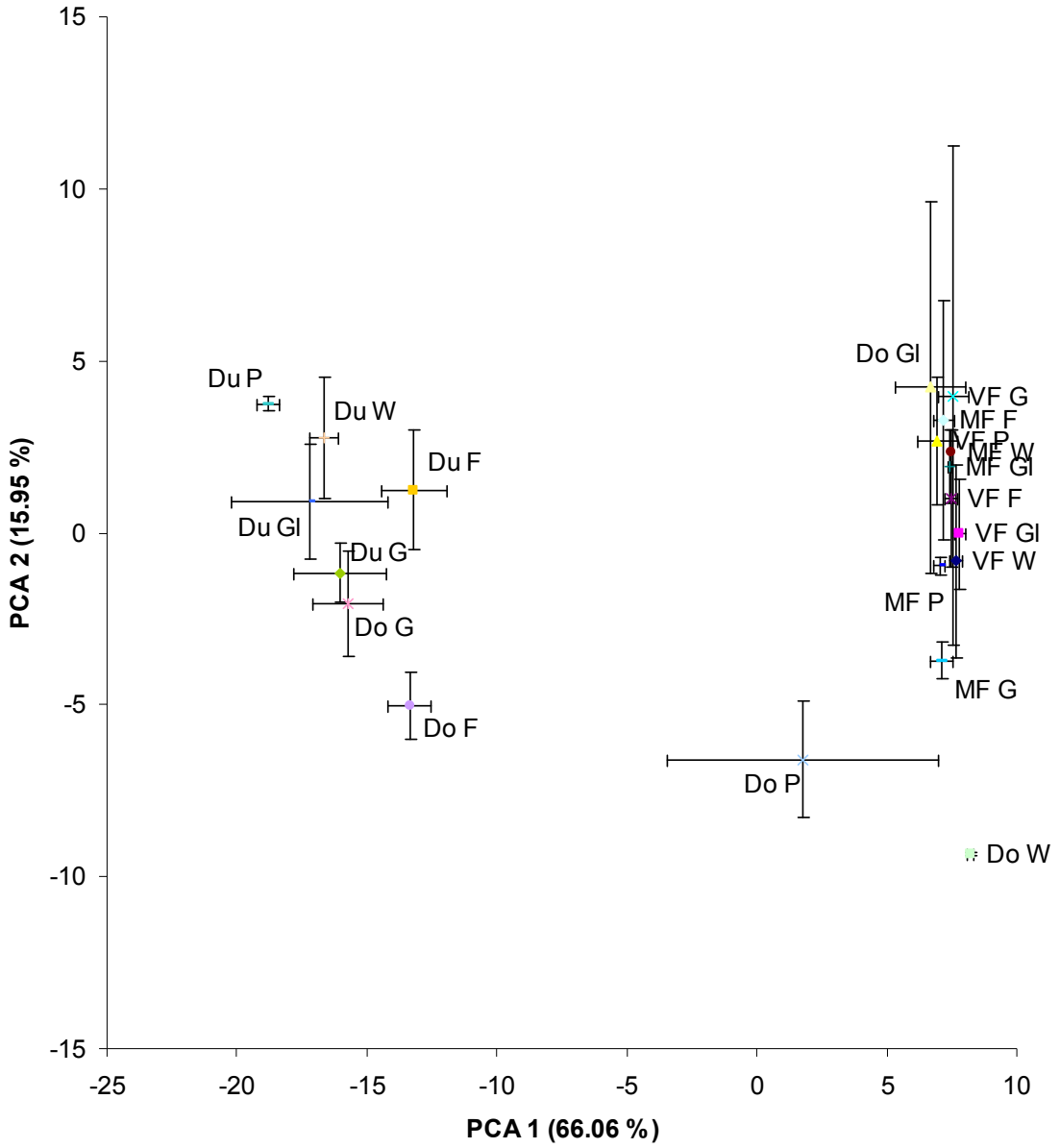


Figure 3.7 Evaluation of principal component analysis using factorial ANOVA for each field and treatment. The bars show standard errors (n = 4).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lowerr Tatras, Du – Slovak Ore Mountains, W – control sample, Gl – glucose, P – polyvinylalcohol, G – Gesagard, F - Fundazol

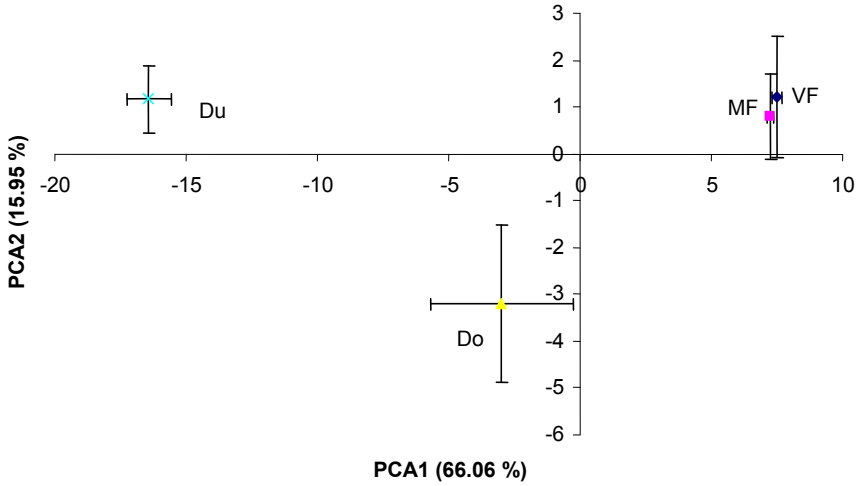


Figure 3.8 Evaluation of principal component analysis using ANOVA for each field. The bars show standard errors (n = 4).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains

A shift in the microbial composition was evident when the PLFA patterns of samples from different sites were compared by the Principal Component Analysis (Figure 3.8). The sites could be separated into three distinct groups, where 66.06 % of the variation was accounted for the PCA 1. The areas Lesser Fatra (MF) and Greater Fatra (VF) were similar, with a greater variation in Greater Fatra. There were significant differences ($P<0.05$) between areas Lower Tatras (Do) and Slovak Ore Mountains (Du) determined by ANOVA.. The PLFAs 16:1ω5 and 19:0c were mainly responsible for the separation of these sites (Figure 3.9). According to the second component PCA 2, which accounted for 15.95 %, the locations Greater Fatra, Lesser Fatra and Slovak Ore Mountains were grouped into one cluster. The separation of site Lower Tatras was confirmed by a statistical Fisher LSD test ($P\leq0.05$).

The PCA of grassland soils with the different treatments, according to the first (66.06 %) and second component (15.95 %) showed that the treatments had no significant effect on the soil microbial community (Figure 3.10). However, the treatment with herbicide Gesagard seemed to be, not significantly, slightly separated from the control sample.

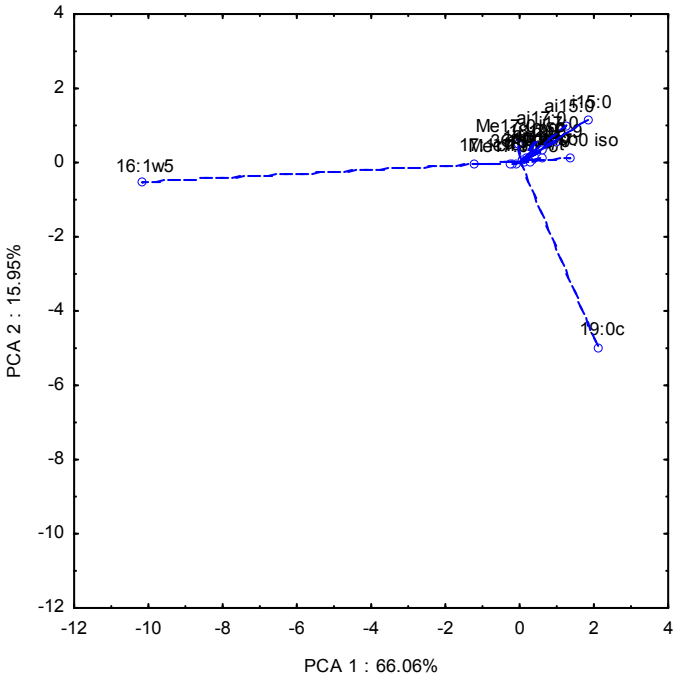


Figure 3.9 Results of the PCA analysis for the PLFA analysis showing the main variables causing the separation of the field data.

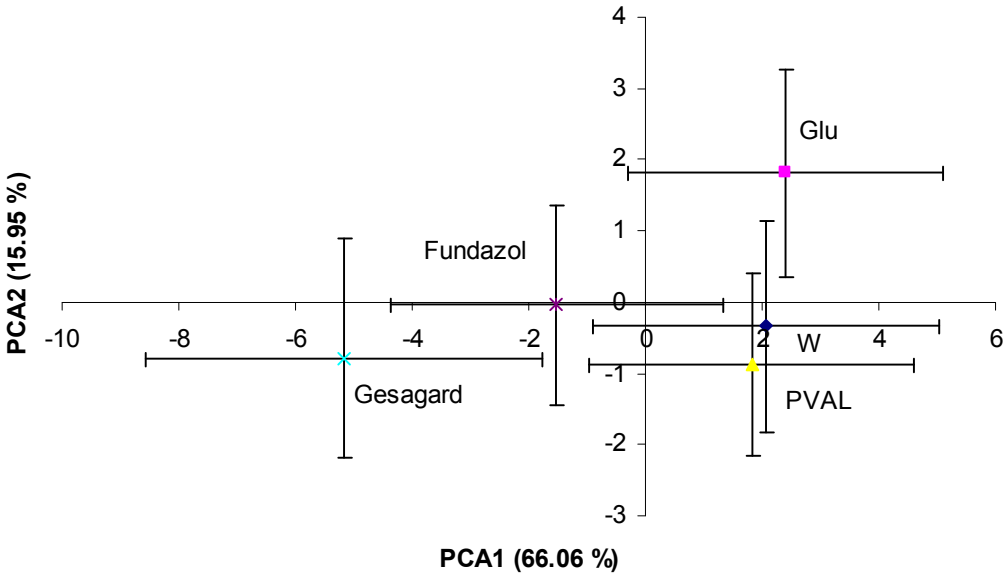


Figure 3.10 Evaluation of principal component analysis using ANOVA for each treatment. The bars show standard errors (n = 4).

Legend: W – control sample, Glu – glucose, PVAL - polyvinylalcohol

The sum of PLFA characteristics of general bacteria, G^+ bacteria, G^- bacteria, actinomycetes, and fungi were used as broad taxonomic microbial groupings. The

PLFAs used for calculation of relative abundance of those groups are present in the Table 2.1. Relative concentrations of these taxonomic groups were analysed using one-way ANOVA. The lowest percentage abundance of almost all the observed groups of microorganisms, with an exception of fungi, could be found in soil sampled from the site Slovak Ore Mountain (Figure 3.11). The group of fungi was present in the lowest abundance in the area Lower Tatras. The high-abundant groups of all the analysed microorganisms were present in the locations Greater and Lesser Fatra. Soil sampled from location Greater Fatra (72.19 %), Lower Tatras (65.35 %) and Lesser Fatra contained the greatest abundant of bacteria. The highest number of the fungi could be found in the locations Greater Fatra (6.48 %) and Lesser Fatra (5.16 %).

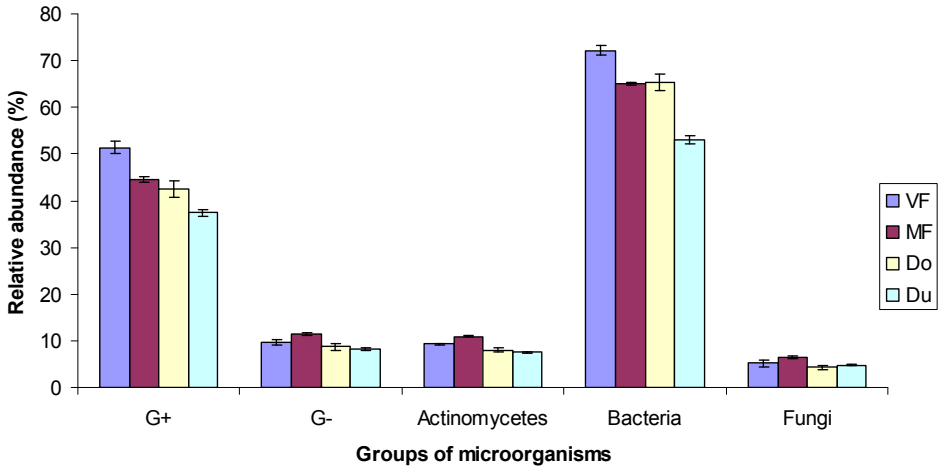


Figure 3.11 Tentative abundance (%) of observed groups of microorganisms for each field. The bars show standard errors (n = 4).

Legend: VF - Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountains, G^+ - gram positive bacteria, G^- - gram negative bacteria

The mol % of PLFAs was similar for the control sample and the sample with added glucose. There was a trend showing a lower relative abundance of all the determined groups of soil microorganisms in samples treated with herbicide Gesagard. The samples treated with Fundazol and PVAL were significantly different ($P<0.05$) in a comparison to the control samples (Figure 3.12). Other groups of microorganisms did not show differences of relative abundance in the samples treated by Fundazol. However, the treatment with PVAL also significantly affected the group of G^- microorganisms.

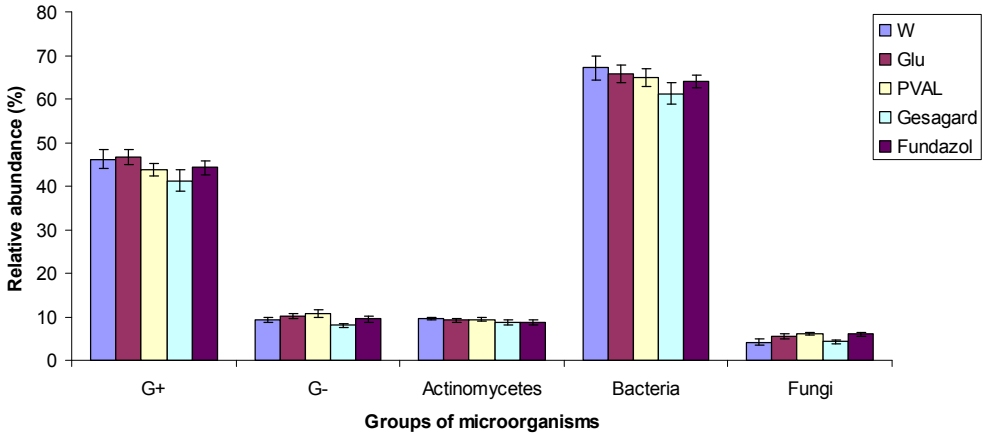


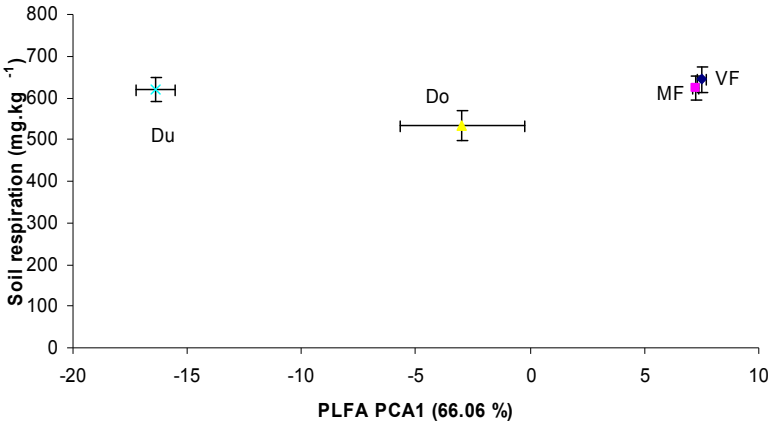
Figure 3.12 Tentative abundance (%) of observed groups of microorganisms for each treatment. The bars show standard errors (n = 4).

Legend:W – control sample, Glu – glucose, PVAL - polyvinylalcohol, G^+ - gram positive bacteria, G^- - gram negative bacteria

Figure 3.13 represents a means plot of the values of soil respiration ($mg.kg^{-1}$) against PLFA PCA1 for each field (Figure 3.13a) and treatment (Figure 3.13b). of different fields, it could be seen that the sites Greater Fatra and Lesser Fatra were still closely grouped together in both variable planes. According to the values of soil respiration, the area Slovak Ore Mountain grouped with the mentioned areas into one cluster. The remaining area (Lesser Tatras) was significantly separated from the all other fields according the both variables – values of soil respiration and PLFA PCA1.

All the analysed treatments (PVAL, Fundazol and Gesagard) were closely grouped, according to both variable planes, into one cluster. The values of soil respiration showed the separation all analysed treatments in comparison to the control sample as well as the sample treated with glucose. However, there was only a little separation between the treatment with Gesagard and the control sample. The remaining treatments were grouped together with the control sample and the sample with the addition of glucose according to the values of soil respiration.

a,



b,

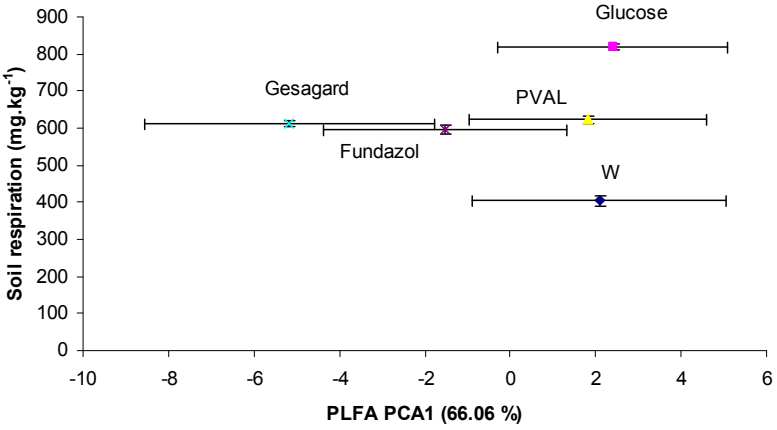


Figure 3.13 Comparison of the values of soil respiration and PLFA PCA1 factor for each field (a) and treatment (b). The bars show standard errors (n = 3).

Legend: VF – Greater Fatra, MF – Lesser Fatra, Do – Lower Tatras, Du – Slovak Ore Mountain

3.2 Experiment 2 (UK)

3.2.1 Soil chemical properties

Silsoe soil was characterised by measurement of pH, soil moisture, WHC, C_{ox}, N_t, the ratio C:N, and soil texture. The soil texture varied from loamy sand soil in the series Cottenham, through sandy clay loam soil in the series Faulkborne, to clay loam soil in the series Denchworth. The moisture content was higher in the sites Faulkborne (21.2) and Denchworth (25.9). The water holding capacity values ranged from 25.56 % (Cottenham) to 31.62 % (Denchworth).

The pH of different areas (Figure 3.14) ranged from neutral to slightly alkalic pH (from pH 6.9 to 8.0). The most alkalic pH was in the series Faulkborne.

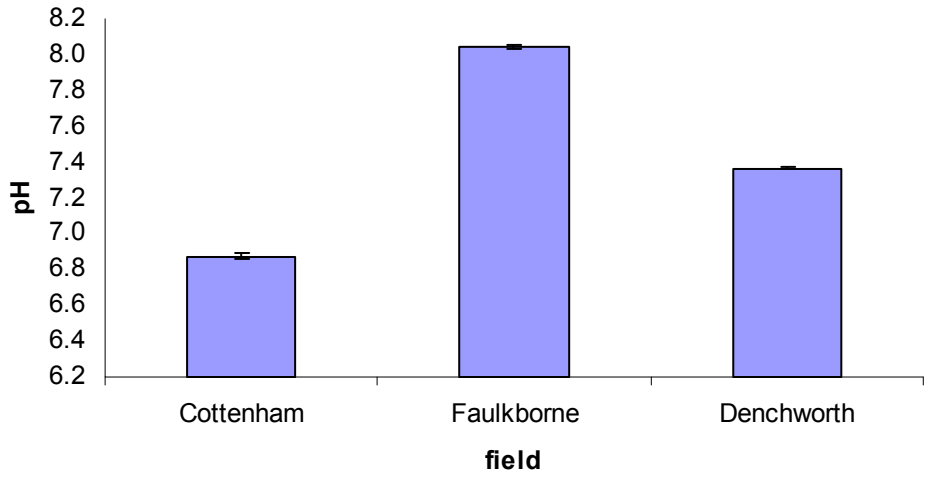


Figure 3.14 The pH for each series. The bars show standard errors (n = 3).

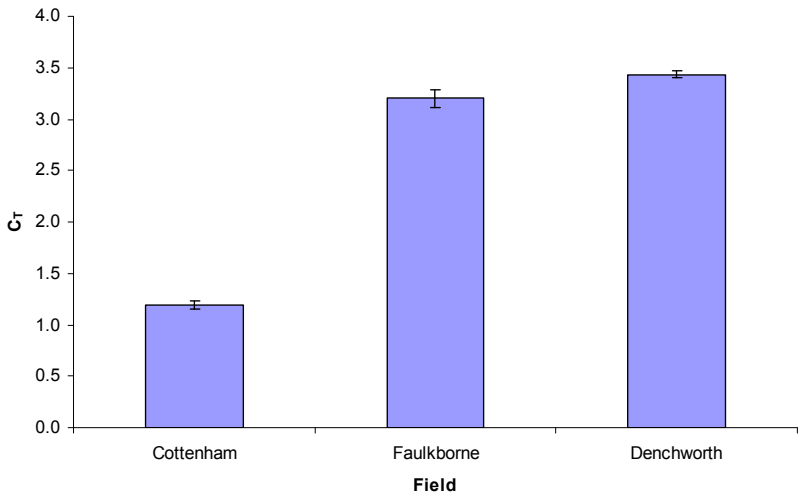


Figure 3.15 The values of total carbon (C_T) in percentage for each series. The bars show standard errors (n = 3).

The values of C_T ranged from 9.7 to 10.1. A significant difference ($P<0.05$) was found between the series Cottenham and the remaining two sites (Figure 3.15). The values were similar in the series Faulkborne and Denchworth which had the higher values than Cottenham. The Figure 3.16 shows statistically significant differences ($P<0.05$) of carbon to nitrogen ratio between all fields. The lowest ratio was found in the series Denchworth.

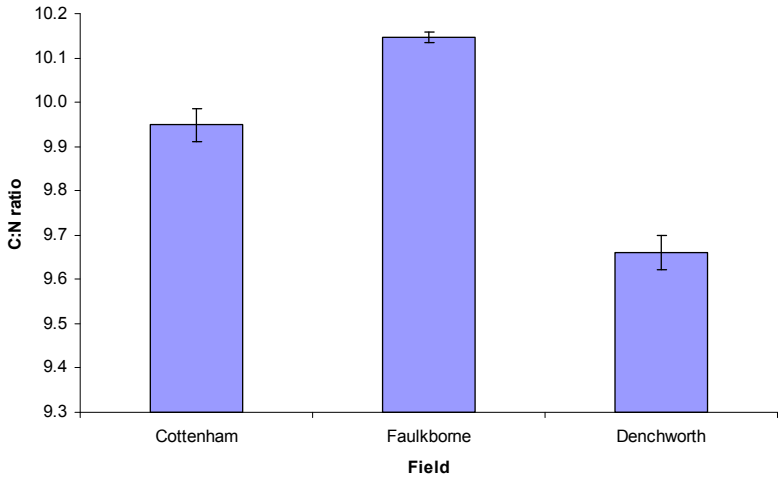


Figure 3.16 The values of ratio carbon to nitrogen (C:N) for each series. The bars show standard errors (n = 3).

3.2.2 Microbial biomass data

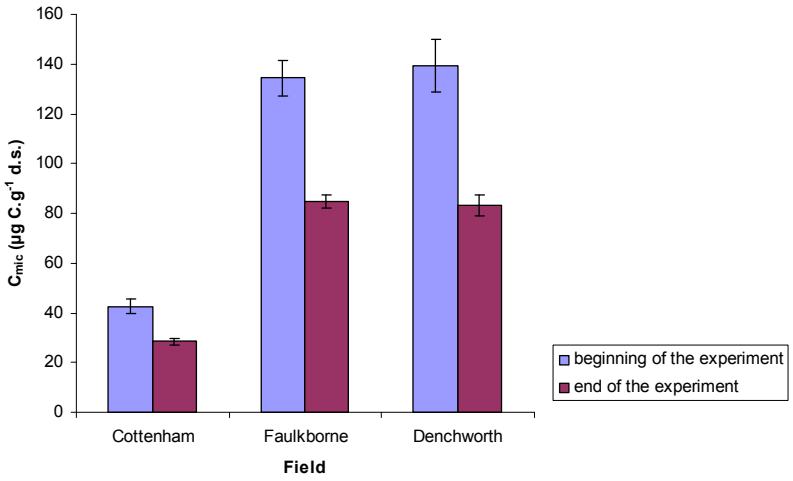
Figure 3.17 shows the values of microbial biomass carbon for each field (Figure 3.17a) and treatment (Figure 3.17b) at the beginning as well as at the end of the experiment.

A significant difference ($P<0.05$) was found between the beginning and the end of the experiment for both treatments and fields. The higher values of microbial carbon were determined at the beginning of the experiment. The series Cottenham had significantly lower values ($P<0.05$) in comparison to Faulkborne and Denchworth, which had similar results.

Microbial biomass in the field soils ranged from 28.36 to 139.38 μg C. g^{-1} d.s. Figure 3.17b shows the variation of microbial biomass values for each treatment, sample with added glucose, and the control sample. The results of microbial biomass ranged from 54.25 to 151.82 μg C. g^{-1} d.s. within the treated samples. The treatment with PVAL and Gesagard affected the values of soil microbial carbon immediately after their addition, which could be seen on increase of the values in comparison to the control sample. The application of Fundazol did not alter the biomass of microbial community. The addition of PVAL slightly increased the microbial biomass after six week incubation. The samples with added Fundazol reached similar values to the control sample. The sampled

treated with Gesagard had lower values in comparison to control sample at the end of the experiment.

a,



b,

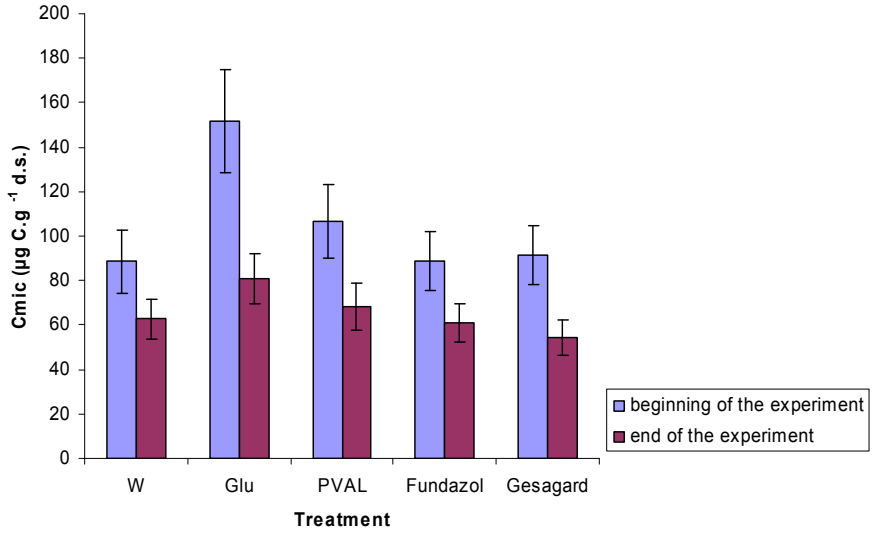


Figure 3.17 The values of microbial carbon for each series (a) and for each treatment (b). The bars show standard errors (n = 3).

Legend: C_{mic} – microbial carbon, W – control sample, Glu - glucose

The ratio of microbial carbon to total carbon ranged from 0.28 to 0.38 %. Statistically significant differences ($P<0.05$) were found between all fields (Figure 3.18). The highest percentage of the ratio was in the soil sampled from the series Faulkborne.

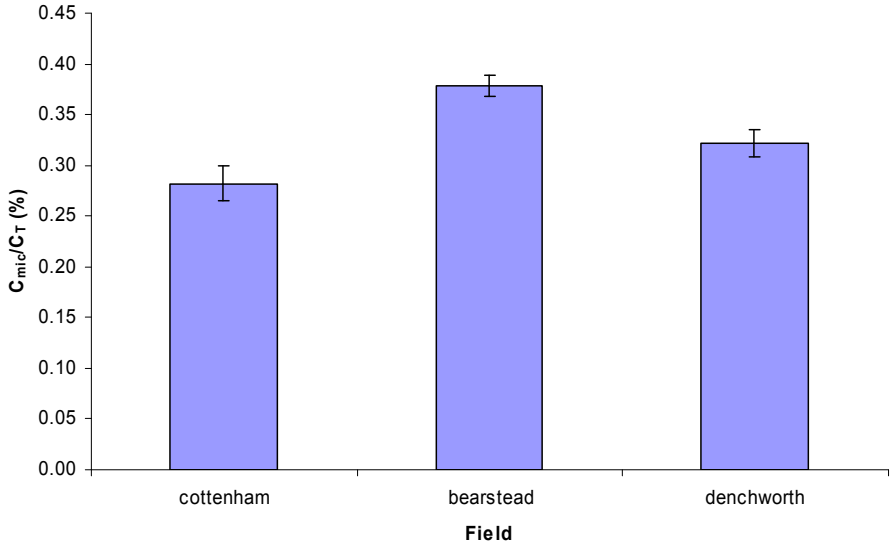


Figure 3.18 The microbial carbon to total carbon ratio (C_{mic}/C_T) for each series. The bars show standard errors (n = 3).

3.2.3 Soil respiration and functional stability

The results of soil respiration obtained after an addition of eight different substrates: water (W), glucose (Glu), arginine (Arg), citric acid (CitA), malic acid (MalA), ketoglutaric acid (aKG), amino butyric acid (aBA), and acetyl glucosamine (AgI), were statistically evaluated by Principal Component Analysis (PCA). The first and second factor scores show a soil textural classification effects on microbial community composition. Soil from the Cottenham series was significantly different to other soils on both PC1 and PC2 (Figure 3.19). The second factor score accounted for 14.94 % of the variation. The Faulkborne and Denchworth soils were separated on the second axis. Mean values of PCA 1 factor (49.19 %) grouped these two fields into one cluster.

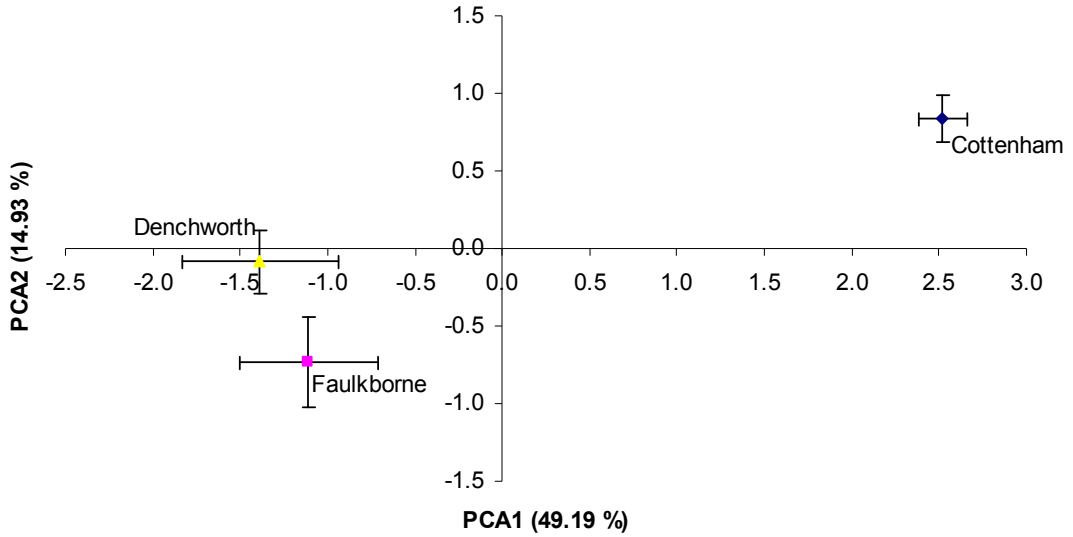


Figure 3.19 Mean PCA values of soil respiration for each series. The bars show standard errors (n = 3).

Figure 3.20 shows the plotting of all carbon substrates which were used for evaluating the obtained data. It can be seen that the separation of series Cottenham as well as the grouping of remaining two fields is caused mainly by glucose values. Malic acid, citric acid and arginine had a lesser effect.

The values of soil respiration were also analysed for each treatment at different stages of experiment. Abbreviations of treatments were used for greater clarity of cases plotting. Abbreviations were W for water, Gl for glucose, P for polyvinylalcohol, F for Fundazol, and G for Gesagard. The number next to the letter represents the date of the experiment, 1 means measurement from fresh soil, 2 is the beginning of the experiment and 3 is for values obtained at the end of the experiment.

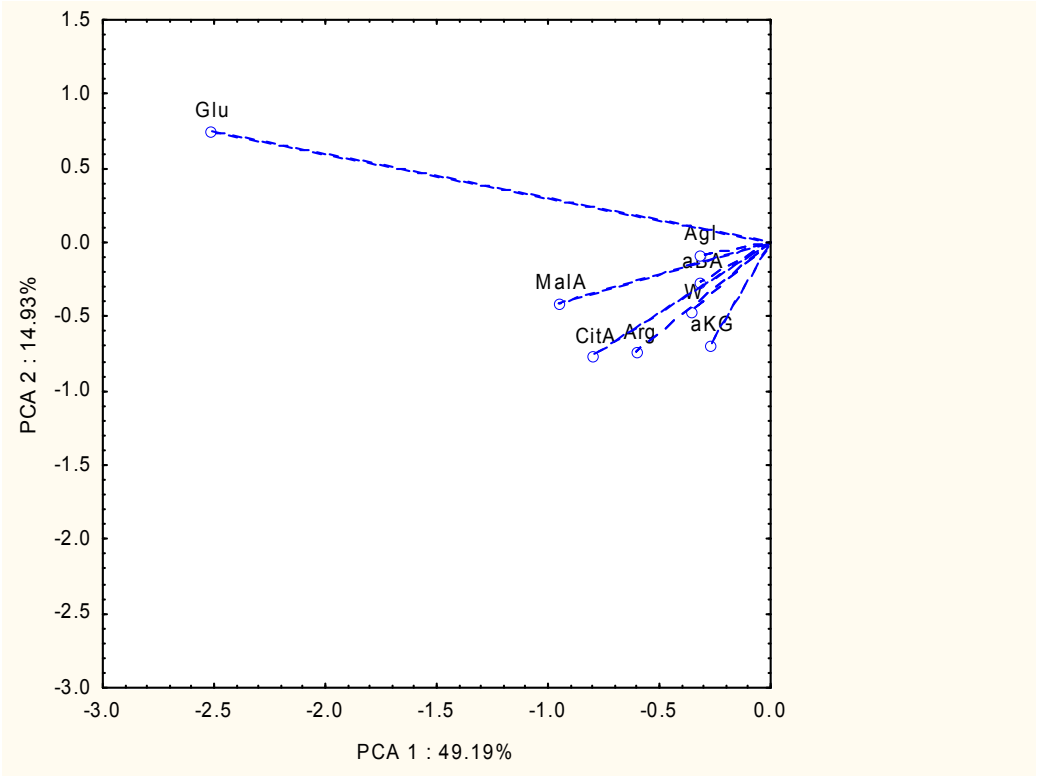


Figure 3.20 Results of the soil respiration PCA analysis showing the variable plot.

Legend: W - water, Glu - glucose, Arg - arginine, CitA - citric acid, MalA - malic acid, aKG - ketoglutaric acid, aBA - amino butyric acid, AgI - acetyl glucosamine

The plot of PCA factor scores for each series (Figure 3.21) show that PC1 accounted for 37.24 % of variation. ANOVA of the first component suggests that the microbial community of Gesagard 2, PVAL 2, PVAL 3, glucose 2, glucose 3, Fundazol 2, and Fundazol 3, are the same as the control sample throughout the experiment. There was a separation of the mean values of the control sample and Gesagard measured at the end of the experiment. A significant separation ($P<0.05$) was found between samples treated with Fundazol, PVAL and glucose, and the control sample. The greatest effect on the separation of mentioned treatments from the control sample had glucose, citric acid and malic acid (Figure 3.22). For this reason, these three substrates are described below in more details (Figure 3.23). Ketoglutaric acid, arginine and water had a lesser effect on the separation.

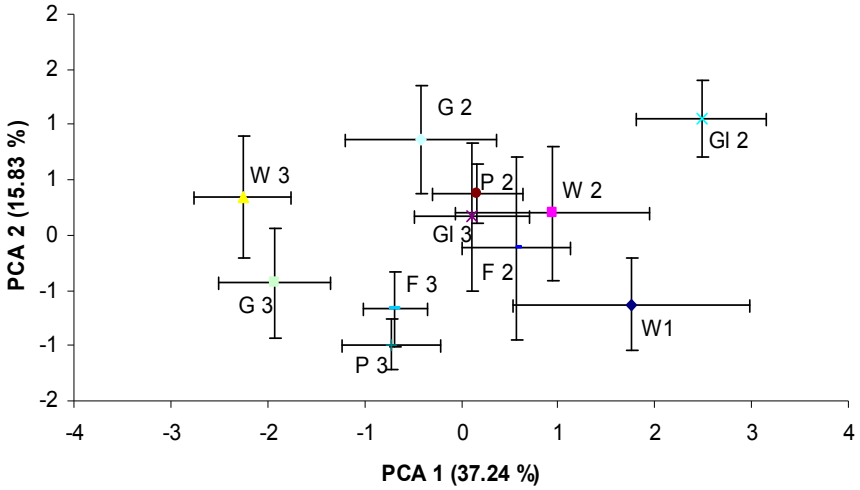


Figure 3.21 Mean PCA values of soil respiration for each treatment determined at the different stages of experiment. These values are average values of all fields. The bars show standard errors (n = 3).

Legend: W – control sample, Gl – glucose, P – polyvinylalcohol, G – Gesagard, F – Fundazol, 1 – measurement from fresh soil, 2 – measurement at the beginning of the experiment, 3 – measurement at the end of experiment

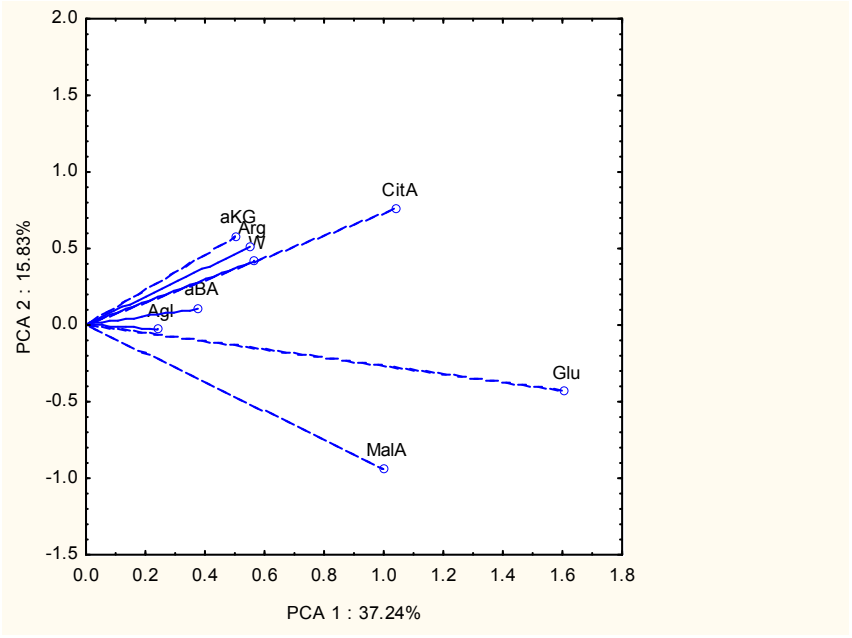
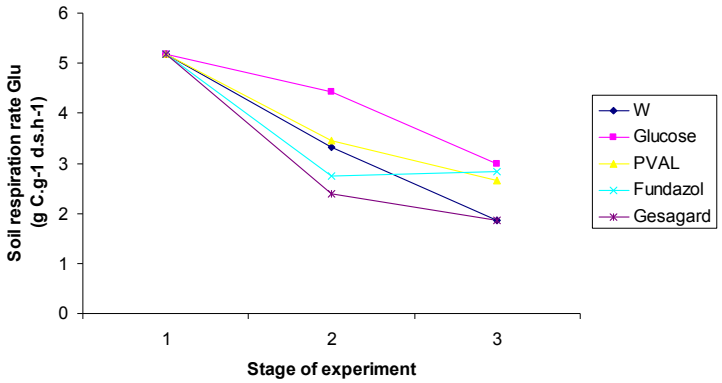


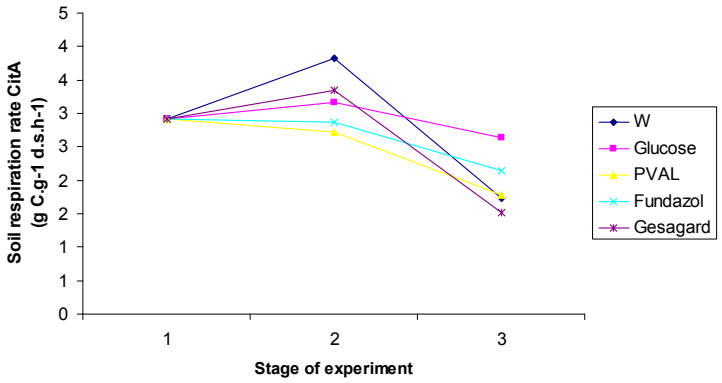
Figure 3.22 Results of the soil respiration PCA analysis showing the variable plot.

Legend: W - water, Glu - glucose, Arg - arginine, CitA - citric acid, MalA - malic acid, aKG - ketoglutaric acid, aBA - amino butyric acid, AgI - acetyl glucosamine

a,



b,



c,

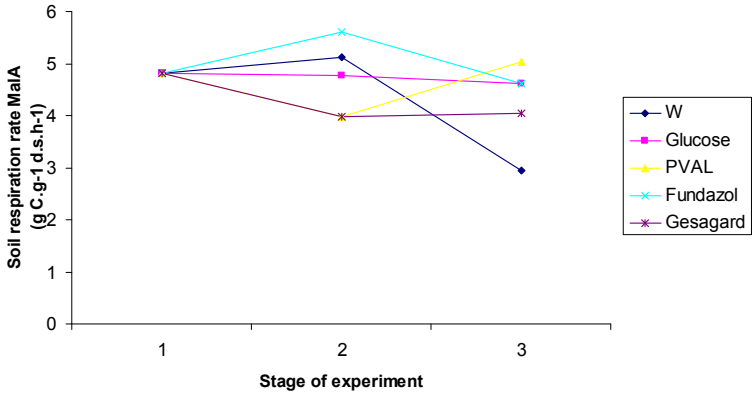


Figure 3.23 The trend lines of the soil respiration rate which was measured after addition of glucose (a), citric acid (b) and malic acid (c) as catabolic substrate for each treatment at the different stages of experiment. Different colours mean different treatment.

Legend: Glu – glucose, CitA – citric acid, MalA – malic acid, W – control sample, PVAL - polyvinylalcohol

Where glucose was used as a carbon substrate in the catabolic profile (Figure 3.23a), a decreasing tendency of soil respiration rate could be seen over time exception Fundazol. There was no significant difference ($P>0.05$) between the treatments and the control sample at the beginning of the experiment. Nevertheless, the treatments Fundazol and PVAL significantly increased ($P<0.05$) the rate of soil respiration at the end of the experiment. Similarly, the rate of soil respiration was increased by the addition of glucose.

Citric acid (Figure 3.23b) resulted in a decrease of the rate of soil respiration at the end of the experiment. There was no significant difference ($P>0.05$) between the treatments and control samples at the beginning of the experiment. The main reason was a great variation (1.20) of the values of the control sample (data not shown). However, the soil respiration rate of the treated samples was lower than of the control sample. At the end of the experiment, the treatment of glucose, PVAL and Fundazol, increased the rate of the soil respiration, although not significantly ($P>0.05$).

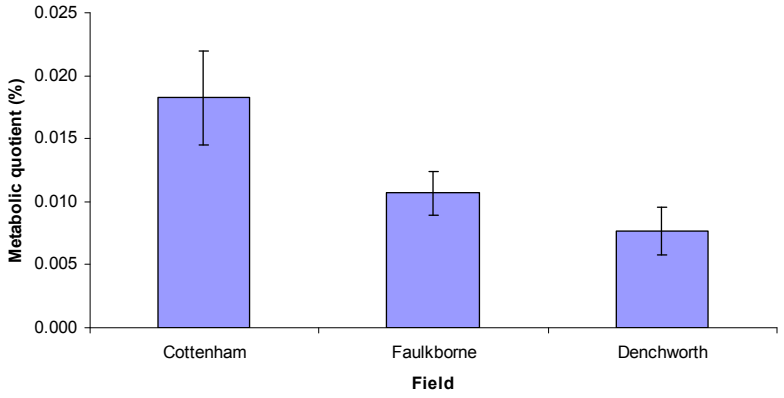


Figure 3.24 The values of the metabolic quotient (%) for each series. The bars show standard errors.

In the case malic acid (Figure 3.23c), an increase of soil respiration in samples treated by PVAL could be seen at the end of the experiment in comparison to the values obtained at the beginning of the experiment. The treatment with Fundazol showed an opposite tendency. The values of soil respiration did not alter after an application of

Gesagard and glucose. All treatments increased the rate of soil respiration at the end of the experiment in comparison to the control sample.

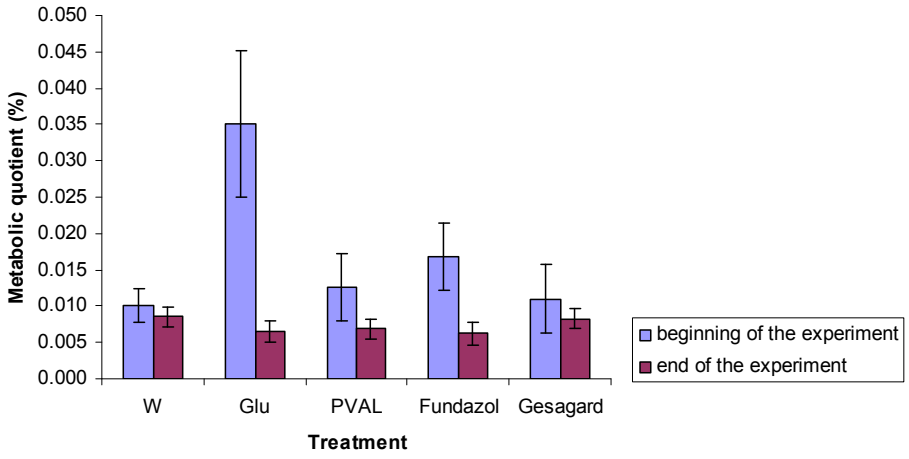


Figure 3.25 The average values of the metabolic quotient (%) for each treatment. The bars show standard values.

Legend: W – water, Glu – glucose, PVAL - polyvinylalcohol

The metabolic quotient reached low values. There was significant difference ($P<0.05$) between the series Denchworth and the remaining two fields (Figure 3.24). The highest amount was found in the series Cottenham. The metabolic quotient showed a decreasing tendency between values measured at the beginning and at the end of the experiment for each treatment. However, a significant difference between the treatments and the control sample was not found. Merely the addition of glucose showed an immediate increase of the soil respiration rate.

The indices of resistance as well as the resilience were calculated for each treatment within the each series (Table 3.3). The soil sampled from series Denchworth seemed to have the lowest resistance within all fields, especially the treatment with Fundazol and Gesagard. The Cottenham soil showed the highest resistance of all of treatments with PVAL and Fundazol. However, the highest resistance against Gesagard was found in the series Faulkborne.

Table 3.3 The resistance index of each treatment within all observed fields

Series	Treatment	Resilience
Cottenham	PVAL	0.76
	Fundazol	0.89
	Gesagard	0.81
Faulkborne	PVAL	0.52
	Fundazol	0.27
	Gesagard	0.88
Denchworth	PVAL	-0.08
	Fundazol	-0.17
	Gesagard	-0.19

3.2.4 Soil enzyme activities data

Eight enzyme substrates were used to analyze the soil sampled from different fields and their treatment variables. The results were evaluated by PCA analysis (Figure 3.26). According to the PCA 1, which covered 41.97 % of variation of the obtained data, the series Cottenham seemed to be separated from the series Denchworth and Faulkborne, which were grouped into one cluster. The greatest effect on the separation of the series Cottenham had the substrates (Figure 3.27) 4-MUF-N-acetyl-β-glucosaminide (S2), 4-MUF-β-D-glucoside (S3), 4-MUF-phosphate (S4). The lesser effect had 4-MUF-β-D-cellobioside, 4-MUF-β-D-xyloside, 4-MUF-β-D-galactopyranoside, and 4-methylumbelliferyl sulphate. The PCA 2 factor (26.69 %) did not show any variation, all series belonged to the same group.

The PCA analysis of hydrolytic enzyme activity was used for determination of the effect of different treatments. However, as can be shown from the Figure 3.28 all treatments as well control sample and samples with addition of glucose are grouped into one cluster. The main reason is the great variation of values of hydrolytic enzyme activity. The standard error of treatments ranged from 78.21 to 198.56 of PCA 1 and from 19.70 to 198.80 of PCA 2.

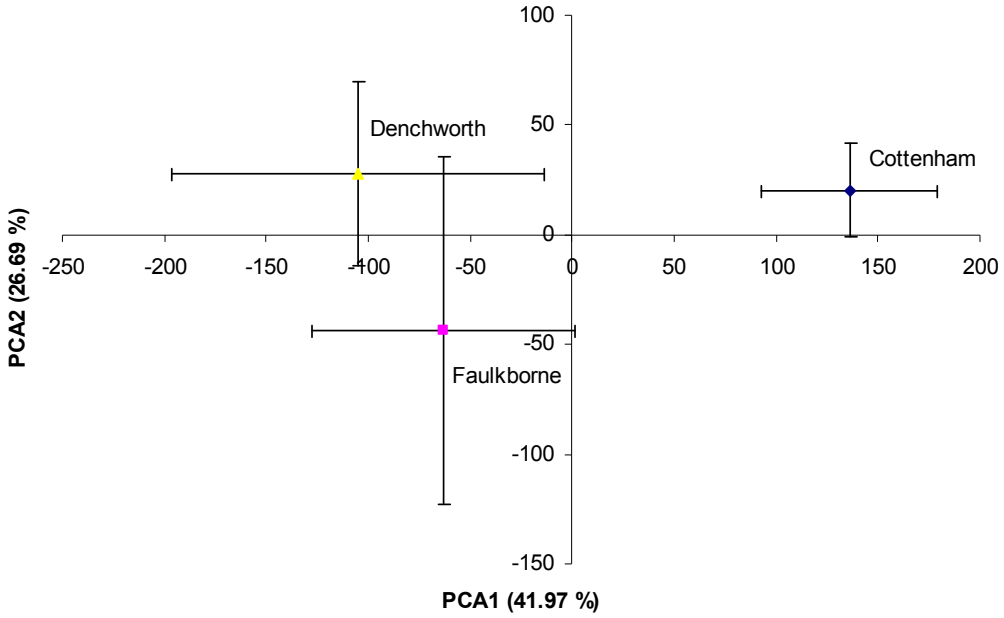


Figure 3.26 Mean PCA values of hydrolytic enzyme activity for each series. The bars show standard errors (n = 3).

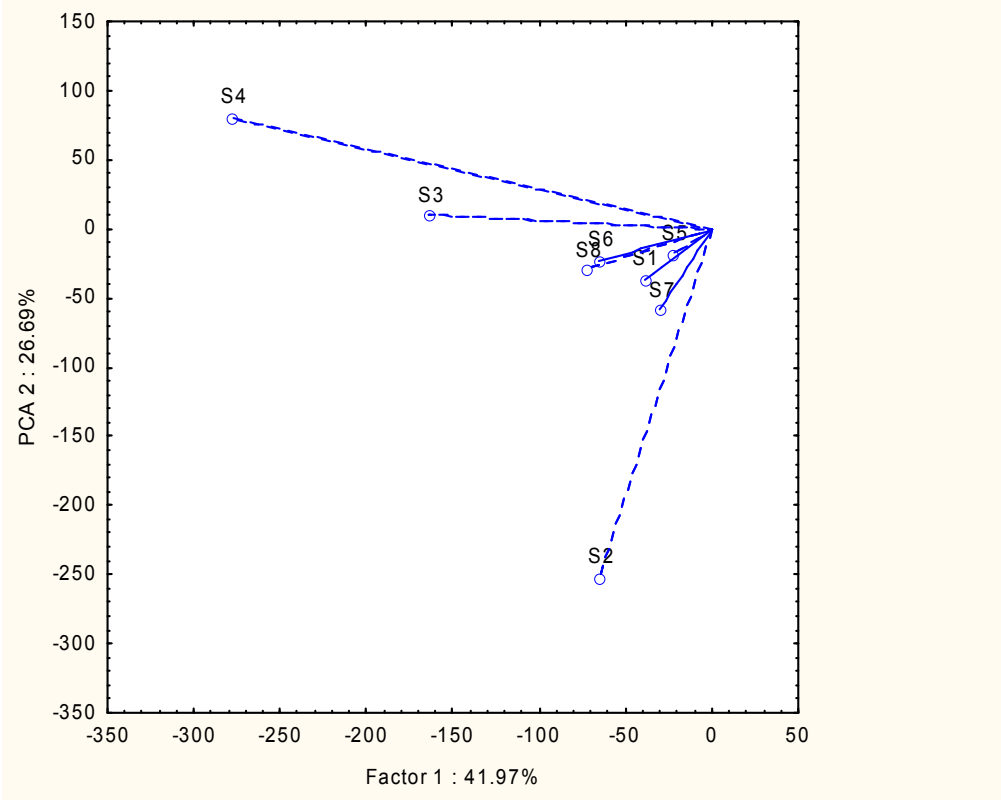


Figure 3.27 The variable plot of the PCA analysis of the hydrolytic enzyme activity.

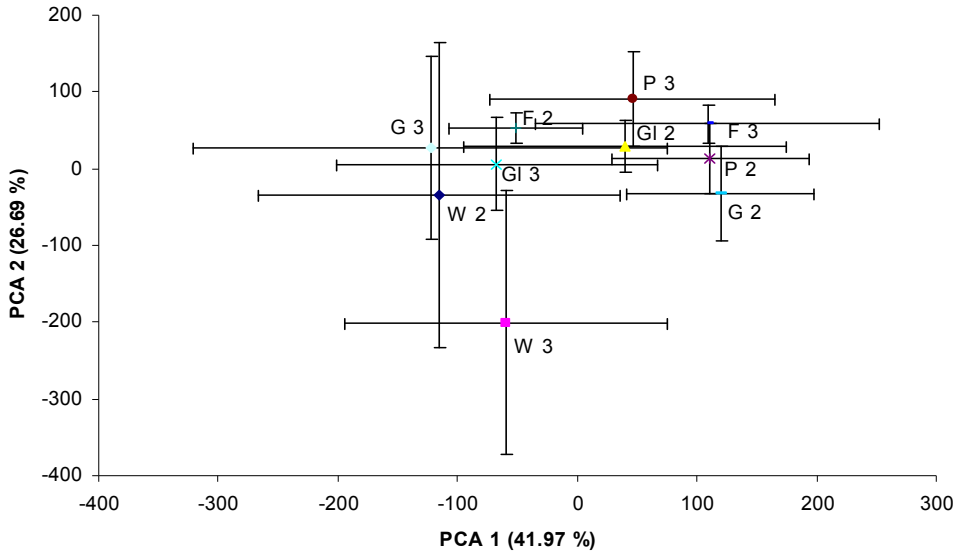


Figure 3.28 Mean PCA values of hydrolytic enzyme activity for each treatment measured at the beginning (2) as well as at the end of the experiment (2). The bars show standard errors (n = 3).

Legend: W – control sample, Gl – glucose, P – polyvinylalcohol, G – Gesagard, F – Fundazol, 2 – measurement at the beginning of the experiment, 3 – measurement at the end of experiment

3.2.5 Microbial community structure

In the study of soils sampled from Cranfield farm in Silsoe, 25 different phospholipid fatty acids (14:0, c14:1, 3OH 14:0, i15:0, ai15:0, i16:1, i16:0, ai16:0, 16:1w7c, 16:1w7t, 16:1w5, 16:0, Me i17:0, cyc i17:0, i17:0, ai17:0, 17:0c, 17:1, 17:0, 18:0, 18:2w6,9, 18:1w9c, 18:1w9t, 18:1w7t, 19:0c) were applied for comparison of different series and treatments.

Figure 3.29 shows PCA analysis of all treatments and fields at the beginning of the experiment. The clear separation of series Denchworth according to PCA 1 could be seen. In Denchworth, the all observed treatments are significantly different from control samples ($P<0.05$). Cottenham soils showed separation of samples treated by Gesagard according to PCA 1 and samples treated by Fundazol and polyvinylalcohol according to both main principal components. In Faulkborne, there was statistically significant ($P<0.05$) separation of samples treated by polyvinylalcohol according to the both PCA.

According to the PCA 2, there was the separation of samples treated by Gesagard from control sample.

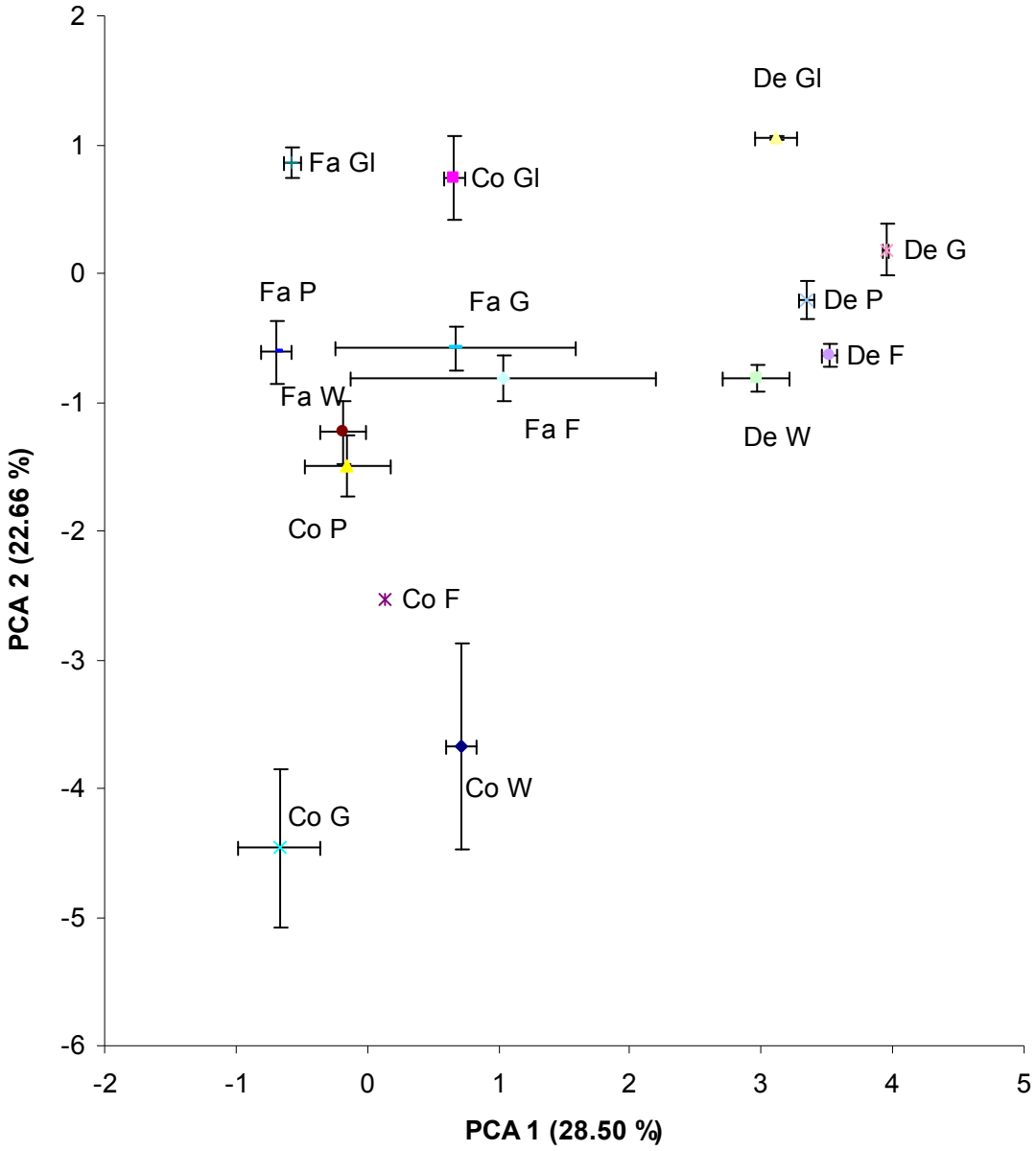


Figure 3.29 The complete variable plot of PLFA PCA analysis for all arable fields and treatments analysed at the beginning of the experiment (acute toxicity). The bars show standard errors (n = 3).

Legend: Co – Cottenham, Fa – Faulkborne, De – Denchworth, W – control sample, Gl – glucose, P – polyvinylalcohol, F – Fundazol, G - Gesagard

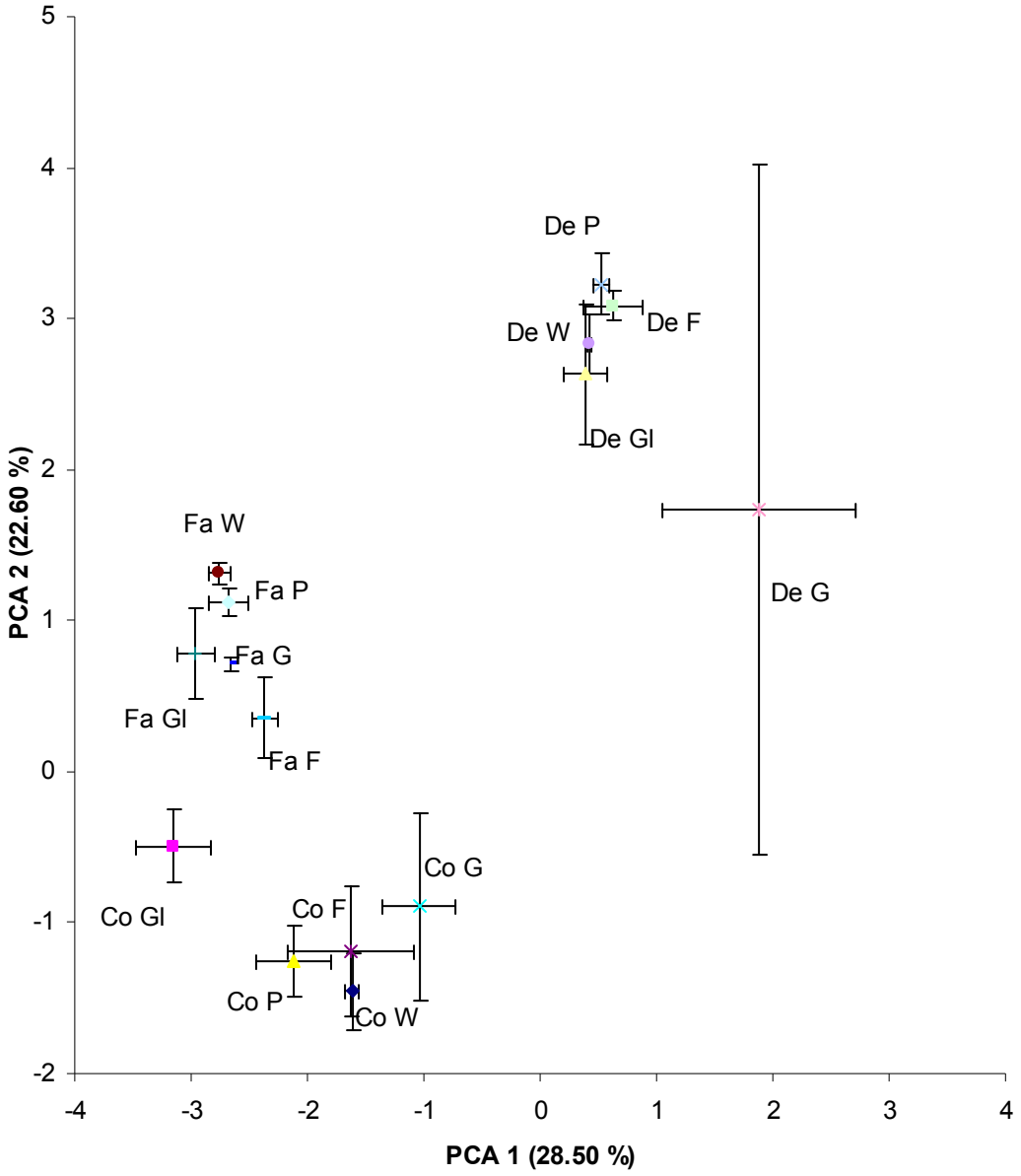


Figure 3.30 The complete variable plot of PLFA PCA analysis for all fields and treatments analysed at the end (b) of the experiment. The bars show standard errors (n = 3).

Legend: Co – Cottenham, Fa – Faulkborne, De – Denchworth, W – control sample, Gl – glucose, P – polyvinylalcohol, F – Fundazol, G - Gesagard

Figure 3.30 shows PCA analysis of all treatments and fields at the end of the experiment. The PCA 1 showed the separation of all samples of series Denchworth. The separation between series Cottenham and Faulkborne could be seen according to the PCA 2. In Denchworth, there was not separation between treatments and control sample. According to PCA 1, Cottenham soils showed the separation of between samples treated by Gesagard and polyvinylalcohol, and control sample. There was statistically significant difference ($P<0.05$) between samples treated by Fundazol and control sample. Results of PLFA analysis is in more detail described separately for series and treatments below.

The plot of mean PLFA PCA values is given in Figure 3.31. According to the PCA1, which covered 50.80 % of data variation, the series Denchworth seemed to be significantly ($P<0.05$) separated from the other fields. The PLFA 16:0 was mainly responsible for the separation of this area (Figure 3.32). The PLFAs 18:1w9t, 16:1w7c, 16:1w9c, 19:0c, 16:1w5 and 18:2w6,9 had a lesser effect on separation. According to the second component PCA 2, which accounted for 15.49 %, all series were grouped into one cluster. The greatest variation of samples was found in series Cottenham and Faulkborne.

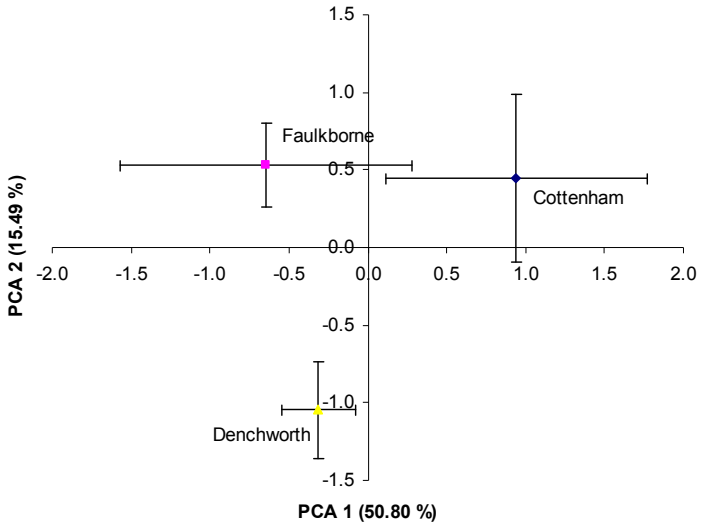


Figure 3.31 The variable plot of the PLFA PCA analysis for each field. The bars show standard errors (n = 3).

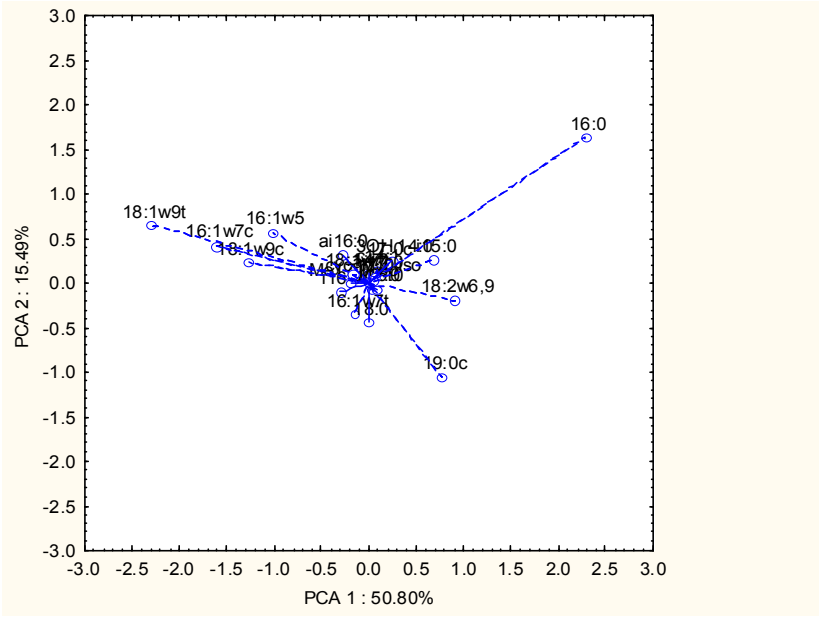


Figure 3.32 Results of the PLFA PCA analysis showing the main variables causing the separation of the field data.

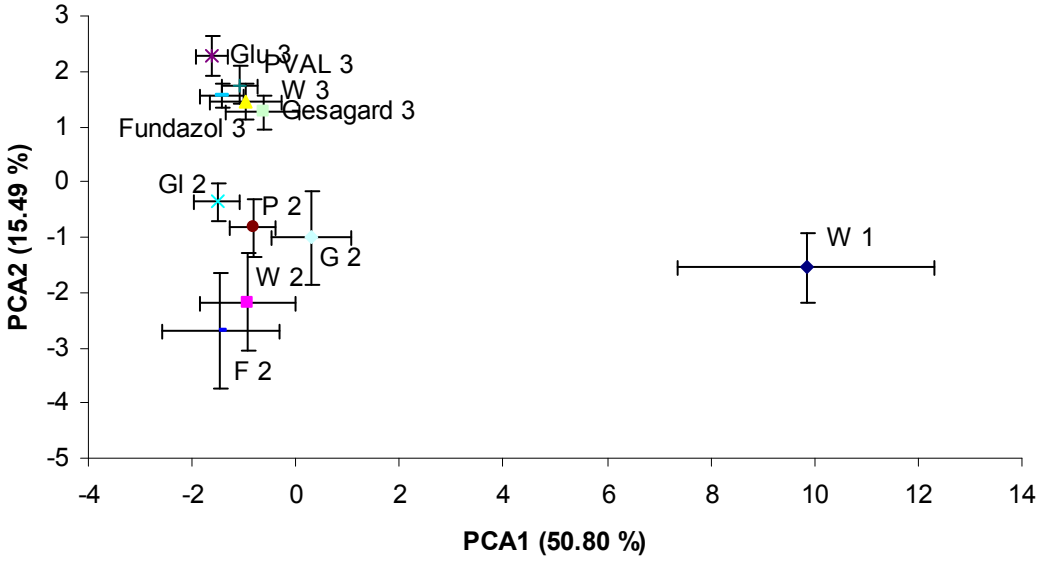


Figure 3.33 The plot of the mean PLFA PCA analysis for each field. The bars show standard errors.

Legend: W – control sample, Gl – glucose, P – polyvinylalcohol, G – Gesagard, F – Fundazol, 1 – measurement from fresh soil, 2 – measurement at the beginning of the experiment, 3 – measurement at the end of experiment

The significant differences ($P<0.05$) between treatments and the control sample were not found according to neither PCA 1 (50.80 %) nor PCA 2 (15.49 %). According to PCA 1, a separation between samples analysed from fresh soil and other samples were found. Similarly, a separation of PLFA composition between samples measured at the beginning and at the end of experiment was detected (Figure 3.33).

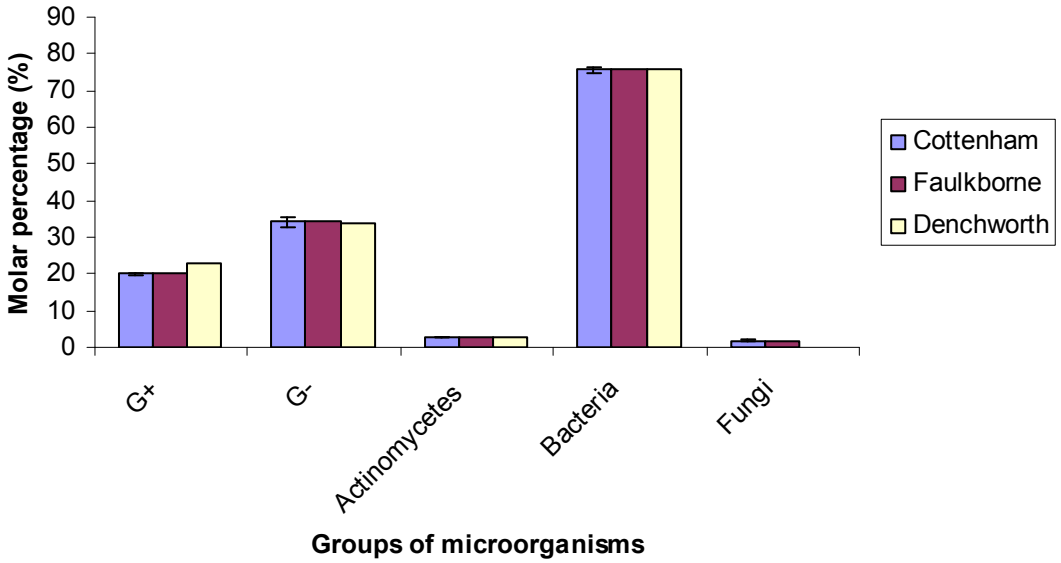


Figure 3.34 The molar percentage of taxonomic groups of microorganisms for each series. The bars show standard errors.

Legend: G^+ - Gram positive bacteria, G^- - Gram negative bacteria

The main groups of microorganisms, G^+ bacteria, G^- bacteria, actinomycetes, bacteria and fungi, were analysed in observed fields and after application of treatments. The relative abundance of these taxonomic groups of microorganisms was calculated by PLFAs present in Table 2.2. The similar values of percentage abundance of G^- bacteria, Actinomycetes and Bacteria were determined in all series (Figure 3.34). However, the percentage amount of the G^+ bacteria and fungi showed slight separation of the series Denchworth. In this series, the highest amount of Gram positive bacteria and the lowest amount of fungi were found.

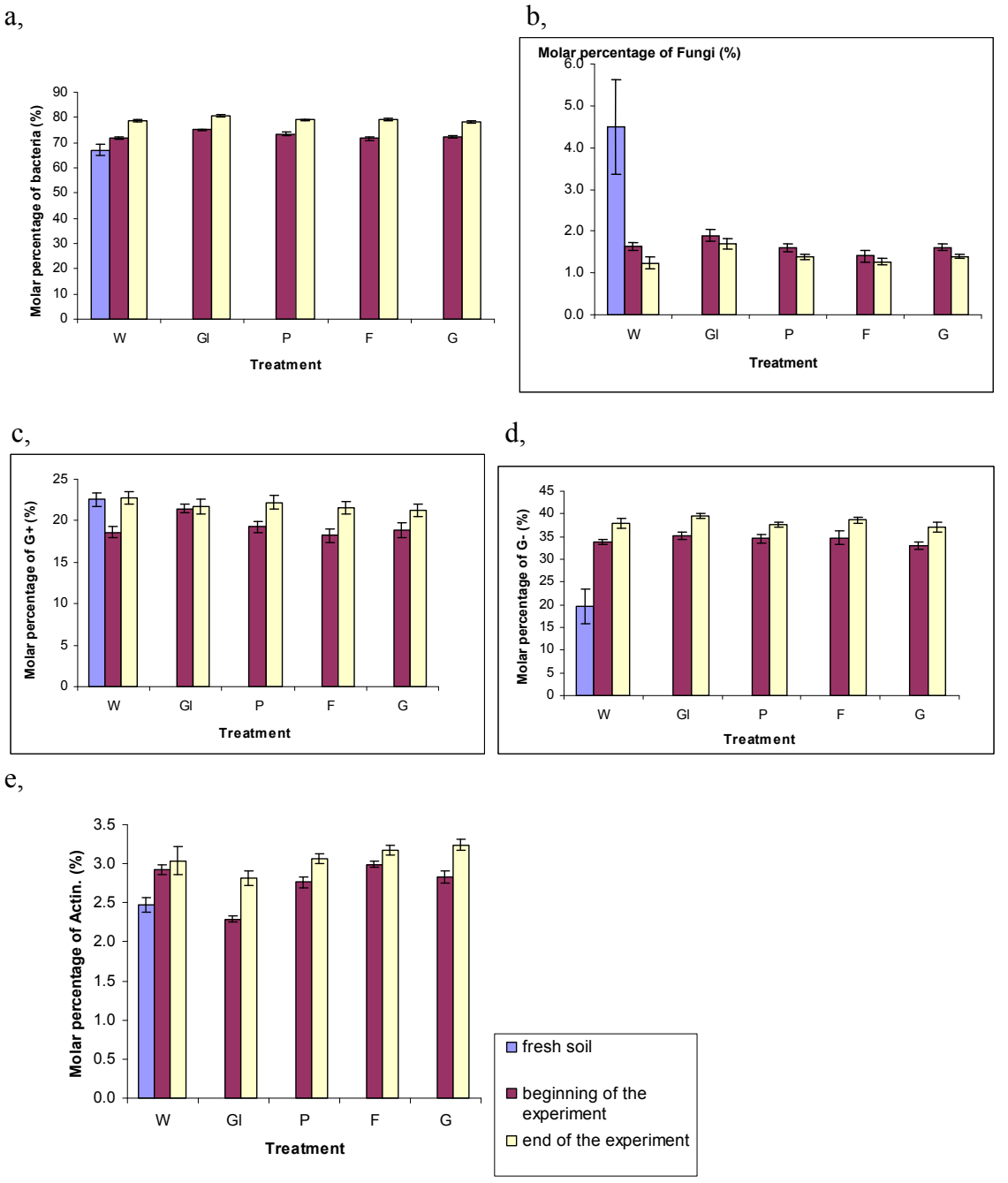


Figure 3.35 The molar percentage of selected groups of microorganisms, Bacteria (a), Fungi (b), G^+ bacteria (c), G^- bacteria (d) and Actinomycetes (e) measured at different time of the experiment. The bars show standard errors.

Legend: W – control sample, Gl – glucose, P – polyvinylalcohol, F – Fundazol, G – Gesagard.

Figure 3.35 shows the mean values of percentage abundance of the control sample, sample with addition of glucose, and samples treated with PVAL, Gesagard and Fundazol. The different colours were given for different stages (fresh soil, beginning of

the experiment, end of the experiment). In almost all observed groups of microorganisms, an increase of values measured at the end of experiment could be seen, in comparison to the values measured at the beginning of the experiment. However, the group of Fungi showed the opposite decreasing tendency of values. In the soil sampled from fresh soil, lower values of abundance of bacteria, G^- bacteria, and Actinomycetes were found than values measured at the beginning of the experiment.

Although not always statistically significant, the addition of glucose affected the abundance of all groups of microorganisms. Specifically, the addition of glucose caused an increase of G^+ bacteria (Figure 3.35c) and significant decrease of Actinomycetes (Figure 3.35e) at the beginning of the experiment. The mol % of PLFA of Bacteria (Figure 3.35a) showed a similarity of treatments to the control sample with an exception of PVAL measured at the beginning of the experiment. The addition of Fundazol (Figure 3.35b) influenced the abundance of Fungi and it caused a significant decrease ($P<0.05$) at the end of the experiment. Geagard influenced the abundance of microscopic fungi and actinomycetes. The remaining groups of microorganisms (Figure 3.35c-e) did not show significant difference ($P>0.05$) between control sample and treatments.

The plot of PCA 1 of soil respiration and PLFA showed that the areas Faulkborne and Denchworth were still closely grouped together in both variable planes. According to the SR PCA 1, which accounted 34.36 % of variation, the area Cottenham was significantly ($P<0.05$) separated from the other fields (Figure 3.36).

Figure 3.37 represents a means plot of the soil respiration PCA 1 against PLFA PCA1 for each treatment. The control sample, sample with added glucose and analysed treatments (PVAL, Fundazol and Gesagard) were closely grouped, according to the PLFA PCA 1, into one cluster. The exception was mean value of control sample measured from fresh soil. The values of SR PCA 1 showed the grouping of all samples measured at the beginning of the experiment into one cluster. However, a separation of samples treated with glucose, PVAL and Fundazol from the control sample was found.

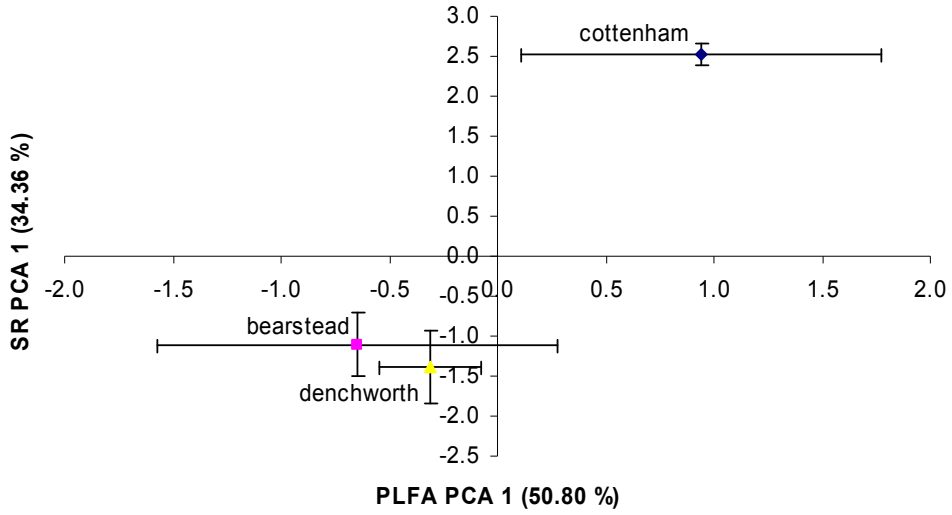


Figure 3.36 The plot of the PCA values of soil respiration and PLFAs for each field. The bars show standard errors (n = 3).

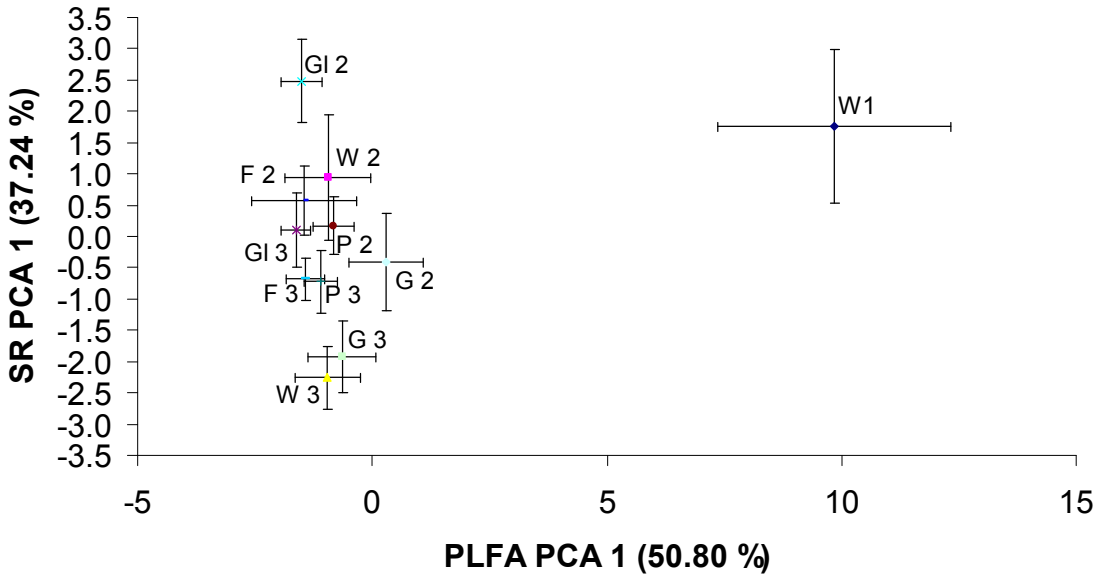


Figure 3.37 The plot of the PCA values of soil respiration and PLFAs for each treatment. The bars show standard errors (n = 3).

Legend: W – control sample, Gl – glucose, P – polyvinylalcohol, G – Gesagard, F – Fundazol, 1 – measurement from fresh soil, 2 – measurement at the beginning of the experiment, 3 – measurement at the end of experiment

4 DISCUSSION

4.1 The effects of site characteristics on soil microbial community

Two different soil types, grassland soils and arable soils were used for analysis of the effect of xenobiotics on soil microbial community.

Chemical characteristics are different between different soil types. The pH is an important indicator of soil fertility. Most microorganisms living in the soil prefer neutral pH. The pH of grassland soils (Sk) was strongly acidic, whereas pH of arable soils (UK) varied from neutral to slightly alkaline. Similar differences in pH of grassland soils and arable soils have been published in the papers of Tscherko and Kandeler (1999). The acidity of grassland soils is caused by dominance of strongly acidic fulvic acids (Javoreková *et al*., 2008). The acidity of grassland soils has been confirmed in studies of Cookson *et al*. (2007), Kuan *et al.* (2006) and Ritz *et al.* (2004).

The amount of C_{ox} in grassland soils (Sk) ranged from 2.49 to 6.75. This research is consistent with the study of Tscherko and Kandeler (1999), which determined the amount of organic carbon in the range from 3.5 to 5.8 %. These values, with the exception of Slovak Ore Mountain C_{ox}, were higher than the total amount of carbon present in arable soils (UK). The main reason of the higher values in grassland soils can be explained by the site characteristic. The mountain grassland soils were developed at lower temperatures, in areas with higher rain fall, which resulted in lower biological activity and higher accumulation of the soil organic matter.

Most microorganisms in soil are chemoheterotrophic. Therefore, input and sufficiency of organic matter are the main factors which affect the presence of microorganisms in soil. Degradation and intensity of the utilisation of organic matters depends on the carbon to nitrogen ratio. The ideal ratio for supporting the microbial activity would be between 20:1 and 25:1. However, this ratio is inappropriate for plant nutrition. For this reason, the right ratio of carbon to nitrogen for equilibrium of the mineralization and degradation processes generally ranges from 10:1 to 12:1 (Prasad and Power, 1997; Pierzynski *et al*., 2005). Similar values of ratio carbon to nitrogen were found in arable

soils (9.66 to 10.14 %). In the grassland soils (Sk), higher values of this ratio were found (10.79 to 13.70 %).

The soil texture (ratio of sandy, silt and clay components) determines the availability of water and nutrients. In general, the soils with dominance of silt and clay components are more appropriate for growth of microorganisms. The main reason is the greater air spaces in these soils than in sandy soils. In this research, different types of soil texture were found. In grassland soils (Sk), only two types, sandy loam soil (Greater Fatra, Lesser Fatra and Lower Tatras) and loamy sand soil (Slovak Ore Mountain), were determined. It was expected that the soil moisture and microbial biomass would be lower in Slovak Ore Mountain because of a higher percentage of sandy particles than clay and silt particles (Landgraf, 2001). Similarly in arable soils (UK), higher values of soil moisture, water holding capacity and microbial biomass were detected in sandy clay loam (Faulkborne) and clay loam (Denchworth) soils than in loamy sand soil (Cottenham).

The C_{mic} is widely used for analysis of the effect of different environmental and anthropogenic factors on soil microbial community (Turgay and Haraguchi, 2000; Filip, 2002; Růžek *et al*., 2004). The expected high values (1341 – 1728 $\mu g.g^{-1}$ d.s.) were present in grassland soils (Sk) of mountain areas. Even higher amount of microbial carbon in upland grassland have been detected in the study of Ritz *et al.* (2004) and Saviozzi *et al*. (2001). Slightly lower values of microbial carbon have been reported in papers of McCulley *et al.* (2004) and Růžek *et al.* (2004). In arable soils (UK), the values of microbial carbon were surprisingly low (28 – 139 $\mu g.g^{-1}$ d.s) in contrast to findings of Joergensen (1996). Landgraf (2001) has determined values of microbial biomass in arable soils ranging from 67.1 to 217.2 $\mu g\ C.g^{-1}$ d.s. Griffiths *et al.* (2001) has detected similar values of C_{mic} (102.5 $\mu g\ C.g^{-1}$ d.s). The most probable reason for the low amount of microbial biomass of arable soils in our study might be that at the date of soil sampling (January) the weather conditions were not optimal for the growth of microorganisms.

The ratio of microbial carbon to organic carbon represents the amount of metabolic active carbon in total soil organic matter. The usual ratio for arable soils is 3 % (Insam and Domsch, 1988). The locations of grassland soils (Sk) reached the values from 2.56 to 3.58, thus the locations were quite metabolically active. Soil from the Slovak Ore Mountains had a ratio of 5.4 %, which caused an increase of nutrients in non-stable part of the organic matter, and therefore increased the sensitivity of this location against environmental factors (Javoreková *et al*., 2008). In contrast to the report of Insam and Domsch (1988), Goodley (2004), and Anderson and Domsch (1993) argue that the ratio typically reaches values 0.1 – 0.5 %. Similar values were detected in the series of arable soil (UK). Joergensen *et al.* (1994) argue that soil with a ratio of less than 1 % has a low carbon turnover.

Enzyme activities have been reported to have a greater correlation to the amount of organic matter than to microbial carbon (Uckam and Okur, 2004). As our results indicated, the hydrolytic activity of grassland soils (Sk) increased from Greater Fatra through Lesser Fatra and Lower Tatras to Slovak Ore Mountain, while the microbial biomass values decreased in these areas in the mentioned order. Soil respiration rate was lower for location Lower Tatras in comparison to other areas. In the arable soil (UK) the PCA showed the separation of series Cottenham.

The metabolic quotient is a reliable eco-physiological indicator (Anderson and Domsch, 1973; Goodley, 2004). High ratios of soil respiration to microbial carbon indicate a deprivation of the ability of microorganisms to utilize the carbon source as the results of the applied stress (Badalucco *et al*., 1992). In our study, the metabolic quotient was very low. Grassland soil (Sk) ratio was less than 0.10 % and in the arable soil (UK) it was less than 0.02%. These low values indicated a high substrate efficiency of soil microorganisms. Our results agree with Landgraf (2001) and they were much lower than that published by Růžek *et al.* (2004).

The PLFA PCA analysis of Slovak grassland soils confirmed that soil pH is a major factor affecting the soil microbial community composition (Bååth *et al*., 1995; Priha *et al*., 2001; Cookson *et al*., 2007). This is due species specific optimal pH for soil

microorganisms (Shah *et al*., 1990). The areas with the highest pH (Greater Fatra, Lesser Fatra, and Slovak Ore Mountain) were separated from the location Lower Tatras, according to both PCA factors. Similarly, Greater Fatra and Lesser Fatra were the most abundant of each observed groups of microorganisms. Surprisingly, the most acidic area (Lower Tatras) had the lowest abundant of microscopic fungi. This result was in contrast to the generally known fact that the microscopic fungi are dominant in acidic environment (Giri et al., 2005). In the arable soils there was a separation of series Denchworth from other series. The main reason was probably the lowest abundance of microscopic fungi in Denchworth. The similar abundance of microscopic fungi was found between the series Faulkborne and Cottenham. The main reason was that the sandy soils are more appropriate for microscopic fungi (Gray, 1985; Bossio *et al*., 1998) because of their lower moisture. Numerous studies in the literature have reported the differences of soil microbial community across land uses (Stevenson *et al*., 2004; Bossio *et al*., 2005; Cookson *et al*., 2007). When comparing both soil types, the findings, which were also probably based on different pH, were detected. The main differences were detected in the group of G^- and G^+ bacteria. G^+ bacteria were present in grassland soils was double that found in arable soils. In contrast, the amount of G^- bacteria was three-times lower in grassland soils than in arable soils. Moreover, abundance of fungi was greater in grassland soils.

In summary, the comparison of the biochemical properties confirmed the differences between arable and grassland soils in this study. For arable soils (UK), the differences associated with the biochemical properties analysed were in agreement with the different textural classifications. As such, soils of the Cottenham series (loamy sand soil) had different microbial properties compared to soils of the Faulkborne and Denchworth series. There was no difference in textural classification of the Slovak grassland soils. A significant difference between area Lower Tatras and other fields was shown in PCA analysis of phospholipid fatty acids and soil respiration. The decrease of the soil respiration was probably caused by the lack of some genus of fungi, which were present in lower values in this location.

4.2 Effect of xenobiotic perturbation on soil microbial community

The addition of xenobiotics (PVAL, Fundazol and Gesagard), and glucose altered the soil microbial community composition and activity, both expressed in the measurement of C_{mic}, soil respiration rate, phospholipids fatty acids and abundance of selected groups of microorganisms. The same finding was determined by Bossio *et al.* (1998) in the experiment where the herbicide treatment was applied on the soil samples. The effect of xenobiotics differed slightly between different soil types, grassland soils (Sk) and arable soils (UK). The PLFA PCA analysis showed (Figure 3.7, Figure 3.29 and Figure 3.30) that the soil type had greater effect on determining the soil microbial community composition than treatment in both soil types, arable soils (UK) and grassland soils (Sk). However, the separation of soil samples treated by glucose (Figure 3.30) was found after six-week incubation in arable soils. This finding showed potential effect not only the fields but also treatments on soil microbial community.

In the grassland soils (Sk), the addition of glucose caused the increase of soil respiration immediately after addition as well as after 21 days of incubation. This increase was significantly ($P \leq 0.01$) different not only from control sample, but also from other treatments. However, the PLFA PCA analysis did not show differences from control sample. In the arable soils (UK), the soil respiration was observed on the best discriminators between treatments – glucose, malic acid and citric acid, which had the greatest effect on the separation of treatments. Similarly as by grassland soils, the soil respiration increased of glucose. The catabolic substrates, malic acid and citric acid did not show the significant increase the soil respiration. The application of catabolic substrate glucose resulted in the decrease of soil respiration rate in comparison to control sample at the beginning of the experiment. It was not surprising that the microbial biomass in arable soil samples was greater than in control sample at the beginning as well as at the end of the experiment. Similar increase of soil respiration and microbial biomass after addition of glucose has already been published (Anderson and Domsch, 1978; Dilly, 2004; Zyakun and Dilly, 2005). The PCA analysis of hydrolytic activity and PLFAs did not show a significance differences from the control sample. However, the addition of glucose provided an increase of abundance of groups Bacteria and Fungi at both stages of the experiment. These results might indicate that

immediately after the addition of glucose the soil microorganisms began to utilize glucose as a source of carbon and energy and their biological activity increased in both soil types. After three weeks of incubation, in grassland soils, the soil microorganisms were still metabolically active, but in consequence of a rapid decline of glucose, the soil microorganisms could not use it for their growth and proliferation. They used it only for their survival at the end of the experiment. In the arable soils, there probably still was enough glucose after six week incubation, which was used by microorganisms for their growth and proliferation. This was confirmed by higher values of soil respiration, microbial biomass and abundance of Bacteria and Fungi than in the control sample. Our findings confirmed that changes in soil metabolic activity are linked to the changes in soil microbial community composition (Zogg *et al*., 1997; Calderón *et al*., 2001; Cookson *et al.*, 2007).

The results of the application of Gesagard on measured parameters in both grassland soils (Sk) and arable soils (UK) confirmed the effect of this herbicide on the soil microbial community. Immediately after the addition of Gesagard to the soil samples, the soil respiration increased in grassland soils. It indicated that some of the microorganisms started to utilize the Gesagard as a carbon source. In contrast to grassland soils, in arable soils, the soil microbial activity decreased at the beginning of the experiment. Similarly, the values of the soil microbial biomass were lower than in control samples. It might indicate that the Gesagard in the arable soils affected the microbial activity and microbial biomass in negative way. However, at the end of the experiment, in grassland soils after a twenty-one-day incubation and in arable soils after a six-week incubation, the soil respiration had again reached higher values than the control sample. These results showed that the application of Gesagard might have caused a reduction of some taxa of microorganisms in the initial stage of experiment, which might have resulted in decreasing of microbial activity and microbial biomass. Consequently, the reduction of these taxa might have resulted in other taxa, which were proportionally of a lower amount, becoming dominant. It was particularly supposed to be the microscopic fungi. The dominant taxa might have been able to degrade this herbicide and to utilize it as a source of energy, nitrogen and sulphur. For this reason, the intensity of soil microbial processes increased. The dominance of these taxa was not

seen from the results of abundance of selected groups of microorganisms analysed in grassland soils. The results showed the lower percentage of all microbial groups in samples treated by Gesagard than in control sample. However, the higher amount of microscopic fungi and, even, actinomycetes was found in arable soils. The similar results of utilization of Gesagard as source for growth and proliferation of soil microorganisms were published in a Herbicide Handbook of Weed Science Society of America (Beste, 1983). The higher level of actinomycetes was found also in the study of areas with alkaline pollution (Bååth *et al*., 1992) and heavy metal contamination (Frostegård *et al*., 1993). However, Frostegård *et al*. (1993) in his research determined the higher level of actinomycetes only in the forest soils, and this group was unaffected in response to metals in the arable soils.

The microbial activity increased in the grassland soils (Sk) immediately after the addition of Fundazol to the soil samples. This probably means that some microorganisms started to utilize Fundazol as a source of nutrient for their growth and proliferation. But after one week, when the soil respiration of arable soil (UK) was measured, the soil microbial activity decreased. Results indicate that Fundazol did not influence the soil microbial community immediately, but after few days. Moreover, it might have killed or even inhibited the activity of specific species of microscopic fungi, particularly saprophytic and parasitic ones. It was expressed in the mentioned decrease of microbial activity. The reduction of the group of microscopic fungi was confirmed by the results of abundance of PLFAs at the beginning of the experiment. Consequently, the remaining microorganisms, especially other species of fungi, might have degraded the dead fungal cells as well as the fungicide, which resulted in increase of microbial activity. These results were confirmed by the increasing abundance of microscopic fungi only in grassland soils. In the literature, there are only few reports focused on an effect of Fundazol on soil microbial community. Chen *et al.* (2001a, 2001b) analysed the effect of benomyl on the soil microbial community by measurement of a substrate-induced respiration, a microbial biomass, an enzyme activity, a nitrogen mineralization and a rate of organic matter degradation. The authors detected the significant effect of the addition of fungicide, which resulted in the decrease of soil microbial activity, but it did not affect the values of microbial biomass. They suppose that the main reason is the

change of dominance from the fungal population to bacterial community. Podio *et al*. (2008) analysed the effect of benomyl using methods of the soil respiration, the microbial biomass, the measurement of ergosterol and the PLFA analysis. The authors did not determine significant influence of benomyl on soil microbial activity and composition. However, the great effect of high doses of benomyl on the fungal biomass was detected by measurement of ergosterol. Demanou *et al*. (2006) and Allison *et al*. (2007) detected the slight effect of fungicide on the fungal community. Bending *et al*. (2007) detected that the application of the benomyl caused the lack of many bands reflected dominant species of soil microbial community by using 18S rDNA DGGE PCR.

A significant effect of polyvinylalcohol on the soil microbial community was found. Most of observed parameters indicated the positive effect of PVAL on the soil microorganisms. In arable soils (UK) the soil respiration in samples treated by PVAL had higher values than the control sample by using the substrate glucose. Similar effect of PVAL on soil respiration was also observed in grassland soils (Sk) at the beginning as well as at the end of the experiment. The microbial biomass measured in arable soil increased in the soil treated by PVAL at the both stages of the experiment. Microscopic fungi and G^- bacteria had higher percentage in grassland soils at the end of the experiment. The results of percentage abundance of observed groups of microorganisms in arable soils showed the increase of abundance of bacteria at the beginning of the experiment, whereas only group of microscopic fungi had greater values of abundance than control sample at the end of the experiment. The possible explanation is that the immediately after the addition of PVAL the appropriate conditions for growth and proliferation of bacteria might have been created. The main reason was that the addition of PVAL might have improved the soil structure and stability of soil aggregates (Özbek, 2004). This fact was confirmed by the increase of soil respiration as well as microbial biomass in comparison to control sample. In the next stage PVAL might have started to be utilized especially by fungi in arable soils and by fungi and G^- bacteria in grassland soils. This degradation of PVAL might have caused still the high level of microbial activity. To the best of our knowledge, there have not been any more scientific papers

which would focus on the relationship between the addition of polyvinylalcohol and the soil microbial community.

4.3 Resistance of soil microbial community to xenobiotic perturbation

Functional stability of an ecosystem, expressed in indices of resistance and resilience, were calculated according to Orwin and Wardle (2004) from the soil respiration rate values. Our findings indicated that the arable soils were more resistant than grassland soils. This could be explained in two ways. Firstly, the higher resistance of the arable soils was affected by differences of soil microbial community composition, especially by different numbers of G^- bacteria, G^+ bacteria, and microscopic fungi between soil types, which were described in more details in chapter 4.1. Second reason is based on the differences in land use. The arable soils have been used for growing and production the crops, which is linked to application of pesticides of conventionally agriculture. Part of applied pesticides could get into soil and affect the soil microbiota. After long-term application, the soil microbiota could become resistant against any pesticides or even could degrade them and utilize them as carbon source. Our results are in contrast to findings Griffiths *et al*. (2000, and 2001) who determined the higher resistance of grassland soils than agricultural soils in their study of copper and heat perturbation.

In grassland soils, the area of Lower Tatras seemed to have the lowest resistance against all treatments. It is probably caused by the low pH of this area, which probably amplified the effect of the treatments on soil microbial community. This combined effect surprisingly resulted in a decrease of amount of microscopic fungi in this location. Other locations had the similar values of resistance against application of selected treatments. The series Denchworth was the less resistant one of arable soils, which was linked to the highest abundance of G^+ bacteria. Our results in both soil types confirmed that the change in soil microbial community structure led to the losses of functional stability (Griffiths *et al*., 2004), in our case to lower levels of soil resistance against application of different treatments.

The resilience to the each treatment was calculated only in the arable soils. The index of resilience for each treatment and each location reached number 0. These results

indicated that the applied xenobiotics were still present in soil samples as persistent stress in that time of measurement of soil resilience.

5 CONCLUSION

The results of this research demonstrated the differences of microbial community structure between different soil types, grassland soils and arable soils, which were probably caused by soil pH. Specifically, the abundance of G^- bacteria, G^+ bacteria and fungi altered between arable soils and grassland soils. The results of soil pH, amount of carbon, ratio carbon to nitrogen, microbial carbon confirmed differences between arable soils and grassland soils. In arable soils (UK), the analysed parameters including pH, total carbon, ratio carbon to nitrogen, and ratio microbial carbon to total carbon indicated a separation of series according to soil texture. The results of microbial biomass, soil respiration rate and hydrolytic enzyme activity showed separation of series Cottenham from Faulkborne and Denchworth. However, the results of PLFA analysis show separation of Denchworth. In grassland soils, the separation between all areas were found according to data of oxidizable carbon, pH, ratio microbial carbon to oxidizable carbon and dehydrogenase activity, The separation of the most acidic area Lower Tatras was significant by soil respiration and PLFA data.

The effect of xenobiotic perturbation altered the soil microbial community structure, microbial activity and microbial biomass. The effect of treatments on soil microbial community was related to the soil type, grassland soils (Sk) and arable soils (UK). In grassland soils, addition of Gesagard resulted in the decrease of all observed taxonomic groups of microorganisms, whereas in arable soils, the increase of abundance of fungi and actinomycetes was found after six-week incubation. The treatment with Fundazol caused the increase of abundance of microscopic fungi in grassland soils, whereas the decrease of abudance of microscopic fungi after addition of fungicide was observed at the end of the experiment. PVAL increased abundance of fungi and G^- bacteria in grassland soils. However, the significance effect of application of PVAL was not found in grassland soils. Therefore, the original hypothesis that the microbial community of soils with different soil types will have differing abilities to resist xenobiotic perturbation was confirmed.

The results of functional stability showed a higher resistance of arable soils to xenobiotic perturbation than grassland soils. These differences in resistance of different

soil types were probably associated with a different soil microbial community composition or with a different land management. Moreover, the loss of resistance corresponded with the shift in soil microbial community structure. In grassland soils (Sk), the area with lowest resistance, Lower Tatras, had the lowest abundance of microscopic fungi. I arable soils (UK), the less resistance of series Denchworth was linked to the highest level of G^+ bacteria. The measured resilience of arable soils reach value 0, which indicates that the xenobiotics are still present in soils as persistent stress.

The findings indicate that in grassland soils (Sk), there might be some groups of microscopic fungi which can degrade the applied fungicide, whereas in arable soils there might be present some species of microscopic fungi which can degrade and utilize the herbicide Gesagard. It would be useful to determine which species are responsible for this degradation and if these species can degrade the herbicides and pesticides with similar chemical composition. The determination of these facts could be then used in the application of species in bioremediation.

6 FUTURE WORK

1. Additional research of the soil microbial community structure using other methods such as traditional cultivation methods, and DGGE would be beneficial for more detailed analysis of the changes in soil microbial community which caused by the selected treatments. After determination of shifts in DGGE profiles in terms of absent or additional bands in samples treated by selected treatments in comparison to control samples, these bands would be further analysed.

2. A more detailed study of functional stability would be necessary to determine whether the soils recover following xenobiotic perturbation in the term of long-term application.

3. Analysis of the effect of the selected xenobiotics in a field experiment would be helpful for the determination of shifts of soil microbial community structure and activity in real conditions. The laboratory experiment ran at a constant temperature and constant soil moisture, whereas variable conditions are present in the field.

REFERENCES

Acton, D. F. and Gregorich, L. J. (1995), "Understanding soil health", in Acton, D. F. and Gregorich, L. J. (eds.) *The health of our soils: Toward sustainable agriculture in Canada,* 1st ed, Centre for land and biological resources research, Research brand, Agriculture and agri-food Canada, Ottawa, Ont., pp. 5-10.

Alef, K. and Nannipieri, P. (1995), *Methods in applied soil microbiology and biochemistry,* 1st ed, Academic press, London.

Alef, K., Beck, T., Zelles, L. and Kleiner, D. (1988), "A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils", *Soil Biology and Biochemistry,* vol. 20, no. 4, pp. 561-565.

Allison, V. J., Rajaniemi, T. K., Goldberg, D. E. and Zak, D. R. (2007), "Quantifying direct and indirect effects of fungicide on an old-field plant community: An experimental null-community approach", *Plant Ecology,* vol. 190, no. 1, pp. 53-69.

Anderson, J. P. E. and Domsch, K. H. (1978), "A physiological method for the quantitative measurement of microbial biomass in soils", *Soil Biology and Biochemistry,* vol. 10, no. 3, pp. 215-221.

Anderson, J. P. E. and Domsch, K. H. (1973), "Quantification of bacterial and fungal contributions to soil respiration", *Archiv fuï Mikrobiologie,* vol. 93, no. 2, pp. 113-127.

Anderson, T. (2003), "Microbial eco-physiological indicators to asses soil quality", *Agriculture, Ecosystems and Environment,* vol. 98, no. 1-3, pp. 285-293.

Anderson, T. and Domsch, K. H. (1993), "The metabolic quotient for CO2 (qCO2) as a specific activity parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass of forest soils", *Soil Biology and Biochemistry,* vol. 25, no. 3, pp. 393-395.

Anderson, T. and Domsch, K. H. (1990), "Application of eco-physiological quotients (qCO2 and qD) on microbial biomasses from soils of different cropping histories", *Soil Biology and Biochemistry,* vol. 22, no. 2, pp. 251-255.

Andrén, O., Brussaard, L. and Clarholm, M. (1999), "Soil organism influence on ecosystem-level processes - Bypassing the ecological hierarchy?", *Applied Soil Ecology,* vol. 11, no. 2-3, pp. 177-188.

APVMA (2005), "Cancellation of product registration at the request of the registrant", *APVMA Gazette,* vol. 1, pp. 32.

Atlas, R. M. and Bartha, R. (eds.) (1998), *Microbial ecology: Fundamentals and applications,* 4th ed, Benjamin-Cummings Pub, Menlo Park, CA.

Bååth, E., Frostegård, A., Pennanen, T. and 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,* vol. 27, no. 2, pp. 229-240.

Bååth, E., Frostegård, A. and Fritze, H. (1992), "Soil bacterial biomass, activity, phospholipid fatty acid pattern, and pH tolerance in an area polluted with alkaline dust deposition", *Applied and Environmental Microbiology,* vol. 58, no. 12, pp. 4026-4031.

Badalucco, L., Grego, S., Dell'Orco, S. and Nannipieri, P. (1992), "Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (Picea abies L.)", *Biology and Fertility of Soils,* vol. 14, no. 2, pp. 76-83.

Bandick, A. K. and Dick, R. P. (1999), "Field management effects on soil enzyme activities", *Soil Biology and Biochemistry,* vol. 31, no. 11, pp. 1471-1479.

Barrios, E. (2007), "Soil biota, ecosystem services and land productivity", *Ecological Economics,* vol. 64, no. 2, pp. 269-285.

Bellinaso, M. D. L., Greer, C. W., Peralba, M. D. C., Henriques, J. A. P. and Gaylarde, C. C. (2003), "Biodegradation of the herbicide trifluralin by bacteria isolated from soil", *FEMS Microbiology Ecology,* vol. 43, no. 2, pp. 191-194.

Bending, G. D., Rodríguez-Cruz, M. S. and Lincoln, S. D. (2007), "Fungicide impacts on microbial communities in soils with contrasting management histories", *Chemosphere,* vol. 69, no. 1, pp. 82-88.

Benedetti, A. and Dilly, O. (2006), "Introduction", in Bloem, J., Hopkins, D. and Benedetti, A. (eds.) *Microbiological methods for assesing soil quality,* CABI, Wallington, pp. 3-14.

Bengtsson, J. (1998), "Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function", *Applied Soil Ecology,* vol. 10, no. 3, pp. 191-199.

Beste, C. E. (1983), *Herbicide handbook of Weed Science Society of America,* 5th ed, The Society, Champaign.

Bisby, F. A. and Coddington, J. (1995), "Biodiversity from a taxonomic and evolutionary perspective", *Global Biodiversity Assessment,* , pp. 27-56.

Bligh, E. G. and Dyer, W. J. (1959), "A rapid method of total lipid extraction and purification", *Canadian Journal of Biochemistry and Physiology,* vol. 37, no. 8, pp. 911-917.

Bloem, J., Schouten, A. and Sørensen, S. J. (2006), "Monitoring and evaluating soil quality", in Bloem, J., Hopkins, D. and Benedetti, A. (eds.) *Microbiological methods for assessing soil quality,* 1st ed, CABI, Wallington, pp. 23-49.

Bollag, J. M. and Liu, S. Y. (1990), "Biological transformation processes of pesticides", in Chen, H. H. (ed.) *Pesticides in the soil environment: Processes, impacts and modeling.* SSSA Book Series 2, Madison, pp. 169-211.

Bossio, D. A., Girvan, M. S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K. M., Ball, A. S., Pretty, J. N. and Osborn, A. M. (2005), "Soil microbial community response to land use change in an agricultural landscape of western Kenya", *Microbial Ecology,* vol. 49, no. 1, pp. 50-62.

Bossio, D. A., Scow, K. M., Gunapala, N. and Graham, K. J. (1998), "Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles", *Microbial Ecology,* vol. 36, no. 1, pp. 1-12.

Botton, S., Van Heusden, M., Parsons, J. R., Smidt, H. and Van Straalen, N. (2006), "Resilience of microbial systems towards disturbances", *Critical Reviews in Microbiology,* vol. 32, no. 2, pp. 101-112.

Briones, A. and Raskin, L. (2003), "Diversity and dynamics of microbial communities in engineered environments and their implications for process stability", *Current Opinion in Biotechnology,* vol. 14, no. 3, pp. 270-276.

British Standard Institution (1998), *Soil quality. Physical methods. Method by sieving and sedimentation.* BS 7755-5.4, ISO 11277, British Standard institution.

British Standard Institution (1995), *Soil quality: Determination of organic and total after dry combustion (elementary analysis),* ISO 10694, British Standard Institution.

British Standard Institution (1993), *Determination of dry matter and water content on a mass basis: Gravimetric method,* ISO 11465, British Standard Institution.

Burrows, L. A. and Edwards, C. A. (2004), "The Use of Integrated Soil Microcosms to Assess the Impact of Carbendazim on Soil Ecosystems", *Ecotoxicology,* vol. 13, no. 1-2, pp. 143-161.

Calderón, F. J., Jackson, L. E., Scow, K. M. and Rolston, D. E. (2001), "Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids after tillage", *Soil Science Society of America Journal,* vol. 65, no. 1, pp. 118-126.

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S. and Potts, J. M. (2003), "A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil", *Applied and Environmental Microbiology,* vol. 69, no. 6, pp. 3593-3599.

Campbell, C. D., Grayston, S. J. and Hirst, D. J. (1997), "Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities", *Journal of Microbiological Methods,* vol. 30, no. 1, pp. 33-41.

Carpenter, S. R. and Kitchell, J. F. (eds.) (1993), *The trophic cascade in lakes,* 1st ed, Cambridge University Press, Cambridge.

Chen, S., Edwards, C. A. and Subler, S. (2001a), "Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations", *Soil Biology and Biochemistry,* vol. 33, no. 14, pp. 1971-1980.

Chen, S., Edwards, C. A. and Subler, S. (2001b), "A microcosm approach for evaluating the effects of the fungicides benomyl and captan on soil ecological processes and plant growth", *Applied Soil Ecology,* vol. 18, no. 1, pp. 69-82.

Chlpík, J. (Department of Soil Science, Slovak Agricultural University, Nitra, 949 01, SR), (2007), Charakterizácia vybraných lokalít Slovenska (*Characterization of soil of selected Slovakian areas)* (unpublished Report).

Ciba-Geigy (1987), *Prometryn: Toxicity and Safety Assessment,* Ciba Geigy Corp., Greensboro.

Coleman, D. C. and Whitman, W. B. (2005), "Linking species richness, biodiversity and ecosystem function in soil systems", *Pedobiologia,* vol. 49, no. 6, pp. 479-497.

Cookson, W. R., Osman, M., Marschner, P., Abaye, D. A., Clark, I., Murphy, D. V., Stockdale, E. A. and Watson, C. A. (2007), "Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature", *Soil Biology and Biochemistry,* vol. 39, no. 3, pp. 744-756.

Crawford, J. W., Harris, J. A., Ritz, K. and Young, I. M. (2005), "Towards an evolutionary ecology of life in soil", *Trends in Ecology and Evolution,* vol. 20, no. 2, pp. 81-87.

De Ruiter, P. C., Neutel, A. M. and Moore, J. C. (2003), "The balance between productivity and community structure in food webs", in Bardgett, R. D., Hopkins, D. W. and Usher, M. B. (eds.) *Biological diversity anf function in soil,* 1st ed, Cambridge University Press, Cambridge, pp. 139-153.

Degens, B. P. and Harris, J. A. (1997), "Development of a physiological approach to measuring the catabolic diversity of soil microbial communities", *Soil Biology and Biochemistry,* vol. 29, no. 9-10, pp. 1309-1320.

Degens, B. P., Schipper, L. A., Sparling, G. P. and Duncan, L. C. (2001), "Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance?", *Soil Biology and Biochemistry,* vol. 33, no. 9, pp. 1143-1153.

Demanou, J., Sharma, S., Dörfler, U., Schroll, R., Pritsch, K., Njine, T., Bausenwein, U., Monkiedje, A., Munch, J. C. and Schloter, M. (2006), "Structural and functional

diversity of soil microbial communities as a result of combined applications of copper and mefenoxam", *Soil Biology and Biochemistry,* vol. 38, no. 8, pp. 2381-2389.

Dick, R. P. (1997), "Soil enzyme activities as integrative indicators of soil health", in Pankhurst, C. E., Doube B.M. and Gupta V.V.S.R. (eds.) *Biological indicators of soil health,* CAB International, Wallingford, pp. 121-156.

Dilly, O. (2004), "Effects of glucose, cellulose, and humic acids on soil microbial eco-physiology", *Journal of Plant Nutrition and Soil Science,* vol. 167, no. 3, pp. 261-266.

Doran, J. W. and Parkin, T. B. (1996), "Defining and assessing soil quality", in Doran, J. W., Coleman, D. C., Bezidicek, D. F., et al (eds.) *Defining soil quality for a sustainable environment,* Soil Science Society of America, Special Pubvlication No. 35, Madison, pp. 3-21.

Doran, J. W. and Safley, M. (1997), "Biological Indicators of Soil Health", in Pankhurst, C. E., Doube B.M. and Gupta V.V.S.R. (eds.) *Defining and Assessing Soil Health and Sustainable Productivity,* CABI, Wallington, pp. 1-29.

Duffy, J. E., MacDonald, K. S., Rhode, J. M. and Parker, J. D. (2001), "Grazer diversity, functional redundancy, and productivity in seagrass beds: An experimental test", *Ecology,* vol. 82, no. 9, pp. 2417-2434.

Dunbar, J., Takala, S., Barns, S. M., Davis, J. A. and Kuske, C. R. (1999), "Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning", *Applied and Environmental Microbiology,* vol. 65, no. 4, pp. 1662-1669.

Edwards, D. E., Kremer, R. J. and Keaster, A. J. (1992), "Characterization and growth response of bacteria in soil following application of carbofuran", *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes,* vol. 27, no. 2, pp. 139-154.

Emmerson, M. C., Solan, M., Emes, C., Paterson, D. M. and Raffaelli, D. (2001), "Consistent patterns and the idiosyncratic effects of biodiversity in marine ecosystems", *Nature,* vol. 411, no. 6833, pp. 73-77.

Extoxnet (1994), *Benomyl,* Pesticide Management Education Program, Cornell University.

Felske, A. and Akkermans, A. D. L. (1998), "Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils", *Microbial Ecology,* vol. 36, no. 1, pp. 31-36.

Felske, A., Wolterink, A., Van Lis, R. and Akkermans, A. D. L. (1998), "Phylogeny of the main bacterial 16S rRNA sequences in drentse A grassland soils (The Netherlands)", *Applied and Environmental Microbiology,* vol. 64, no. 3, pp. 871-879.

Fernandez, A. S., Hashsham, S. A., Dollhopf, S. L., Raskin, L., Glagoleva, O., Dazzo, F. B., Hickey, R. F., Criddle, C. S. and Tiedje, J. M. (2000), "Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose", *Applied and Environmental Microbiology,* vol. 66, no. 9, pp. 4058-4067.

Fierer, N. and Jackson, R. B. (2006), "The diversity and biogeography of soil bacterial communities", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 103, no. 3, pp. 626-631.

Filip, Z. (2002), "International approach to assessing soil quality by ecologically-related biological parameters", *Agriculture, Ecosystems and Environment,* vol. 88, no. 2, pp. 169-174.

Finlay, B. J., Maberly, S. C. and Cooper, J. I. (1997), "Microbial diversity and ecosystem function", *Oikos,* vol. 80, no. 2, pp. 209-213.

Fisher, R. A., Corbet, A. S. and Williams, C. B. (1943), "The relation between the number of species and the number of individuals in a random sample of an animal population", *Journal of Animal Ecology,* vol. 12, pp. 42-58.

Folke, C., Holling, C. S. and Perrings, C. (1996), "Biological diversity, ecosystems, and the human scale", *Ecological Applications,* vol. 6, no. 4, pp. 1018-1024.

Frostegård, A. and Bååth, E. (1996), "The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil", *Biology and Fertility of Soils,* vol. 22, no. 1-2, pp. 59-65.

Frostegård, A., Tunlid, A. and Bååth, E. (1993), "Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally

exposed to different heavy metals", *Applied and Environmental Microbiology,* vol. 59, no. 11, pp. 3605-3617.

Frostegård, A., Tunlid, A. and Bååth, E. (1991), "Microbial biomass measured as total lipid phosphate in soils of different organic content", *Journal of Microbiological Methods,* vol. 14, no. 3, pp. 151-163.

Gardiner, D. T. and Miller, R. W. (2008), *Soils in our environment,* 11th ed, Pearson Prentice Hall, Upper Saddle River, N.J.

Garland, J. L. and 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,* vol. 57, no. 8, pp. 2351-2359.

Gaston, K. J. and Spicer, J. I. (1998), *Biodiversity: an introduction,* 1st ed, Blackwell Scientific, Oxford, UK.

Giller, K. E., Beare, M. H., Lavelle, P., Izac, A. -. N. and Swift, M. J. (1997), "Agricultural intensification, soil biodiversity and agroecosystem function", *Applied Soil Ecology,* vol. 6, no. 1, pp. 3-16.

Gil-Sotrés, F., Trasar-Cepeda, C., Leirós, M. C. and Seoane, S. (2005), "Different approaches to evaluating soil quality using biochemical properties", *Soil Biology and Biochemistry,* vol. 37, no. 5, pp. 877-887.

Giri, B., Giang, P. H., Kumari, R., Prasad, R. and Varma, A. (2005), "Microbial diversity in soil", in Buscot, F. and Varma, A. (eds.) *Soil biology, vol 3: microorganisms in soil: role in genesis and functions,* Springer, Berlin, pp. 19-49.

Glowka, L., Burhenne-Guilmin, F. and Synge, H. (1994), *A guide to the Convention on biological diversity,* IUCN, Gland & Cambridge.

Godley, A. R. (2004), "Factors affecting the soil microbial quality measurements of biomass quotient and respiration quotient", *The Journal,* vol. 18, no. 2, pp. 73-79.

Gray, N. F. (1985), "Ecology of nematophagous fungi: Effect of soil moisture, organic matter, pH and nematode density on distribution", *Soil Biology and Biochemistry,* vol. 17, no. 4, pp. 499-507.

Griffiths, B. S., Bonkowski, M., Roy, J. and Ritz, K. (2001), "Functional stability, substrate utilisation and biological indicators of soils following environmental impacts", *Applied Soil Ecology,* vol. 16, no. 1, pp. 49-61.

Griffiths, B. S., Kuan, H. L., Ritz, K., Glover, L. A., McCaig, A. E. and Fenwick, C. (2004), "The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil", *Microbial Ecology,* vol. 47, no. 1, pp. 104-113.

Griffiths, B. S., Ritz, K., Bardgett, R. D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S. J., Bååth, E., Bloem, J., De Ruiter, P. C., Dolfing, J. and Nicolardot, B. (2000), "Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: An examination of the biodiversity-ecosystem function relationship", *Oikos,* vol. 90, no. 2, pp. 279-294.

Griffiths, B. S., Ritz, K. and Wheatley, R. E. (1997), "Relationship between functional diversity and genetic diversity in complex microbial communities", in Insam, H. and Rangger, A. (eds.) *Microbial communities,* 1st ed, Springer-Verlag, Heidelberg, pp. 1-9.

Grime, J. P. (2001), *Plant strategies, vegetation processes and ecosystem proporties,* 2nd ed, John Wiley and Sons, Chichester.

Harrison, I., Laverty, M. and Sterling, E. (2004a), "Genetic diversity", *Connexions Website,* [Online], pp. July 28 available at: http://cnx.org/content/m12158/1.2/.

Harrison, I., Laverty, M. and Sterling, E. (2004b), "Species diversity", *Connexions Website,* [Online], pp. 29 July available at: http://cnx.org/content/m12174/1.3/.

Harrison, I., Laverty, M. and Sterling, E. (2004c), "Ecosystem diversity", *Connexions Website,* [Online], pp. 29 July available at: http://cnx.org/content/m12156/1.2/.

Hawksworth, D. L. (2001), "The magnitude of fungal diversity: the 1.5 millions species estimate revisited", *Mycological Research,* vol. 105, pp. 1422-1432.

Head, I. M., Saunders, J. R. and Pickup, R. W. (1998), "Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms", *Microbial Ecology,* vol. 35, no. 1, pp. 1-21.

Herbert, D. A., Fownes, J. H. and Vitousek, P. M. (1999), "Hurricane damage to a Hawaiian forest: Nutrient supply rate affects resistance and resilience", *Ecology,* vol. 80, no. 3, pp. 908-920.

Heuer, H. and Smalla, K. (1997), "Evaluation of community-level catabolic profiling using BIOLOG GN microplates to study microbial community changes in potato phyllosphere", *Journal of Microbiological Methods,* vol. 30, no. 1, pp. 49-61.

Heywood, V. H. and Bates, I. (1995), "Introduction", in Heywood, V. H. and Watson, R. T. (eds.) *Global biodiversity assessment,* 1st ed, Cambridge University Press, Cambridge, pp. 1-21.

Holling, C. S. (1973), "Resilience and stability of ecological systems", *Annual Review of Ecology and Systematics,* vol. 4, pp. 1-23.

Hooper, D. U. and Vitousek, P. M. (1997), "The effects of plant composition and diversity on ecosystem processes", *Science,* vol. 277, no. 5330, pp. 1302-1305.

Hughes, R. G. (1984), "A model of the structure and dynamics of benthic marine intervertebrate communities", *Marine Ecology Progress Series,* vol. 23, pp. 1-11.

Insam, H. (2001), "Developments in soil microbiology since the mid 1960s", *Geoderma,* vol. 100, no. 3-4, pp. 389-402.

Insam, H. and Domsch, K. H. (1988), "Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites", *Microbial Ecology,* vol. 15, no. 2, pp. 177-188.

Javoreková, S., Kraliková, A., Labuda, R., Labudová, S. and Maková, J. (eds.) (2008), *Biológia pôdy v agroekosystémoch (Soil biology in agricultural ecosystems),* Slovak Agricultural University, Nitra.

Jenkinson, D. S. (1988), "Determination of microbial biomass nitrogen and carbon in soil", in Wilson, J. T. (ed.) *Advances in nitrogen cycling in agricultural ecosystems,* Commonwealth Agricultural Bureau International, Wallingford, pp. 368-386.

Jenkinson, D. S. and Ladd, J. N. (1981), "Microbial biomass in soil: measurement and turnover", in Paul, E. A. and Ladd, J. N. (eds.) *Soil biochemistry,* Marcel Dekker, New York, pp. 415-475.

Joergensen, R. G. (1996), "The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value", *Soil Biology and Biochemistry,* vol. 28, no. 1, pp. 25-31.

Joergensen, R. G., Meyer, B. and Mueller, T. (1994), "Time-course of the soil microbial biomass under wheat: A one year field study", *Soil Biology and Biochemistry,* vol. 26, no. 8, pp. 987-994.

Johnson, D. W. and Todd Jr., D. E. (1998), "Harvesting effects on long-term changes in nutrient pools of mixed oak forest", *Soil Science Society of America Journal,* vol. 62, no. 6, pp. 1725-1735.

Johnson, K. H., Vogt, K. A., Clark, H. J., Schmitz, O. J. and Vogt, D. J. (1996), "Biodiversity and the productivity and stability of ecosystems", *Trends in Ecology and Evolution,* vol. 11, no. 9, pp. 372-377.

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline, R. G., Harris, R. F. and Schuman, G. E. (1997), "Soil quality: A concept, definition, and framework for evaluation: (A guest editorial)", *Soil Science Society of America Journal,* vol. 61, no. 1, pp. 4-10.

Kibblewhite, M. G., Ritz, K. and Swift, M. J. (2008), "Soil health in agricultural systems", *Philosophical Transactions of the Royal Society B: Biological Sciences,* vol. 363, no. 1492, pp. 685-701.

Krebs, C. J. (1989), *Ecological methodology,* 1st ed, Harper Collins, Inc., New York.

Kuan, H. L., Fenwick, C., Glover, L. A., Griffiths, B. S. and Ritz, K. (2006), "Functional resilience of microbial communities from perturbed upland grassland soils to further persistent or transient stresses", *Soil Biology and Biochemistry,* vol. 38, no. 8, pp. 2300-2306.

Lal, R. (1998), *Soil quality and agricultural sustainability,* CRC Press.

Landgraf, D. (2001), "Dynamics of microbial biomass in Cambisols under a three year succession fallow in North Eastern Saxony", *Journal of Plant Nutrition and Soil Science,* vol. 164, no. 6, pp. 665-671.

Lawton, J. H. (1994), "What do species do in ecosystems?", *Oikos,* vol. 71, no. 3, pp. 367-374.

Lee, S., Bollinger, J., Bezdicek, D. and Ogram, A. (1996), "Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method", *Applied and Environmental Microbiology,* vol. 62, no. 10, pp. 3787-3793.

Liesack, W., Janssen, P. H., Rainey, F. A., Ward-Rainey, N. L. and and Stackebrandt, E. (1997), "Microbial diversity in soil: The need for a combined approach using molecular and cultivation techniques", in Van Elsas, J. D., Trevors, J. T. and Wellington, E. M. H. (eds.) *Modern soil microbiology,* Marcel Dekker, Inc., New York, pp. 375-439.

MacArthur, R. (1955), "Fluctuations of animal populations and a measure of community stability", *Ecology,* vol. 36, no. 3, pp. 533-536.

MacArthur, R. H. (1960), "On the relative abundance of species", *American Naturalist,* vol. 94, pp. 25-36.

Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. (2002), "Soil fertility and biodiversity in organic farming", *Science,* vol. 296, no. 5573, pp. 1694-1697.

Magurran, A. E. (1988), "Diversity indices and species abundance models", in Magurran, A. E. (ed.) *Ecological diversity and its measurement,* 1st ed, Princeton University Press, Princeton, New Jersey, pp. 7-46.

Margalef, R. (1968), *Perspectives in ecological theory,* University of Chicago Press, Chicago.

Marx, M., Wood, M. and Jarvis, S. C. (2001), "A microplate fluorimetric assay for the study of enzyme diversity in soils", *Soil Biology and Biochemistry,* vol. 33, no. 12-13, pp. 1633-1640.

May, R. M. (1975), "Patterns of species abundance and diversity", in Cody, L. M. and Diamond, J. L. (eds.) *Ecology and evolution of communities,* 1st ed, Harvard University Press, Cambridge, Massachussets, USA, pp. 81-120.

McCulley, R. L., Archer, S. R., Boutton, T. W., Hons, F. M. and Zuberer, D. A. (2004), "Soil respiration and nutrient cycling in wooded communities developing in grassland", *Ecology,* vol. 85, no. 10, pp. 2804-2817.

McGrady-Steel, J., Harris, P. M. and Morin, P. J. (1997), "Biodiversity regulates ecosystem predictability", *Nature,* vol. 390, pp. 162-165.

McIntosh, R. P. (1967), "An index of diversity and the relation of certain concepts to diversity", *Ecology,* vol. 67, pp. 1115-1126.

McNaughton, S. J. (1994), "Biodiversity and function of grazing ecosystems", in Schulze, E. D. and Mooney, H. A. (eds.) *Biodiversity and ecosystem function*, Springer-Verlag, London, pp. 361-383.

McNaughton, S. J. (1977), "Diversity and stability of ecological communities: a comment on the role of empiricism in ecology", *American Nature,* vol. 111, pp. 515-525.

Millenium Ecosystem Assessment (2003), *Ecosystem and Human Well-Being: Synthesis,* 1st ed, Island Press, Washington, DC.

Moore, J. C. and De Ruiter, P. C. (2000), "Intervertebrates in detrital food webs along gradients of productivity", in Coleman, C. D. and Hendrix, P. F. (eds.) *Intervertebrates as webmasters in ecosystem,* 1st ed, CABI Publishing, , pp. 161-184.

Müller, A. K., Westergaard, K., Christensen, S. and Sørensen, S. J. (2002), "The diversity and function of soil microbial communities exposed to different disturbances", *Microbial Ecology,* vol. 44, no. 1, pp. 49-58.

Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H. and Woodfin, R. M. (1994), "Declining biodiversity can alter the performance of ecosystems", *Nature,* vol. 368, no. 6473, pp. 734-737.

Nannipieri, P. and Badalucco, L. (2002), "Biological processes", in Kenbi, D. K. and Nieder, R. (eds.) *Handbook of processes and modelling in the soil-plant system,* The Haworth Press Inc., New York, pp. 57-82.

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G. and Renella, G. (2003), "Microbial diversity and soil functions", *European Journal of Soil Science,* vol. 54, no. 4, pp. 655-670.

Odum, E. P. (1975), *Ecology,* 1st ed, Holt Saunders, London.

Oldeman, L. R. (ed.) (1994), *The global extent of soil degradation,* CAB International, Wallingford.

Orwin, K. H. and Wardle, D. A. (2004), "New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances", *Soil Biology and Biochemistry,* vol. 36, no. 11, pp. 1907-1912.

Orwin, K. H., Wardle, D. A. and Greenfield, L. G. (2006), "Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences", *Oikos,* vol. 112, no. 1, pp. 196-208.

Øvreås, L. and Torsvik, V. (1998), "Microbial diversity and community structure in two different agricultural soil communities", *Microbial Ecology,* vol. 36, no. 3, pp. 303-315.

Özbek, A. (2004), "Effects of polyvinyl alcohol (PVA) on some soil-water parameters", *Proceedings of ICID Interregional Conference on food production and water: social and economic issues of irrigation and drainage,* Moscow, Soil Science Society of Turkey International Symposium, Moscow.

Öztas, T., Özbek, A. and Aksakal, E. (2004), "Structural developments in soil treated with Polyvinyl alcohol", *International Conference on Sustainable Land Use and Management,* 11 September, Moscow, Soil Science Society of Turkey International Symposium, Moscow, pp. 143.

Pankhurst, C. E., Yu, S., Hawke, B. G. and Harch, B. D. (2001), "Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia", *Biology and Fertility of Soils,* vol. 33, no. 3, pp. 204-217.

Pascual, J. A., Garcia, C., Hernandez, T., Moreno, J. L. and Ros, M. (2000), "Soil microbial activity as a biomarker of degradation and remediation processes", *Soil Biology and Biochemistry,* vol. 32, no. 13, pp. 1877-1883.

Pawlett, M. (2002), *The interaction between earthworms, liming and soil microbial community diversity and function in upland grassland,* University of East London, London.

Peijnenburg, W. (2000), "Fate of contaminants in soil", in Doelman, P. and Eijsackers, H. (ed.) *Vital soil,* Elsevier, Oxford, pp. 245-280.

Pennanen, T. (2001), "Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH - A summary of the use of phospholipid fatty acids, Biolog® and 3H-thymidine incorporation methods in field studies", *Geoderma,* vol. 100, no. 1-2, pp. 91-126.

Petchey, O. L., Casey, T., Jiang, L., McPhearson, P. T. and Price, J. (2002), "Species richness, environmental fluctuations, and temporal change in total community biomass", *Oikos,* vol. 99, no. 2, pp. 231-240.

Pielou, E. C. (1975), *Ecological diversity,* 1st ed, Wiley, New York.

Pierzynski, G. M., Sims, J. T. and Vance, G. F. (2005), *Soils and environmental quality,* CRC Press, Boca Raton, Fl.

Pimm, S. L. (1984), "The complexity and stability of ecosystems", *Nature,* vol. 307, pp. 321-326.

Piotrowska-Seget, Z. and Mrozik, A. (2003), "Signature Lipid Biomarker (SLB) analysis in determining changes in community structure of soil microorganisms", *Polish Journal of Environmental Studies,* vol. 12, no. 6, pp. 669-675.

Plaza, C., Senesi, N., Polo, A., Brunetti, G., García-Gil, J. C. and D'Orazio, V. (2003), "Soil fulvic acid properties as a means to assess the use of pig slurry amendment", *Soil and Tillage Research,* vol. 74, no. 2, pp. 179-190.

Power, M. E. and Mills, L. S. (1995), "The Keystone cops meet in Hilo", *Trends in Ecology and Evolution,* vol. 10, no. 5, pp. 182-184.

Prasad, R. and Power, J. F. (1997), *Soil fertility management for sustainable agriculture,* CRC Press, Boca Raton, Fl.

Preston, F. W. (1962), "The canonical distribution of commonness and rarity", *Ecology,* vol. 43, pp. 410-432.

Priha, O., Grayston, S. J., Hiukka, R., Pennanen, T. and Smolander, A. (2001), "Microbial community structure and characteristics of the organic matter in soils under

Pinus sylvestris, Picea abies and Betula pendula at two forest sites", *Biology and Fertility of Soils,* vol. 33, no. 1, pp. 17-24.

Richardson, M. (1996), *Environmental Xenobiotics,* 1st ed, CRC Press, Abingdon.

Ritz, K., Harris, J. A., Pawlett, M. and Stone, D. (2006), *The use of catabolic profiles as an indicator of soil microbial functional diversity,* SC040063/SR, Environment Agency Science Report.

Ritz, K., McNicol, J. W., Nunan, N., Grayston, S., Millard, P., Atkinson, D., Gollotte, A., Habeshaw, D., Boag, B., Clegg, C. D., Griffiths, B. S., Wheatley, R. E., Glover, L. A., McCaig, A. E. and Prosser, J. I. (2004), "Spatial structure in soil chemical and microbiological properties in an upland grassland", *FEMS Microbiology Ecology,* vol. 49, no. 2, pp. 191-205.

Růžek, L., Voříšek, K., Strnadová, S., Nováková, M. and Barabasz, W. (2004), "Microbial characteristics, carbon and nitrogen content in cambisols and luvisols", *Plant, Soil and Environment,* vol. 50, no. 5, pp. 196-204.

Šantrůčková, H. (1993), "Soil respiration as a measure of biological activity", *Rostlinná Výroba,* vol. 39, pp. 769-778.

Saviozzi, A., Levi-Minzi, R., Cardelli, R. and Riffaldi, R. (2001), "A comparison of soil quality in adjacent cultivated, forest and native grassland soils", *Plant and Soil,* vol. 233, no. 2, pp. 251-259.

Sayler, G. S. and Layton, A. C. (1990), "Environmental application of nucleic acid hybridization", *Annual Review of Microbiology,* vol. 44, pp. 625-648.

Saxena, S. K. (2004), *Polyvinylalcohol (PVA): Chemical and technical assessment (CTA),* FAO.

Schulze, E. D. and Mooney, H. A. (1994), *Biodiversity and Ecosystem Function,* 1st ed, SpringerSpringer-Verlag Berlin and Heidelberg GmbH & Co. K, Berlin, Germany.

Schwartz, M. W., Brigham, C. A., Hoeksema, J. D., Lyons, K. G., Mills, M. H. and Van Mantgem, P. J. (2000), "Linking biodiversity to ecosystem function: Implications for conservation ecology", *Oecologia,* vol. 122, no. 3, pp. 297-305.

Seybold, C. A., Herrick, J. E. and Brejda, J. J. (1999), "Soil resilience: a fundamental component of soil quality", *Soil Science,* vol. 164, pp. 224-234.

Shah, Z., Adamst, W. A. and Haven, C. D. V. (1990), "Composition and activity of the microbial population in an acidic upland soil and effects of liming", *Soil Biology and Biochemistry,* vol. 22, no. 2, pp. 257-263.

Shannon, C. E. (1948), "A mathematical theory of communication", *Bell System Technical Journal,* vol. 27, pp. 379-423.

Shaw, L. J. and Burns, R. G. (1996), "Enzyme activity profiles and soil quality", in Bloem, J., Hopkins, D. and Benedetti, A. (eds.) *Microbiological methods for assessing soil quality,* CABI Publishing, Wallington, pp. 158-183.

Simpson, E. H. (1949), "Measurement of diversity", *Nature,* vol. 163, pp. 688.

Smit, E., Leeflang, P. and Wernars, K. (1997), "Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis", *FEMS Microbiology Ecology,* vol. 23, no. 3, pp. 249-261.

Stachowicz, J. J., Fried, H., Osman, R. W. and Whitlatch, R. B. (2002), "Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process", *Ecology,* vol. 83, no. 9, pp. 2575-2590.

Staddon, W. J., Duchesne, L. C. and Trevors, J. T. (1997), "Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns", *Microbial Ecology,* vol. 34, no. 2, pp. 125-130.

Steinman, A. D., Mulholland, P. J. and Palumbo, A.V. et. al. (1990), "Resistance of lotic ecosystems to a light elimination disturbance: a laboratory streams study", *Oikos,* vol. 58, pp. 80-90.

Stevenson, B. A., Sparling, G. P., Schipper, L. A., Degens, B. P. and Duncan, L. C. (2004), "Pasture and forest soil microbial communities show distinct patterns in their catabolic respiration responses at a landscape scale", *Soil Biology and Biochemistry,* vol. 36, no. 1, pp. 49-55.

Storch, D., Marquet, P. A. and Brown, J. H. (2007), "Introduction: scaling biodiversity - what is the problem?", in Storch, D., Marquet, P. A. and Brown, J. H. (eds.) *Scaling biodiversity,* 1st ed, Cambridge University Press, Cambridge, pp. 1-11.

Susilo, F. X., Neutel, A. M., Van Noordwijk, M., Hairiah, K., Brown, G. and Swift, M. J. (2004), "Soil biodiversity and food web", in Van Noordwijk, M., Cadisch, G. and Ong, C. K. (eds.) *Below-ground interactions in tropical agroecosystems,* 1st ed, CAB Internetional, Wallingford, pp. 285-302.

Suzuki, C., Kunito, T., Aono, T., Liu, C. and Oyaizu, H. (2005), "Microbial indices of soil fertility", *Journal of Applied Microbiology,* vol. 98, no. 5, pp. 1062-1074.

Swift, M. J., Izac, A. -. N. and Van Noordwijk, M. (2004), "Biodiversity and ecosystem services in agricultural landscapes - Are we asking the right questions?", *Agriculture, Ecosystems and Environment,* vol. 104, no. 1, pp. 113-134.

Thomas, M. R. and Garthwaite, D. G. (1993), *Outdoor Bulbs and Flowers in Great Britain,* Pesticide Usage Survey Report 121, Central Science Laboratory.

Tilman, D., Wedin, D. and Knops, J. (1996), "Productivity and sustainability influenced by biodiversity in grassland ecosystems", *Nature,* vol. 379, no. 6567, pp. 718-720.

Tomlin, C. (1994), *The pesticide manual,* British Crop Protection Council, Surrey.

Torsvik, V. and Øvreås, L. (2002), "Microbial diversity and function in soil: From genes to ecosystems", *Current Opinion in Microbiology,* vol. 5, no. 3, pp. 240-245.

Torsvik, V., Øvreås, L. and Thingstad, T. F. (2002), "Prokaryotic diversity - Magnitude, dynamics, and controlling factors", *Science,* vol. 296, no. 5570, pp. 1064-1066.

Tscherko, D. and Kandeler, E. (1999), "Classification and monitoring of soil microbial biomass, N-mineralization and enzyme activities to indicate environmental changes", *Bodenkultur,* vol. 50, no. 4, pp. 215-226.

Tunlid, A., Ringelberg, D., Phelps, T. J., Low, C. and White, D. C. (1989), "Measurement of phospholipid fatty acids at picomolar concentrations in biofilms and deep subsurface sediments using gas chromatography and chemical ionization mass spectrometry", *Journal of Microbiological Methods,* vol. 10, no. 2, pp. 139-153.

Turco, R. F., Kennedy, A. C. and Jawson, M. D. (1994), "Microbial indicators of soil quality", in Doran, J. W., Coleman, D. C., Bezdicek, D. F., et al (eds.) *Defining soil quality for sustainable environment,* Soil Science Society of America, Madison, pp. 73-90.

Turgay, O. C. and Haraguchi, A. (2000), "Different indices of soil biological activities; Measurement of soil microbial biomass carbon, nitrogen and NRN", *International Symposium on Desertification,* Konya.

Uckam, H. S. and Okur, N. (2004), *Seasonal changes in soil microbial biomass and enzyme activity in arable and grassland soil,* Finnish Environment Institute Laboratory, Helsinki.

Van Der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. and Sanders, I. R. (1998), "Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity", *Nature,* vol. 396, no. 6706, pp. 69-72.

Van Elsas, J. D., Duarte, G. F., Keijzer-Wolters, A. and Smit, E. (2000), "Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis", *Journal of Microbiological Methods,* vol. 43, no. 2, pp. 133-151.

Vance, E. D., Brookes, P. C. and Jenkinson, D. S. (1987), "An extraction method for measuring soil microbial biomass C", *Soil Biology and Biochemistry,* vol. 19, no. 6, pp. 703-707.

Verma, K. S. and Bradley, R. I. (1988), *Soils of the College Farm, Silsoe, Bedfordshire,* Soil Survey and Land Research Centre (SSLRC) Report, Silsoe.

Vitousek, P. M. and Hooper, D. U. (1993), "Biological diversity and terrestrial ecosystem biochemistry", in Schulze, E. D. and Mooney, H. A. (eds.) *Biodiversity and ecosystem function,* 1st ed, Springer-Verlag, Berlin, pp. 3-14.

Voroney, R. P. (2007), "The soil habitat", in Paul, E. A. (ed.) *Soil microbiology, ecology and biochemistry,* 3rd ed, Elsevier, Oxford, pp. 25-52.

Walker, B. H. (1992), "Biodiversity and ecological redundancy", *Conservation Biology,* vol. 6, no. 1, pp. 18-23.

Wardle, D. A., Bonner, K. I. and Barker, G. M. (2000), "Stability of ecosystem properties in response to above-ground functional group richness and composition", *Oikos,* vol. 89, pp. 11-23.

Weaver, R. W., Angle, S., Bottomley, P., Bezdicek, P., Smith, S., Tabatabai, A. and Wollum, A. (1994), *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties,* Soil Science Society of America Book, Madison.

Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Guillaumaud, N. and Le Roux, X. (2007), "Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance", *Environmental Microbiology,* vol. 9, no. 9, pp. 2211-2219.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D. and Bobbie, R. J. (1979), "Determination of the sedimentary microbial biomass by extractible lipid phosphate", *Oecologia,* vol. 40, no. 1, pp. 51-62.

Whitman, W. B., Coleman, D. C. and Wiebe, W. J. (1998), "Perspective: Prokaryotes: the unseen majority", *Proceedings of the National Academy of Sciences,* vol. 95, no. 12, pp. 6578-6583.

Whittaker, R. H. (1972), "Evolution and measurement of species diversity", *Taxon,* vol. 21, pp. 313-351.

Winding, A., Hund-Rinke, K. and Rutgers, M. (2005), "The use of microorganisms in ecological soil classification and assessment concepts", *Ecotoxicology and Environmental Safety,* vol. 62, no. 2 SPEC. ISS., pp. 230-248.

Woodwell, G. M. and Smith, H. H. (1969), *Diversity and stability in ecological systems,* 1st ed, Brookhaven National Laboratory, Associated Universities, Inc., New York.

World Health Organization (1993), *Environmental Health Criteria 148,* WHO, Geneva.

Xu, Q. and Jiang, P. (2005), "Microbial development in soils under intensively managed bamboo (Phyllostachys praecox) stands", *Pedosphere,* vol. 15, no. 1, pp. 33-40.

Yao, H., He, Z., Wilson, M. J. and Campbell, C. D. (2000), "Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use", *Microbial Ecology,* vol. 40, no. 3, pp. 223-237.

Young, I. M. and Ritz, K. (2005), "The habitat of soil microbes", in Bardgett, R. D., Usher, M. B. and Hopkins, D. W. (eds.) *Biological diversity and function in soils,* Cambridge University Press, Cambridge, pp. 31-43.

Zak, J. C., Willig, M. R., Moorhead, D. L. and Wildmand, H. G. (1994), "Functional diversity of microbial communities: a quantitative approach", *Soil Biology and Biochemistry,* vol. 26, pp. 1101-1108.

Zelles, L. (1999), "Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review", *Biology and Fertility of Soils,* vol. 29, no. 2, pp. 111-129.

Zogg, G. P., Zak, D. R., Ringelberg, D. B., MacDonald, N. W., Pregitzer, K. S. and White, D. C. (1997), "Compositional and functional shifts in microbial communities due to soil warming", *Soil Science Society of America Journal,* vol. 61, no. 2, pp. 475-481.

Zyakun, A. M. and Dilly, O. (2005), "Use of carbon isotope composition for characterization of microbial activity in arable soils", *Applied Biochemistry and Microbiology,* vol. 41, no. 5, pp. 512-520.