UCD SCHOOL OF BIOLOGY AND ENVIRONMENTAL SCIENCE

Postgraduate seminar day

Dec 2009

UCD SCHOOL OF BIOLOGY AND ENVIRONMENTAL SCIENCE

POSTGRADUATE SEMINAR DAY
Agriculture and Food Science Centre, FSG01
Thursday, 3rd December 2009

PROGRAMME

9:15 Opening remarks: Dana Miller (Postgraduate representative)

Session 1 **Chair: *Dr. Matt Saunders***

9:20 – 9:40 *Carol Fitzroy*
Proteomic characterisation of pea aphid salivary proteins: unveiling hidden molecular interactions between aphids and plants.

9:40 – 10:00 *Edel Hannigan*
An investigation of seasonal variation in the macroinvertebrate communities of the open-water habitats in Irish peatlands.

10.00 – 10.20 *Thorsten Knipfer*
Whole-plant water flow in barley – the role of apoplastic and cell-to-cell (aquaporins) root water flow in regulating shoot water supply?

10:20 – 10:40 *Simone Hepp*
Impacts of the soil moisture on trace gas emissions from grassland: a case study in Northern Ireland

10.40 – 11.00 *Nizar Ibrahim*
Fossil vertebrates from the Early Late Cretaceous of Morocco.

COFFEE BREAK (tea, coffee and biscuits provided)

Session 2 **Chair: *Bruno Simões***

11:25 – 11:45 *Louise Deering*
The effects of anthropogenic and environmental factors on microbial communities in a raised bog.

11:45 – 12:05 *Burkart Dieterich*
Application of digestate on permanent grassland – fertiliser value and environmental impact.

12:05 – 12:25 *Aoife Corrigan*
Microbial population dynamics of poultry cecal contents over time and with respect to dietary supplementation with Bio-Mos®.

12:25 – 12.45 *Bas Boots*
Ants and microbial diversity in Irish grasslands.

12.45 – 1:05 *Sara Hayden*
Ecological adaptation detemines functional mammalian olfactory subgenomes.

LUNCH BREAK

Session 3 **Chair: *Dr. Emma Teeling***

2:30 – 2.50 *Jayne Fitch*
Combined effects of inorganic nutrients and organic enrichment on littoral benthic macro-fauna: an experimental approach.

2.50 – 3:10 *Serena Dool*
Phylogeography and population genetics of the lesser horseshoe bat (*Rhinolophus hipposideros*).

3:10 – 3:30 *Shahin Ali*
Cellulosic-ethanol: simultaneous saccharidification and fermentation of wheat straw and bran.

3:30 – 3.50 *John Kirwan*
The molecular evolution of hearing in mammals.

3.50 – 4.10 *Luke Mander*
Reconstructing vegetation cover in deep time: macrofossil leaves, pollen and spores, or both?

4:10 – 4:30 *Przemyslaw Sawulski*
Effect of polycyclic aromatic hydrocarbons (PAHs) on microbial community dynamics in soil.

Close of seminar: Prof. Tom Bolger

Reception to follow in common area, SCRC West

Proteomic characterisation of pea aphid salivary proteins: unveiling hidden molecular interactions between aphids and plants.

Carol Fitzroy*, James Carolan[1] and Tom Wilkinson

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Aphids are major pests of temperate agriculture, causing economic damage through direct infestation and the transmission of plant viruses. Aphid mouthparts are adapted to pierce through plant tissue to feed on phloem sap within an individual sieve element. This intimate relationship requires the aphid to overcome many defense mechanisms employed by their plant host, and bioactive components within aphid saliva may play a crucial role in this interaction. The aim of this study was to identify secreted salivary proteins from the pea aphid (*Acyrthosiphon pisum*) using mass spectrometry and resources resulting from the recently completed genome sequence of the pea aphid. To obtain protein of sufficient quantity and quality for mass spectrometry required the development of a novel collection protocol using an artificial feeding system. Nine proteins were identified in the secreted saliva. Four proteins were identified by their sequences: a homolog of angiotensin converting enzyme (an M2 metalloprotease), an M1 zinc-dependant metalloprotease, a glucose-methanol-choline oxidoreductase and regucalcin (a calcium-binding protein also known as senescence marker protein 30). The other five proteins were not homologous to any previously described sequence. One of these novel proteins (termed SHP-1) is thought to be a major constituent of the salivary sheath, a structure essential for successful aphid feeding. Genomic expression of the four identified proteins and SHP-1 was confirmed following mRNA extraction, cDNA synthesis and PCR. The putative role of the salivary proteins in the aphid-plant interaction will be discussed.

Keywords: Aphid-plant interactions, aphid saliva, mass spectrometry, novel proteins, SHP-1.

An investigation of seasonal variation in the macroinvertebrate communities of the open-water habitats in Irish peatlands.

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The present study is part of a national project developing a protocol for sustainable peatland management (www.ucd.ie/bogland). The hydrochemical and biological characteristics of open-water habitats in Irish peatlands are being examined. In the present study seven sites were chosen to represent all categories of peatland and included two raised bogs, one fen, two Atlantic blanket bogs and two mountain blanket bogs. The hydrochemical parameters measured were pH, alkalinity, conductivity, nutrients (nitrate, phosphate and ammonia), total organic carbon and the major cations and anions. The macroinvertebrate samples were taken using both sweep netting and activity traps in spring and autumn 2006. The hydrochemical result showed that Scragh bog, which is a fen, was minerotrophic while the other six sites were all characteristic of ombrotrophic bogs. In terms of the macroinvertebrate communities present, species richness generally decreased in autumn in all sites while species abundance increased in the majority of sites. This paper further explores seasonal changes in species composition and considers its implications for future monitoring of such habitats.

Keywords: Macroinvertebrates, peatlands, open-water habitats, seasonal variation.

Whole-plant water flow in barley – the role of apoplastic and cell-to-cell (aquaporins) root water flow in regulating shoot water supply?

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The present research aims at a detailed analysis of whole-plant hydraulic properties in barley with focus on the implication of the root-system in regulating shoot water supply, i.e. by the activity of root aquaporins. Root aquaporins have been studied extensively in the context of limiting water transport through plants. The implicit assumption has been that the biophysical hydraulic properties of roots actually limit whole-plant water flow. In the present study we test this assumption on hydroponically-grown barley plants which are 14–18 days old at the time of analyses. A range of techniques are used (cell pressure probe, root pressure probe, vacuum pump, gravimetric analyses) to measure the hydraulic properties of the main components along the transport path of water through plants and to assess the significance of cell-to-cell water flow (involving aquaporins) in roots, next to apoplastic root water flow along the cell wall. To quantify the relative contribution of root aquaporins on overall root water flow, the ratio of apoplastic *vs.* cell-to-cell water flow is crucial to determine. Based on these ratios, experiments show that inhibition of root aquaporins by H_2O_2 (OH^-) and $HgCl_2$ (Hg^{2+}) result in an up to 40 – 50% decrease of root hydraulic conductivity and transpirational water loss. The data suggests that root aquaporins can regulate significantly shoot water transport next to biophysical limitations, e.g. xylem development.

Keywords: Whole-plant water flow, root hydraulics, aquaporins, transpiration.

Impacts of soil moisture on trace gas emissions from grassland: a case study in Northern Ireland.

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With rising global demand for food production, cultivated land and inputs of chemical fertilisers into agricultural ecosystems are expected to further increase. As a result, the emission of greenhouse gases (GHGs) from agriculture may be intensified and their impact on global climate may become more serious. Therefore, greater understanding of the processes of trace gas emissions from agriculture must be developed. This understanding will result in the development of practical measures for reducing trace gas emissions from agriculture. In this study, the impacts of soil moisture on trace gas emissions from grassland were investigated with an in situ measurement which was conducted over two sampling periods from 2008–2009 in a typical agricultural ecosystem of Northern Ireland. This study is part of the project 'Evaluating Irish grassland as a source for bioenergy' where environmental impacts as well as long-term sustainability are examined. Gas samples were taken with the closed chamber technique and analysed on a gas chromatographer for carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). As a result wet soil conditions after a long period of rainfall led to high emissions of CH_4 were there was an uptake measured before during drier periods. Wet conditions also led to high emissions of N_2O and a loss of applied nitrogen (N) fertilizer. These results are valuable toward the development of practical measures for reducing greenhouse gas emissions from agriculture.

Keywords: Irish grasslands, soil moisture, trace gas emissions.

Fossil vertebrates from the Early Late Cretaceous of Morocco.

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Recent and historical discoveries from the Kem Kem beds of South Eastern Morocco, located in a remote area close to the Algerian border, have yielded one of the world's richest early Late Cretaceous terrestrial vertebrate assemblages. This sequence is important because, in contrast to other landmasses, terrestrial vertebrate evolution in the Cretaceous of Africa remains poorly understood. As part of an ongoing research project, which combines fieldwork in the Sahara with extensive work in museum collections, the diversity, evolutionary significance and geological context of the Kem Kem assemblage are explored. This work, which has led to new discoveries, including some spectacular fossil specimens and new kinds of crocodiles, pterosaurs and dinosaurs, reveals that the assemblage is far more diverse than previously thought. Particular attention is given to the diversity and abundance of theropod dinosaurs: surprisingly, remains of these predators, which include extremely large forms such as *Carcharodontosaurus* and *Spinosaurus*, appear to be unusually common in museum collections as well as in the field. Herbivorous dinosaurs, such as ornithopods and sauropods, however, appear to be rare, although new elements of large sauropods found in 2008 suggest greater diversity.

Keywords: Dinosaurs, pterosaurs, cretaceous, North Africa, Morocco.

The Effects of Anthropogenic and Environmental Factors on Microbial Communities in a Raised Bog.

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Peatlands are unique ecosystems supporting important and rare wildlife habitats. They cover more than 4 x 10^6 km^2 (approx. 3%) of the earth's total land and freshwater area and are responsible for 40-60% of global methane emissions of which around 74% is derived from microbial activity. Atmospheric concentrations of methane are increasing globally by approximately 1% yr^{-1}. Practices of cutting and draining Ireland's peatlands, although being economically viable, have resulted in extensive losses of raised bog, blanket bog and fens. The objectives of this study are to assess diversity of the main microbial groups active in Irish peatlands, including bacteria, archaea, and specifically methanogenic archaea and methanotrophic bacteria. Such analyses will endeavour to relate microbial community structure to peatland type, human activity and methane fluxes. Molecular community analysis approaches such as terminal restriction fragment length polymorphism (TRFLP) are being applied, using specific functional and universal PCR primer sets to fingerprint target communities and specific microbial diversity. Organisms associated with methane production (methanogenic and methanotrophic communities) are being targeted by using the functional molecular markers, *mcr* (methyl-coenzyme M reductase) and *mxaF* (methanol dehydrogenase) respectively. The effects of environmental parameters and human influences on the microbial community structure of a raised bog will be discussed.

Keywords: Raised bog, T-RFLP.

Application of digestate on permanent grassland – fertiliser value and environmental impact.

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Digestate from anaerobic digestion of biomass may be used as a fertiliser in agriculture. One option would be applying it to grass that is to be used as a biogas feedstock whereby agricultural nutrient cycles can be closed. However, as the digestate carries an active population of methanogenic micro-organisms, negative environmental impacts in the form of methane emissions are possible. Our goal is to quantify the fertiliser replacement value and environmental impact of digestate application in Ireland. We are conducting a trial on long-term set-aside grassland at the Oak Park station of Teagasc, Republic of Ireland. Treatments comprise four levels of mineral N fertiliser, four levels of digestate matched to the mineral treatments, a combined mineral / digestate treatment and a control receiving no N fertiliser. The two fertilisers are compared with respect to: the efficiency of N utilisation by grass, and the environmental impact from trace gas emissions (CO_2, CH_4, N_2O) after fertilizer application. The data obtained so far (2009) suggest that the "available" N in digestate is less efficient for the support of plant growth than mineral fertiliser but digestate may have environmental advantages.

Keywords: Biogas, grassland, digestate, fertiliser, greenhouse gases.

Microbial population dynamics of poultry cecal contents over time and with respect to dietary supplementation with Bio-Mos®.

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Antibiotics were used traditionally for their prophylactic benefits and with this an improvement in performance was noticed which lead the excessive and unregulated use of antibiotics.in animal feeds. This imposed a selective pressure on bacteria and resulted in resistance to antibiotics developing, which lead to the EU ban on their use in feed. The EU ban on antibiotics has emphasised the need for natural alternatives to antibiotics as growth promoters. Bio-Mos®, a yeast cell wall mannan oligosaccharide, are one such alternative. This study was undertaken to investigate whether dietary supplementation with Bio-Mos® altered the microbial populations of poultry caeca. Efforts were made to elucidate what bacteria were present, their relative quantities and also to investigate if Bio-Mos® has an effect on the levels of antibiotic resistance genes. The techniques employed included Automated Ribosomal Intergenic Spacer Analysis (ARISA), 16S gene clone library analysis and quantitative real time PCR. Results from ARISA analysis showed that significant microbial population shifts were clearly evident as a result of dietary supplementation with Bio-Mos®. 16S gene clone library analysis revealed that the bacteria present in caecal contents are predominantly from three phyla, with Firmicutes being the most abundant followed by the Bacteroidetes and the Proteobacteria. Quantification of specific bacterial groups indicated the bacteria were influenced either positively or negatively by Bio-Mos® supplementation. qPCR of antibiotic resistance genes also revealed that Bio-Mos® had an effect at reducing the levels of certain antibiotic resistance genes present. In conclusion it was found that dietary supplementation with Bio-Mos® causes microbial population shifts in the caecal contents of poultry.

Keywords: Bio-Mos®, microbial population, poultry.

Ants and microbial diversity in Irish grasslands.

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In the soil ants, as earthworms, are seen as ecosystem engineers for their burrowing and nest building activity, which can significantly alter soil characteristics. Ant densities are highest where grasslands are managed in an extensive fashion. Ant species in Irish meadows may have different ecological behaviour, ranging from root-aphid farming (e.g. *Lasius flavus*) to hunting and gathering (e.g. *Myrmica sabuleti*). This study aimed to explore the microbial assemblages associated with ants that display different ecological behaviour. We hypothesised that different ant species harbour different microbial assemblages in their gut systems and nests compared to surrounding non-ant-worked soil. Additionally, nests built by *L. flavus* were expected to harbour similar microbial assemblages regardless of the environment (soil parent material). We selected nests of three different ant species in Special Areas of Conservation, and assessed ant ecosystem engineering effects. Associated nest bacteria (16S rRNA) and fungi (ITS) gene assemblages, as well as some functional genes (nitrogen fixing, *nifH*) were examined utilising molecular approaches. We found that mainly *L. flavus* displayed significant alterations of the soil environment and all ant species harboured different microbial assemblages in their gut system and nest. Interestingly, nitrogen fixing communities were found in ant gut systems, which were highly diverse and 5% was unique to ant abdomen. *Lasius flavus* nests built in different environments harboured extremely different microbial assemblages. However, surprisingly, their gut systems contained similar bacterial and nitrogen fixing assemblages regardless of the environment, suggesting that ant species favour certain microbial gut flora.

Keywords: Irish Grasslands, soil ants.

Ecological adaptation detemines functional mammalian olfactory subgenomes.

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Olfaction is considered as one of the most important modes of sensory perception in animals and provides the basis for the extraordinary sensitivity required for the discrimination of environmental and sexual cues. The olfactory receptors (ORs) are essential for the sense of smell; accordingly there are over 1,000 different OR genes in the mouse genome, and by far, the ORs form the largest gene superfamily in the mammalian genome. Increasingly, environmental niche specialisation is found to be reflected in mammalian genomes. To investigate if the evolution of the OR gene repertoire has been influenced by rudiments such as habitat, we surveyed the published genome sequences from 32 mammals, and amplified and sequenced the OR genes from 18 additional mammals. All study species were classified into four ecogroups; Terrestrial, Aquatic, Semi-Aquatic and Volant. Using PCA and Bayesian methods, we found the distribution of functional OR genes into the OR gene families to be significantly different between terrestrial mammals and all other ecogroups, also between volant and aquatic mammals. This indicates that environmental niche specialization has indeed driven the evolution of olfaction in mammals and that these ecogroups have a unique OR signature.

Keywords: Olfaction, mammals, bats, olfactory receptors.

Combined effects of inorganic nutrients and organic enrichment on littoral benthic macro-fauna: an experimental approach.

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Many marine environments are affected by multiple anthropogenic stressors. Research to date has focused on impacts resulting from individual stressors. Nutrient inputs and organic enrichment are important threats to biodiversity and the provision of goods and services by ecosystems. Nutrient and organic enrichment can occur separately or in combination with each other, but their combined effects are not fully understood. Three levels of nutrient pollution (200, 100 and 0 g/m²) were crossed with two levels of organic matter (200 and 0g/m²) in 0.25m² plots, in Galway Bay, Ireland. The treatments were applied over an 11 month period and plots were sampled after 3 months and 11 months. Organic matter caused greater impacts on benthic communities than nutrients. Their influence on multivariate patterns was largely underpinned by changes in abundance of *Capitella*, which responded primarily to organic matter, but was mediated by levels of nutrients in the system. *Capitella* is an accepted bio-indicator, but our results show that its abundance varies in response to complex interactions among stressors, so cannot be easily interpreted. These finding make it clear that effective management of coastal environments must take account of complex combined effects of multiple stressors.

Keywords: Multiple stressors, nutrients, organic matter, benthic, littoral, macro-fauna.

Phylogeography and population genetics of the lesser horseshoe bat (*Rhinolophus hipposideros*).

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Twenty thousand years ago, the earth was undergoing an ice-age with climatic oscillations between glacial and interglacial periods. Following the last glacial maximum 10,000 years ago, in which much of northern Europe was covered by dense ice-sheets, displaced flora and fauna gradually began to repopulate the Eurasian landmass from glacial refugia. The aim of this study was to retrace the origin of European lineages of lesser horseshoe bat and to look at the population structure within Ireland in detail. Tissue or DNA samples from across the range of this species were either collected through collaborations with European researchers, or during fieldwork in Ireland. DNA was extracted and amplified for mitochondrial (Cytochrome b, D-loop 1740bp in total). Two divergent clades, one in the east of Europe, and one in the west emerge with a contact zone in the east of central Europe. There is a loss of genetic diversity due to founder effects in the north-west of Europe. Phylogeography can help in the prioritization of areas of high value for conservation, that is, the areas used as glacial refugia which retain the highest genetic diversity for the species.

Key words: phylogeography, population genetics, bats, *Rhinolophus.*

Cellulosic-ethanol: simultaneous saccharidification and fermentation of wheat straw and bran.

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Fusarium oxysporum is a good candidate for simultaneous saccharification and fermentation (SSF) of lignocellulosic-agricultural waste to ethanol. We studied 18 *F. oxysporum* strains collected from Ireland to evaluate their SSF ability in solid state fermentation of wheat straw and bran. Based on preliminary studies, 5 strains were selected and their solid state cultural conditions were optimised in terms of media, pH, moisture content and temperature. Among the six different standard fungal growth media tested, production of ethanol was highest in the media earlier described by Mishra *et al.* (1984). The best pH, moisture content and temperature for ethanol production by these fungi were found to be 7, 90% and 30^0C respectively. Under these optimum growth conditions, the *F. oxysporum* isolates produced up to 57 and 136 mg ethanol per gram of wheat straw and bran, respectively. We studied the inter-strain variation in the activity of the enzymes involved in saccharification of cellulose and hemicellulose with lignocellulosic material. Of the 5 strains tested, *F. oxysporum* strain 11C had highest β-glucosidase, endoglucanase and exoglucanase activities. But *F. oxysporum* strain 27E had highest β-xylosidase and endoxylanase activities. Suppressive Subtractive Hybridization (SHH) was carried out to identify the genes of *F. oxysporum* strain 11C involved in such SSF.

Keywords: *F. oxysporum*, saccharification, fermentation.

The molecular evolution of hearing in mammals.

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The ecological importance of hearing as well as the great variety of ear morphology and other adaptations suggests an important role for hearing in mammalian evolution. But how is this manifested at the molecular level? In humans, congenital deafness affects millions. The central processes of hearing are beginning to be understood at the molecular level. Here we examine the role of adaptation at the molecular level in a number of hearing genes, to better understand these genes, how they have evolved and how this can be applied to molecular medicine. We analyse 11 hearing genes involved in different aspects of the hearing process for evidence of natural selection (positive, neutral and purifying) across the mammal taxa, focusing on the 'auditory specialists', the bats. We investigate congenital deafness mutations in some of these genes to develop predictive disease diagnostics. Orthologous gene sequences from mammal taxa are derived from publically available data and experimental amplification and aligned. Tests of selection and polymorphism are applied to these alignments. We find that most hearing genes are under purifying selection and evolutionarily conserved. We find evidence of positive selection in mammals in the gene Myo15a and in the echolocating bats in the gene Tecta. We find that predictive disease diagnostics can be applied to some hearing genes effectively but not in all cases.

Keywords: hearing, mammals.

Reconstructing vegetation cover in deep time: macrofossil leaves, pollen and spores, or both?

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Reconstructions of ancient vegetation are often based on either plant macrofossils or pollen and spores (sporomorphs) alone. In such instances, interpretations may be limited by a lack of understanding of how accurately the fossil record of a particular plant, or group of plants, reflects the original standing vegetation. To explore this issue, the relative proportional representation of three botanical groups has been compared in the macrofossil and sporomorph fossil records across a Triassic–Jurassic (*c*.200Ma) boundary section in east Greenland. These groups are: (1) ferns, comprising all representatives of the class Filicopsida; (2) conifers and seed-ferns, comprising all representatives of the class Coniferopsida and the order Corystospermales; (3) monosulcate producers, comprising all representatives of the classes Cycadopsida, Ginkgopsida, Bennettitopsida and the order Peltaspermales. Ferns appear relatively over-represented in the sporomorph record, whereas monosulcate producers appear relatively under-represented in the sporomorph record. Conifers and seed-ferns appear relatively over-represented in the sporomorph record, although mass occurrences of *Stachyotaxus* and *Podozamites* leaves result in considerable relative over-representation of conifers and seed-ferns in the macrofossil record at certain horizons. The results of this study indicate that vegetation reconstructions based on a single fossil group may be misleading, and should be interpreted with caution.

Keywords: Macrofossil leaves, pollen, spores.

Effect of polycyclic aromatic hydrocarbons (PAHs) on microbial community dynamics in soil.

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Polycyclic aromatic hydrocarbons (PAHs) are rated priority pollutants by environmental protection agencies worldwide due to their abundance and persistence in natural environments, as well as their deleterious effects on living organisms. Bioremediation is a potentially cost effective and safe approach for the rehabilitation of PAH contaminated sites. However, it remains an unpredictable technology due mainly to a lack of understanding of the microbial communities involved in bioremediation and the factors affecting these communities. The aim of this study was to examine the effect of different PAHs at various concentrations and in selected mixtures on microbial communities in soil. The soil used in the experiment was obtained from a former creosote manufacturing facility. Creosote is composed mainly of PAHs and has been extensively used as a commercial timber preservative A microcosm experiment was set up containing soil amended with a 3 ring (phenanthrene), a 4 ring (fluoranthene) and a 5 ring (benzo[a]pyrene) PAH. The rate of disappearance of PAHs was measured by gas chromatography. Dehydrogenase activity and MPN of bacteria and fungi were used as an indication of general microbial activity in the soil. The influence of different PAHs on soil microbial community structure was assessed using culture independent techniques, T-RFLP and ARISA. The effect of the PAH on the abundance of key functional genes involved in bacterial PAH degradation was also assessed by qPCR. Both 3-ring and 4-ring PAHs were readily removed from the pre-exposed soil whereas the more toxic 5 ring PAH benzo[a]pyrene was still present after 40 days. Microbial community profiles differed in amended and non-amended soil. Introduction of PAH into the soil caused short toxic effect shown by dehydrogenase activity assay and MPN The abundance of key functional genes varied over time, but highest levels of the gene encoding a PAH dioxygenase from Gram positive bacteria were detected during the period associated with maximum degradation.

Keywords: Polycyclic aromatic hydrocarbons, microbial community, soil