Actinobacterial community dynamics in long term managed grasslands

Sasha Jenkins[A], Ian Waite[A], Adrian Blackburn[B], Rebecca Husband[B], Steven Rushton[B] and Anthony O'Donnell[A]

[A]Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley WA 6009, Australia Email sasha.jenkins@uwa.edu.au
[B]Institute for Research on Environment and Sustainability, Newcastle University, Newcastle upon Tyne, NE1 7RU, England, UK

Abstract
Organic management is believed to 'enhance' nutrient cycling and microbial diversity in soil, yet the extent of this enhancement remains largely unknown. Actinobacteria play a major role in organic matter turnover, carbon cycling and suppression of some fungal pathogens. Thus, we investigated the long-term impact of organic and mineral fertilisers on the actinobacterial community structure in grassland soils using terminal restriction length polymorphism (T-RFLP). Multivariate statistics was then used to investigate the relationship between community structure, seasonality and fertiliser management. Fertilisers have had a profound impact on soil pH with the organically fertilised plots showing a significantly higher pH than those receiving mineral fertilisers. Consequently, soil pH was the most significant edaphic factor influencing actinobacterial communities with *Arthrobacter* and *Micrococcus* more abundant in soils receiving organic inputs whilst *Streptomyces, Acidimicrobium* and *Actinospica* more prevalent in acid soils. Soil water content was also influential, with major changes evident over the summer months between May and September. Quantitative PCR of the actinobacterial and fungal 16S and 18S rRNA genes, respectively suggested that fungal gene abundance was negatively correlated ($P = 0.0131$) with increasing actinobacterial gene abundance. The importance of these findings in terms of fungal abundance and potential disease suppression are discussed.

Key Words
Actinobacteria, fertiliser management, 16S rRNA genes, quantitative PCR, disease suppression, C cycling.

Introduction

Actinomycetes are an important and functionally diverse group of organisms that are known to be involved in carbon cycling and nutrient transformation in soils (Goodfellow and Williams 1983). This role in sustaining soil processes reflects their metabolic diversity, their ability to produce secondary metabolites and their mycelial growth habit which, like soil fungi, makes it possible to explore the bulk soil in search of water and nutrients (McCarthy and Williams 1990). There are increasing concerns over the impacts of climate and land use change on the maintenance of soil functions (O'Donnell *et al.* 2001) and the need to increase crop production to meet the diverse demands of food, fibre and biofuel production. To meet these demands requires a better understanding of how soils function and ultimately, the ability to engineer diversity:function relationships so as to maintain and even enhance key system processes (O'Donnell *et al.* 2001). The use of organic inputs in the form of farm yard manure (FYM) and composts are considered to be important in increasing microbial diversity and enhancing nutrient cycles in soils (Mader *et al.* 2002). However, the real extent to which organic and inorganic fertilisers 'enhance' the diversity of different functional and taxonomic groups in soils remains largely unexplored.

Long-term field sites such as the Palace Leas Hay Meadow Experiment, Northumberland, UK, are ideal sites on which to study the impacts of extended fertiliser use on the structure and activity of soil microbial communities since they offer the opportunity of separating the long-term effects of management from shorter-term, temporal variability. The site was established in winter 1896-97 since then the 13 plots have received regular and virtually unchanged mineral fertiliser and FYM inputs. In this study, we have used a combination of molecular fingerprinting using terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene cloning to investigate the structure and seasonal dynamics of actinobacterial communities. Our aim was to investigate whether the different fertiliser regimes had impacted on the actinobacterial community structure and to identify using multivariate statistical techniques the relative importance of environmental parameters such as pH, time of sampling, and the form and rate of fertiliser addition in shaping these communities.

1 – 6 August 2010, Brisbane, Australia. Published on DVD.

Methods

Sampling regime and environmental variables

To differentiate fertiliser effects from seasonal variation, soils were sampled in triplicate from each of the 13 plots three times over one growing season between May and September 2004 and January 2005. Soil pH and water content were also measured which together with data on the fertiliser inputs and sampling time (January, May or September) provided the environmental variables used in the multivariate statistical analyses

Actinobacterial community profiling and cloning

Total nucleic acids were extracted from 0.5 g soil using previously described methods (Griffiths *et al.* 2000). Actinobacterial community composition was profiled using T-RFLP, where individual peaks represent taxon (OTU) richness and the height of the peaks gives an indication of their relative abundance. Community DNA was amplified using actinobacterial specific primers targeting the 16S rRNA gene where the 5'end was labelled with 6-carboxy-fluorescein (FAM). Following amplification PCR product were digested with restriction enzyme HhaI, desalinated and denatured prior to injecting the samples into the MegaBace 1000 DNA Sequencing System. To assess changes in actinobacterial community composition, reference clone libraries were generated for each plot at Palace Leas in order to identify TRF peaks found in the community profiles of all samples. The TRF of each clone was determined experimentally as described for the soil DNA and also checked *in silico* using published procedures (Egert and Friedrich 2003). Cloned 16S rRNA genes were then amplified, labelled, restricted and run on the MegaBACE as described for the soil DNA. The resulting T-RFLP profiles were analyzed using MegaBACE Genetic Profiler Version 1.5 and used to generate a data matrix of relative taxon abundances for each of the actinobacterial communities. A corresponding matrix of the environmental variables (time of sampling, soil pH, soil water content and the rates and forms of fertiliser inputs) for each of the plots was also prepared. To explore which of these parameters best explained the differences in actinobacterial communities between plots, canonical correspondence analysis (CCA) was used to model the changes in the community profile of the different plots relative to the measured environmental variables.

Fungal and actinobacterial abundance using quantitative PCR

Total gene abundances for actinobacterial and fungal communities in each plot were quantified by real-time PCR (qPCR) using actinobacterial specific and fungal specific primer sets. qPCR was performed using a Roche LightCycler instrument and a SYBR Green I fluorophore protocol. The relationship between actinobacterial and fungal population sizes were investigated using actinobacterial abundance as a predictor of fungal abundance by fitting generalised linear models (GLM). Residuals were assessed for normality using quantile plots and all regression analyses were performed using the R statistical package version 2.0.

Results and discussion

In Figure 1a, the highest correlation between environmental variables and the differences in actinobacterial community structure between plots was obtained with soil pH on axis 1. This means that of the measured variables, soil pH was the most important driver of changes in actinobacterial community composition in the plots at Palace Leas and this has been shown in previous studies (O'Donnell *et al.* 2001; Fierer and Jackson 2006). Figure 1b showed that the increase in pH due to the addition of FYM (contains liming properties, such as Ca^{2+} or Mg^{2+}) to plots 1-5 is accompanied by a change in actinobacterial community structure and an increase in the relative abundance of *Micrococcus, Norcardia, Mycobacterium* and *Arthrobacter* and these taxa are particularly associated with manures and organic compost (Atagana 2004). Figure 1 also showed that the extended use of NH_2SO_4 as a source of inorganic N has had a major impact on the actinobacterial community structure in plots 7 and 11, with an increase in the relative abundance of acidophilic taxa such as *Acidimicrobium, Streptomyces* and *Actinospica* (Figure 1b). These results provide good support for the hypothesis that long-term fertilizer inputs and pH in particular have not only changed the community structure but also the taxonomic diversity of actinobacteria in these soils.

As seen in Figure 1, time of sampling was also identified as an important covariate and therefore actinobacterial community structure changes during the year. Since, time of sampling was correlated to soil water content these changes are most likely a consequence of seasonal differences in rainfall that result in changes in soil water content. This suggests that soil water content acts selectively on actinobacterial communities, possibly favouring desiccation tolerant taxa during the drier months, such as, *Arthrobacter* that can exist for long periods as resting cocci to avoid desiccation (Goodfellow and Williams 1983). When the

soil water content was at its highest in January there was a marked increase in the relative abundance of *Mycobacterium, Streptomyces* and *Micrococcus* in all plots. Perhaps these taxa are drought intolerant and periods of soil moisture limitation may affect the physiological status of these bacteria through starvation, induced osmotic stress, and resource competition.

Figure 2 shows the variation in fungal and actinobacterial biomass between plots as estimated using qPCR amplification of the 16S rRNA and the 18S rRNA genes respectively. One of the benefits claimed for organic agriculture is that organic inputs help limit the growth of soil fungal pathogens (Bailey and Lazarovits 2003). The data presented here supports this view since fungal abundance was lowest in those plots receiving annual inputs of FYM (plots 1 and 2) but that the effect was lost when FYM was applied in alternate years with NPK fertilizer (plot 3). If one considers what was happening in plots 4 and 5, where FYM was added in alternate years but without NPK (plot 4) and at double the rate followed by 4 years of NPK (plot 5) it appears that these management induced changes in actinobacterial and fungal dynamics are related to both the amounts and timing of FYM inputs. Although the high correlation between the abundance of actinobacteria and fungi was interesting in terms of the potential control of soil-borne fungal diseases, there was yet no direct evidence that there are any differences in disease suppression between treatments or that there was any direct interaction or antagonism between actinomycetes and fungi.

Conclusion

The data presented in this study show clearly that soil pH is a major driver of change in actinobacterial communities. The data also suggests that at the level of individual taxa these responses are consistent with previously published results of isolation studies with genera such as *Arthrobacter* and *Micrococcus* more abundant in soils receiving organic inputs and others such as *Streptomyces, Acidimicrobium* and *Actinospica* more prevalent in acid soils. The actinobacteria have also been shown to respond to other environmental drivers such as time (seasonal variation) in a manner consistent with changes in soil water content throughout the year. Again these findings are consistent with published reports of isolation studies and could have an important bearing on the design and optimisation of selective isolation and screening programmes. In addition to these abiotic interactions the T-RFLP and qPCR data have shown that management and pH in particular have impacted on the biological interactions in these soils with elevated actinomycete numbers consistent with a decrease in soil fungal propagules. However, the data also show that this effect can be lost where organic fertiliser inputs are not applied annually as in plots 4 and 5. The reasons for this are unknown and need to be further investigated.

References

Atagana HI (2004) Co-composting of PAH-contaminated soil with poultry manure. *Letter of Applied Microbiology* **39,** 163-168.

Bailey KL, Lazarovits G (2003) Suppressing soil-borne diseases with residue management and organic amendments. *Soil Tillage Research* **72,** 169-180.

Egert M, Friedrich MW (2003) Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. *Applied and Environmental Microbiology* **69,** 2555–2562.

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of National Academy of Science USA* **103,** 626-631

Goodfellow M, Williams ST (1983) Ecology of actinomycetes. *Annual Reviews in Microbiology* **37,** 189-216.

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and RNA-based microbial community composition. *Applied and Environmental Microbiology* **66,** 5466-5491.

Mader P, Fliebbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. *Science* **296,** 1694-1697.

McCarthy AJ, Williams ST (1990) Methods for studying the ecology of actinomycetes. *Methods in Microbiology* **22,** 533-563

O'Donnell AG, Seasman M, Macrae A, Waite I, Davies JT (2001) Plants and fertilisers as drivers of change in microbial community structure and function in soils. *Plant and Soil* **232,** 135-145.

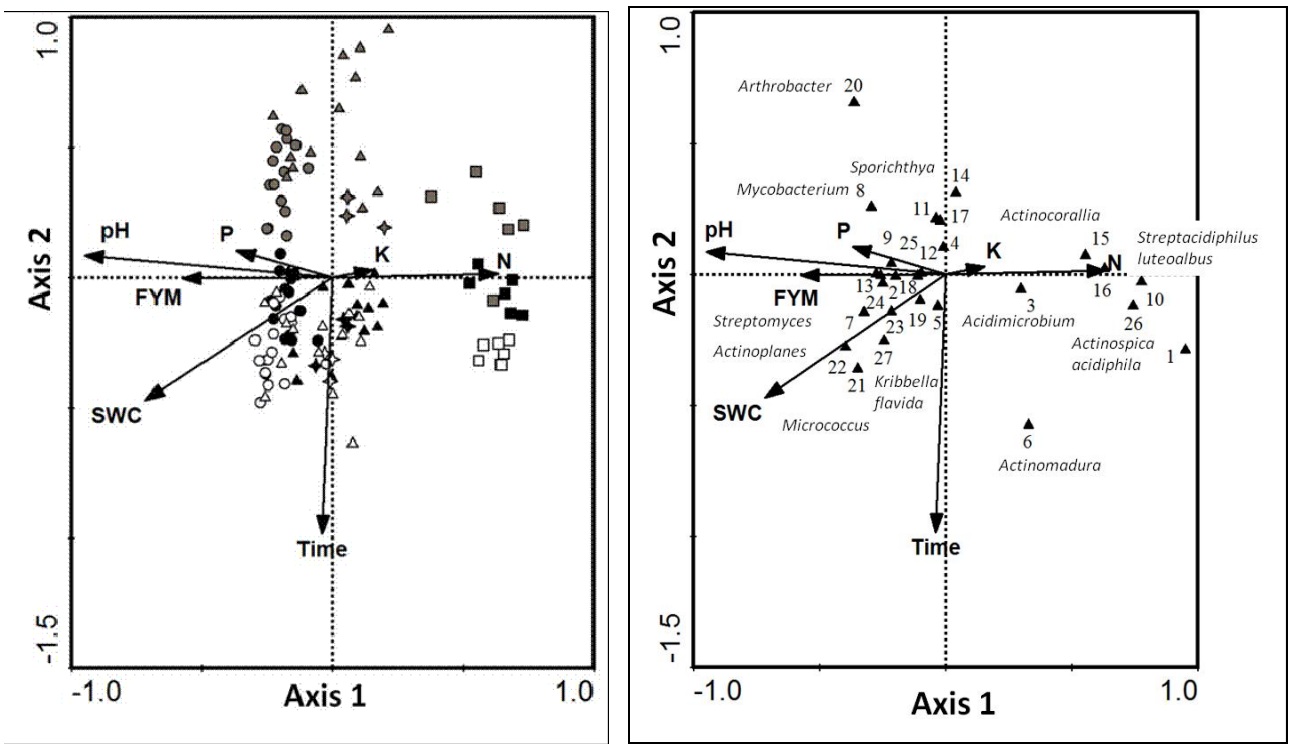

Figure 1. Bi-plots from canonical correspondence analysis (CCA) showing the relationship between a) actinobacterial community composition (as judged by T-RFLP profiles), fertiliser management and time of sampling at Palace Leas [first two axes account for 44% of total variance, axis 1 (32%) and axis 1 (12%)]. Points on the graph represent actinobacterial communities sampled from the soil plots under different management regimes [plots 1-5 receive organic inputs (●) plots 8, 9, 10, 12 & 13 receive mineral fertilisers inputs including P (▲), plots 7 & 11 receive mineral fertiliser inputs without P (■) and plot 6 the control receives no fertiliser inputs (✦). Arrows represent the environmental variable [inorganic inputs (FYM), inorganic inputs (N, P, K), pH, soil water content (SWC) and time of sampling (Time)]. In order to assess temporal changes in community structure throughout the year the May (grey ■), September (black ■) and January (white □) samples were assigned as 0, 4 and 8 months, respectively, which represents the number of months after initial sampling in May.
b) the distribution of different taxa (as judged by terminal restriction fragments, TRFs) with management and time of sampling. Triangles on the graph represent TRFs derived from T-RFLP analysis on the soil plots under different management regimes at Palace Leas.

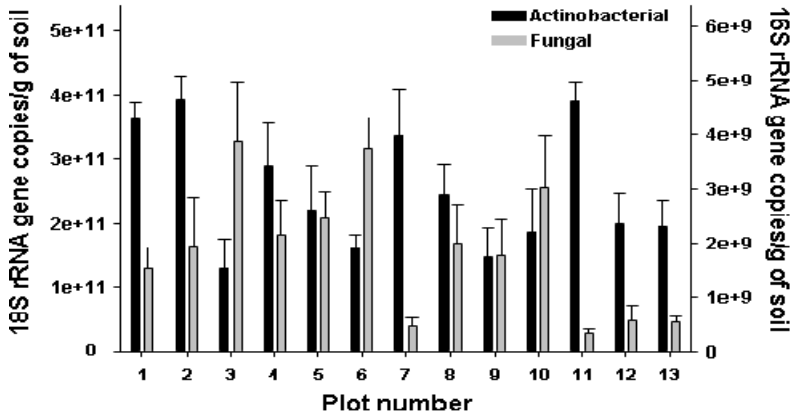

Figure 2. Actinobacterial and fungal abundances in the 13 plots at the Palace Leas Hay Meadow Experiment estimated using real time qPCR of 16S rRNA and 18S rRNA genes respectively. Plots 1-5 have received organic inputs (FYM) either singly or in combination with inorganic fertilisers since 1896. Plots 7 to 13 have received only inorganic fertilisers over the same period. The control (plot 6) has received no fertiliser input since 1896. All qPCR reactions were done in triplicate on separate soil samples. Error bars indicate the standard error where n=3