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The impact of grassland management regime on the community structure of selected bacterial groups in soils

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Abstract

The impact of long-term grassland management regimes on microbial community structure in soils was assessed using multivariate analysis of polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) banding patterns of selected bacterial groups and PLFA (phospholipid fatty acid) profiling. The management regimes assessed were inorganic nitrogen (N) fertiliser application and soil drainage. PCR–DGGE profiles of the eubacteria, actinomycetes, ammonia oxidisers and pseudomonads were assessed by principal co-ordinate analysis of similarity indices which were generated from binary data using both Dice and Jaccard coefficients. The analysis of binary DGGE data revealed significant impacts of N fertiliser on the eubacterial and actinomycete community structure using the Jaccard coefficient, whilst N fertiliser had a significant impact on the actinomycete community structure only when using similarity indices generated from the Dice coefficient. Soil drainage had a significant impact on the community structures of the actinomycetes and the pseudomonads using both Dice and Jaccard derived similarity indices. Multivariate analysis of principal components derived from PLFA profiling revealed that N fertiliser had a significant impact on the microbial community structure. Although drainage alone was not a significant factor in discriminating between PLFA community profiles of the different treatments, there was a significant interaction with N fertiliser. Analysis of principal component analysis (PCA) loadings revealed that PLFAs i15:0 and i17:0 were partly responsible for the clustering away of the undrained–N fertilised treatment. Although soil management regime influenced some background soil data, correlation analysis using PC1 from PLFA data revealed no significant relationship with soil organic matter, pH, total C and total N. These results provide evidence that grassland management practices impact on the community composition of specific microbial groups in soils.

Keywords: DGGE profiling; 16S rDNA; Grassland; Multivariate analysis; Phospholipid fatty acid

1. Introduction

In recent times, land use in the United Kingdom has undergone considerable changes due to social and economic pressures, leading to a fine balance between the demands of highly productive intensive systems and practices which are perceived to be more environmentally acceptable. Plant productivity is governed by the supply of nutrients from the soil, which in turn is dependent on the activity of soil micro-organisms. Microbial communities in soils play a key role in grassland ecosystems through regulating the dynamics of organic matter decomposition and plant nutrient availability.

Considerable information is available concerning the impact of management practice on communities of macro-fauna and flora, but the effects on specific microbial communities in soils are less clear. Traditional approaches to the study of microbial communities in soils have entailed the use of culture based protocols. These approaches are useful for isolation purposes, but are limited in their scope and applicability to forwarding our understanding of microbial ecology as it was suggested in a recent review that less than 1% of the bacteria present in soils may be readily isolated [1]. Community based methodologies such as phospholipid fatty acid (PLFA) profiling and DNA approaches circumvent the problems associated with culturing. PLFA profiling [2,3] and broad-scale DNA ap-

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proaches, such as reassociation kinetics and % G+C profiling of community DNA [4–6] have previously revealed shifts in the microbial community structure of soils. These approaches inform us about general community changes that are occurring through management practice, whilst more detailed analyses can be performed using 16S ribosomal DNA (rDNA) techniques. The utilisation of 16S rDNA approaches in studying microbial ecology has revealed that a vast diversity of unknown and uncultured bacterial species exists in soils (e.g. [7–10]). Advances in sequence analysis and also the application of polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) profiling to microbial communities in soils now allows an increased resolution in our studies from a broad-scale through to profiling specific groupings of bacteria. PCR–DGGE profiling is a powerful tool to apply to microbial ecology, and whilst there is some concern about the use and interpretation of banding intensity data, the analysis of binary data from the banding patterns offers much appeal in microbial ecology. Binary data informs us of the presence–absence status of specific members of a community profile and provides a more objective view of the population structure.

The objective of this study was to determine the impact of long-term management status on the community structure of selected bacterial groups in grassland soils. Specifically, the impacts of inorganic N fertiliser and drainage on the PCR–DGGE 16S rDNA banding patterns of eubacteria, actinomycetes, ammonia oxidisers and the pseudomonads, and microbial community PLFA profiles were determined.

2. Materials and methods

2.1. Soils

Soils were sampled from the Rowden long-term experimental site at the Institute of Grassland and Environmental Research, North Wyke Research Station, Devon, in S.W. England. These were grazed swards that had been under long-term pasture for the previous 50 years and under the current management regime for the past 15 years. The soil is a poorly drained silty clay loam of the Hallsworth series. Four treatments were analysed: undrained and unfertilised (UO), drained and unfertilised (DO), drained and fertilised at 200 kg inorganic N ha^{-1} yr^{-1} (DN), and undrained and fertilised at 280 kg inorganic N ha^{-1} yr^{-1} (UN). Drained plots consisted of 55 cm deep mole drains crossing 85 cm deep permanent pipe drains. Soil samples were taken as follows; about 10–12 25 mm × 50 mm deep cores were taken from each of 40 cm × 40 cm random triplicate quadrats from each of the four different management treatments. Soils were sieved using a 4 mm mesh. The unfertilised plots were typically dominated by *Agrostis* spp., *Anthoxanthum odoratum* L. and *Holcus lanatus* L.) whilst the fertilised plots were dominated by *Lolium perenne* L. Total C and total N were obtained using an automated Dumas procedure on a Carlo Erba NA2000 elemental analyser (CE Instruments) and soil pH (water), organic matter content (loss of weight on ignition) and bulk density were also determined [11].

2.2. Isolation of total DNA from soils

To 1.0 g (fresh weight) soil were added 1.0 g 0.1 mm diameter zirconia/silica beads (BioSpec. Products, Inc.) and 400 μl guanidinium thiocyanate solution (4M guanidinium thiocyanate, 0.5% sarkosyl, 0.25 M sodium citrate, pH 7.0). Samples were then processed in a Mini-beadbeater (Biospec Products, Inc.) at 5000 rpm for 60 s and the soil suspension centrifuged at 13 000 rpm (MSE Micro Centaur, UK) for 1 min. The supernatant was removed and mixed by vortexing with an equal volume of phenol (pH 8.0), centrifuged at 13 000 rpm for 5 min. The upper aqueous phase was removed and mixed by vortexing with an equal volume of chloroform and centrifuged at 13 000 rpm for 5 min. To precipitate out nucleic acids, 0.1 volumes of 5 M NaCl and 2.5 vol of 100% ethanol were added to the aqueous phase and centrifuged at 13 000 rpm for 30 min. The resultant pellet was then washed twice in 70% ethanol and dried. The dried pellet was resuspended in sterile distilled water. The yields of DNA extracted from the different soils were determined colorimetrically by the diphenylamine reaction [12].

2.3. PCR amplification of 16S rDNA

PCR amplification was carried out to generate products for molecular population profiling of the eubacteria, actinomycetes, ammonia oxidisers and pseudomonads in each of the soils. All PCR amplifications were carried out in a 40 μl reaction volume containing 20 pmol of each primer, 250 μM dNTPs, 3 U Expand High Fidelity polymerase (Roche Diagnostics, UK), 4 μl 10× reaction buffer. Where applicable, PCR mixes used about 50 ng genomic DNA isolated from soil as the template for amplification and each reaction also contained 16 μg bovine serum albumin (Roche Diagnostics, UK). The following primers and cycles were used in the amplification of eubacterial, actinomycete, ammonia oxidiser and pseudomonad 16S rDNA; PCR amplification of the 16S rRNA eubacterial genes was carried out using the primers 341f-GC and 534r [13]. The GC clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) was attached to the 5′ end of the forward primer. PCR was performed using the following cycle: 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min. A final extension time of 10 min at 72°C was included. The actinomycete, ammonia oxidiser and pseudomonad populations in the soils were PCR amplified using a nested approach. The first round of PCR was carried out using

primers specific to the groupings and the second round of PCR used eubacterial primers [13] to provide a product of a fragment size suitable for DGGE analysis. This was because the products from the first round group specific PCR reactions were too long for complete resolution through DGGE. The first round PCR products for the actinomycetes, ammonia oxidisers and pseudomonads were generated using the following primer sets and thermal cycles. For the actinomycetes, the primers F243 and R1378 [14] were used in conjunction with the following cycle of 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 63°C for 1 min, 72°C for 2 min. This was followed by a final extension time of 10 min at 72°C. For the ammonia oxidisers, the first round of PCR was carried out using the CTO primers [15] with a cycle of 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 10 min. Pseudomonad (sensu stricto) 16S rDNA was amplified using the primers Ps-for and Ps-rev [16], and the cycle, 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 62°C for 1 min, 72°C for 2 min., and a final extension time of 10 min at 72°C was employed. The products from the first round of PCR were then run on a 1% low melting point agarose gel (Roche Diagnostics), stained in ethidium bromide and visualised under ultraviolet light. Bands of the estimated product sizes were excised from the gel, melted at 70°C and 1 μl was added to the reaction mix for the second round of PCR using the primers 341f-GC and 534r [13]. The cycle used for the second round of PCR was the same as that described earlier for the eubacterial primers. All PCR samples were amplified using a Primus Thermocycler (MWG-Biotech, UK), and final PCR products were checked on a 1.2% agarose gel stained with ethidium bromide.

2.4. Molecular profiling of soil bacterial population structure

DGGE was employed to resolve the PCR products obtained from each of the different soils. Polyacrylamide gels (8% acrylamide, 0.5×TAE, 37:1 acrylamide:bisacrylamide) were cast using 40–55% denaturant [100% denaturant was defined as 7 M urea with 40% (vol/vol) formamide]. PCR products were loaded into the lanes of the gel in a random order to avoid any potential bias in the later analysis. The gel was run using a DCode system (Bio-Rad Laboratories) at a constant temperature of 60°C and at 60 V for 16 h. After electrophoresis, gels were fixed for at least 2 h in a solution of 10% ethanol and 0.5% acetic acid. The gels were then stained in a solution of 0.1% (wt/vol) silver nitrate for 20 min before briefly rinsing and developing [0.02 g $NaBH_4$, 0.8 ml formaldehyde, 200 ml 1.5% (wt/vol) NaOH] until the bands appeared. Gels were then fixed in 0.75% Na_2CO_3 for 10 min before scanning using a Hewlett Packard Scanjet 5370C. Banding patterns were determined using Phoretix 1D Gel Analysis Software, Version 4.0 (Phoretix International, Newcastle Upon Tyne, UK).

2.5. PLFA analysis

PLFAs were extracted from replicate 2 g soil samples using the method of Bardgett et al. [17], which is based on the method of Bligh and Dyer [18]. Fatty acid nomenclature was as follows: fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, cis18:1ω7 and cy19:0 represented current known bacterial PLFAs [2,19,20], 10Me18:0 was indicative of actinomycetes and 18:2ω6 was used as an indicator of fungal biomass [19]. Other PLFAs determined were i14:0, 14:0, 16:0, 16:1ω7, 17:1ω6, nMe18:0 (C17 chain with methyl group at unknown position), 18:0, trans18:1ω9 and 20:0.

2.6. Banding pattern analysis and statistics

Similarity indices of PCR–DGGE molecular profiles for each of the bacterial groupings were determined using binary data only. Similarity matrices were generated from binary data for each lane using both the Dice and Jaccard coefficients as follows: Dice coefficient $C_D = 2j/(a+b)$, and Jaccard coefficient $C_J = j/(a+b-j)$, where j = number of bands in common between lanes A and B, a = the total number of bands in lane A, and b = the total number of bands in lane B. The similarity indices were used in principal co-ordinate analysis (PCO) and the first six PCOs (accounting for 70–80% of the variability) were then analysed by two-way multivariate analysis of variance (MANOVA, Genstat 5th edition) to determine statistical differences in the microbial community structure profiles. PLFA data were log transformed and analysed initially by principal component analysis (PCA) to reduce the dimensionality. The first three components of the PCA (accounting

Table 1
Means (±standard error) of background soil characteristics ($n = 3$)

Treatment	Soil pH	Bulk density (g cc^{-1})	Organic matter content (%)	Total carbon (%)	Total nitrogen (%)	C:N
(UO)	5.85 (0.01)	0.68 (0.06)	13.9 (1.13)	6.65 (0.41)	0.66 (0.02)	10.1
(DO)	6.33 (0.09)	0.71 (0.04)	14.4 (0.42)	6.57 (0.20)	0.64 (0.01)	10.3
(DN)	6.33 (0.18)	0.83 (0.03)	14.6 (0.58)	7.21 (0.35)	0.73 (0.02)	9.9
(UN)	5.72 (0.12)	0.85 (0.04)	14.2 (0.43)	7.32 (0.35)	0.71 (0.04)	10.3

Percentage of organic matter, carbon and nitrogen expressed on a dry soil basis.

Table 2
P values for different PCR–DGGE banding patterns of bacterial groupings under different grassland management regimes obtained from the MANOVA of PCOs derived from Dice and Jaccard coefficient similarity indices

Bacterial group	Treatment	Dice coefficient	Jaccard coefficient
Eubacteria	N	0.11	0.03
	D	0.24	0.11
	N×D	0.42	0.28
Actinomycetes	N	0.02	0.003
	D	0.02	0.02
	N×D	0.01	0.004
Ammonia oxidisers	N	0.10	0.10
	D	0.12	0.06
	N×D	0.11	0.29
Pseudomonads	N	0.07	0.11
	D	0.001	0.02
	N×D	0.22	0.22

N, nitrogen fertiliser; D, drainage; N×D, nitrogen×drainage interaction.

for 96.6% of the variability) were analysed by two-way MANOVA to determine treatment effects on the PLFA composition of the soils. Correlation analysis was performed on background soil data and PLFA PC1. Results were considered significant at $P < 0.05$.

3. Results

Management practice in the different grassland treatments had a significant impact on some of the soil characteristics determined. Treatment induced effects were seen in both DNA and PLFA data, and to a lesser extent in the background soil characteristics (Table 1). Soil pH was lowest in the undrained plots ($P = 0.003$), bulk density was highest in the N fertilised plots ($P = 0.006$) and there was no significant difference in the organic matter content of the four treatments. Total carbon was not significantly different ($P = 0.087$) for all the treatments; however, total nitrogen was highest in the N fertilised plots ($P = 0.02$). The C:N ratios remained similar in all four treatments.

PCR amplifiable DNA was isolated from all soil samples in this study. Quantities of DNA extracted from the soils ranged from 70 μg^{-1} dry soil to 96 μg^{-1} dry soil, with significantly greater amounts ($P = 0.037$) recovered in soils from the undrained, than the drained treatments.

Community composition determined using PCR–DGGE profiles was conducted using binary data and this revealed a total number of different banding positions of 133 for the eubacteria, 118 for the actinomycetes, 43 for the ammonia oxidisers and 65 for the pseudomonads. MANOVA of PCO data derived from both Dice and Jac-

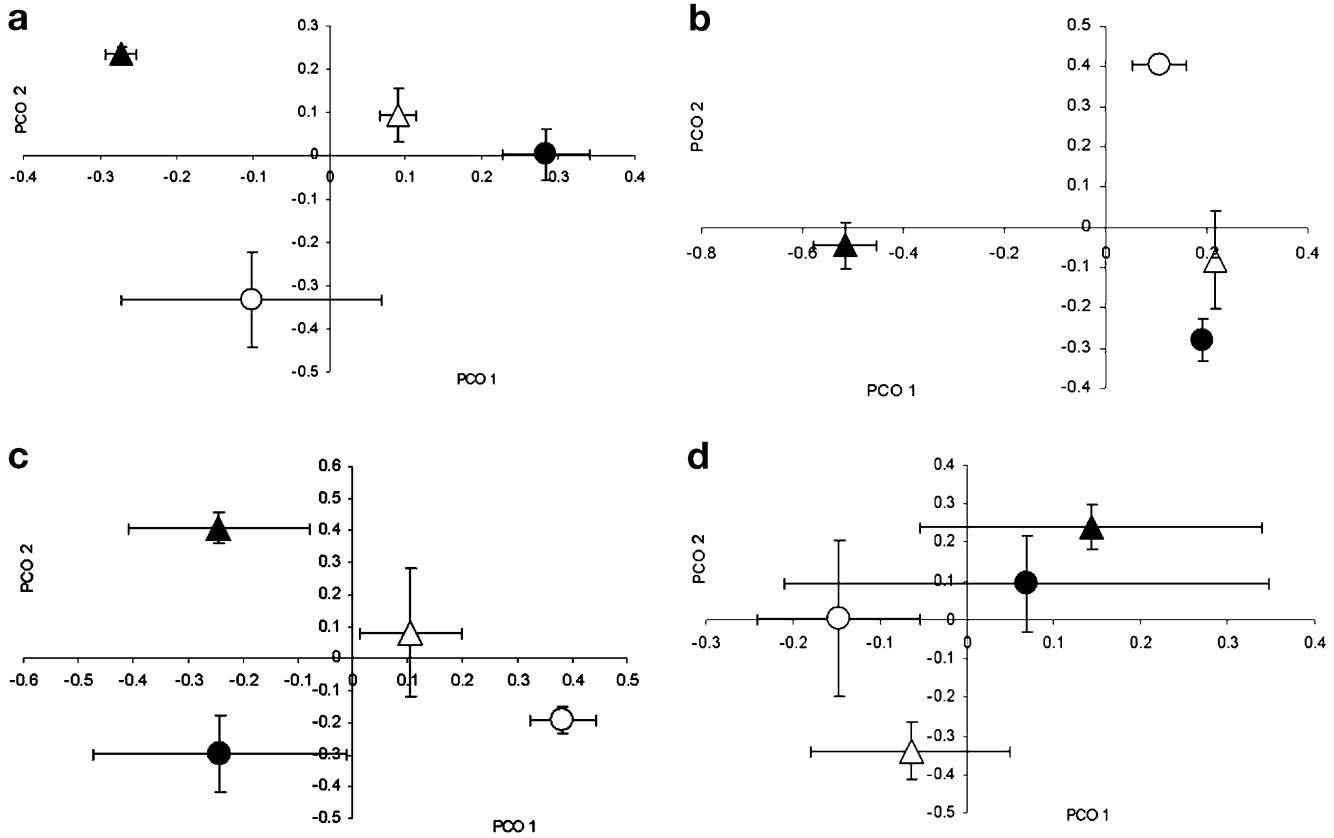

Fig. 1. a–d: Plots of ordination of means (±standard error) of PCO1 and PCO2 produced from the multivariate analysis of (a) eubacterial, (b) actinomycete, (c) ammonia oxidiser and (d) pseudomonad PCR–DGGE banding profiles from undrained–unfertilised (○), drained–unfertilised (●), drained–N fertilised (△) and undrained–N fertilised (▲) grassland soils.

card coefficients for the four different bacterial groupings revealed some significant treatment related effects (Table 2). Ordination plots for each of the bacterial communities are shown in Fig. 1a–d. These plots were generated using PCO1 and PCO2 derived from Jaccard similarity indices, whereby PCO1 and PCO2 generally accounted for 14–17% and 13–15% of the variability respectively. The eubacterial community structure was significantly effected by N fertiliser according to the analysis using Jaccard, but not Dice, derived similarity indices. Analysis of Dice and Jaccard similarity data revealed that N fertiliser, drainage and the fertiliser × drainage interaction impacted significantly on the actinomycete community structure. The ammonia oxidiser banding patterns were not significantly different from each other; however, P values derived from Jaccard similarity data for N and drainage effects were less than 0.1. The community structure of the pseudomonads in soils under different treatments clustered significantly as a result of drainage.

Total PLFAs extracted from soils were highly variable and ranged from 38 nmol g^{-1} dry soil up to 320 nmol g^{-1} dry soil. Quantities of PLFAs extracted from soils were not significantly different between treatments; however, the highest amount of total PLFA was extracted from the undrained–unfertilised treatment. Total mass of PLFAs extracted from the different soils did not correlate with amounts of DNA isolated from soils ($r^2 = 0.02$).

PCA was used to determine treatment related impacts on the community profiles of 21 PLFAs in the different soil treatments. MANOVA of the first three PCs indicated that N fertiliser had a significant impact on the PLFA community profiles of the grassland soils ($P = 0.01$). Whilst drainage alone was not a significant factor impacting on the PLFA community composition ($P = 0.1$), the drainage × nitrogen fertiliser interaction was a significant factor ($P = 0.005$). The ordination plot in Fig. 2 illustrates the difference in the PLFA composition of the four treatments, where PC1 and PC2 account for 84.1% and 9.2% of the variation respectively. The undrained and N fertilised treatment clusters away from the other three treatments.

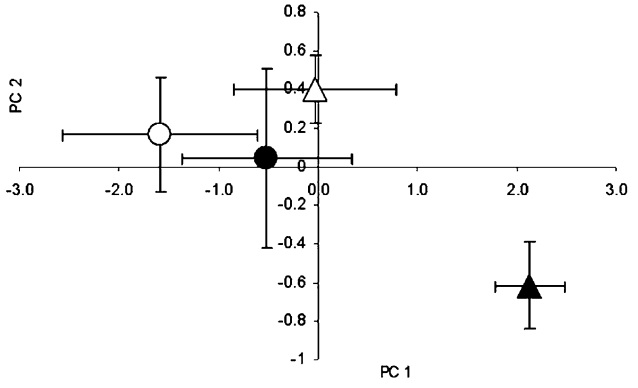

Fig. 2. Plot of ordination of means (± standard error) of PC1 and PC2 produced from the multivariate analysis of extracted microbial community PLFAs from undrained–unfertilised (○), drained–unfertilised (●), drained–N fertilised (△) and undrained–N fertilised (▲) grassland soils.

The loadings for PC1 and PC2 revealed those outlier PLFAs responsible for the treatment separation; these were PLFAs i15:0 and i17:0 which are both indicative of Gram positive bacteria and these were lowest and highest respectively in the undrained–N fertilised treatment. PLFA trans 18:1, indicative of Gram negative bacteria, was variable, but highest in the undrained–unfertilised treatment. The amount of the actinomycete PLFA 10Me18:0 was highest in the unfertilised plots ($P = 0.04$), and the recovered amount of fungal PLFA 18:2ω6 was not effected by treatment. Correlation analysis using PC1 revealed no significant relationship between PLFA community structure and organic matter ($P = 0.92$), pH ($P = 0.1$), total C ($P = 0.69$) and total N ($P = 0.95$).

4. Discussion

Through the use of MANOVA of binary PCR–DGGE banding patterns and PLFA profiles, the main finding of this study was that grassland management practice has an impact on the community structure of specific bacterial groups. The ability to analyse binary data and generate similarity indices for the multivariate analysis of communities is ideal for the study of microbial ecology. Several different coefficients have often been used in determining the levels of association between different microbial strains or communities. In this study we used both the Dice and Jaccard coefficients to generate similarity indices for banding patterns between different treatments. The simple matching coefficient is also often used to generate similarity indices; however, we considered that it was inappropriate to use in the analysis of PCR–DGGE banding data. This was because this coefficient takes into account negative matches, and whilst these are crucial when, for example, comparing the utilisation of a range of substrates between a number of different bacterial strains, we considered it inappropriate for PCR–DGGE banding analysis. P values obtained from the MANOVA of Dice and Jaccard derived data differed only slightly in their outcome of significance; however, only the Jaccard coefficient derived data was able to discriminate the effects of N on the eubacterial community structure.

The quantification of DGGE data is possible and has been applied to both simple communities such as the ammonia oxidisers [21] and more complex bacterial populations in upland grassland soils [22]. PCR–DGGE profiling generally reveals the most dominant members of mixed microbial populations [13,23], and whilst biases can occur during the PCR amplification of 16S rDNA [24–27] it is unclear to what extent any preferential bias does occur. In addition to PCR generated bias, there are other factors to be taken into consideration when analysing PCR–DGGE banding patterns. For example, using the approach

adopted here, reliance is placed on the specificity of the primer sets. The primer sets used in this study for the generation of eubacterial, actinomycete, ammonia oxidiser and pseudomonad PCR products were considered specific enough for the purposes of this approach and were chosen because of their availability in the literature and applicability to soil communities. Furthermore, the identification of the bands through DNA sequencing was beyond the scope of this study, which focused on the impact of management on bacterial community structure. The analysis of binary data from DGGE banding patterns is a powerful and relatively easy way of comparing microbial communities from different samples. However, it is possible that this approach may underestimate the number of species, assuming that each band represents a different species, through the co-migration of bands through the gel. Whilst it is unrealistic to assume that absolute values for the number of species within a community can be obtained through PCR–DGGE profiling, it is a suitable approach in comparative analyses of the most abundant members of communities. Although we consider that there is probably a limit in the resolution for the determination of richness through DGGE profiling, banding patterns in this study for the actinomycetes, ammonia oxidisers and pseudomonads were generally clear and distinct. However, those of the eubacterial profiles were highly complex with many faint bands remaining undetected due to the limits of resolution of the analysis software and staining procedure. Eubacterial populations in soils are clearly more abundant and diverse than the resolution of the eubacterial profiling approach adopted here; however, on the basis that statistical differences were obtained using this approach we can assume that real differences do exist.

Diversity indices for complex microbial populations in soils, where most of the members remain uncultured, are currently practically impossible to determine. For example, previous cloning and sequencing approaches have provided evidence on the diversity of bacteria in soils where little or no sequence repetition was found after the analysis of hundreds of clones of 16S rDNA PCR products [8,10]. Indeed, cloning and sequence analysis was unable to discriminate bacterial communities from different grasslands whereas the use of multivariate analysis of PCR–DGGE banding patterns was able to do so [22]. Using a more broad-scale approach of DNA reassociation kinetics, Torsvik et al. [1] estimated that there were approximately 10 000 bacterial genome types in 100 g of soil. In this study a statistical interpretation of the apparent differences from the PCO analysis was undertaken through MANOVA of the first six PCs which accounted for about 70% of the variation for banding patterns for each of the bacterial groupings. It is unclear to what extent the PCR–DGGE banding patterns reflect the true community structure in the different grasslands, but it is likely that the differences between treatments are real because the potential for PCR bias is common to all samples. In this study similarity indices and PCO analysis were determined using unweighted binary data; however, a previous study reported that treatment related discrimination of PCR–DGGE profiling was possible with both weighted (band intensity) and unweighted (binary) data using the more subjective canonical variate analysis [22].

Changes in the community structure of the actinomycetes and pseudomonads were brought about by soil drainage, and significant changes in the PLFA profiles were driven by the interaction of drainage with N application. Drainage increases the aerobicity of soils with resulting increases in the rates of N mineralisation [28], nitrate leaching [29] and a decrease in the amount of dissolved organic carbon in runoff and drainage water in studies on the same site [30]. Soil drainage results in a shift in the N balance, whereby nitrate can account for about 46% and less than 26% of mineral N in drained and undrained soils respectively [28]. Changes in the chemical composition of soils are both facilitated by, and impact on, indigenous soil micro-organisms.

The application of N to grassland soils resulted in significant changes to the PLFA community profiles and differences in the PCR–DGGE population structures of the eubacteria and actinomycetes, and to a lesser extent the ammonia oxidisers. There was wide variation in the community structure of the pseudomonads and this may have masked any treatment related effects. Grayston et al. [3] suggested that differences, based on plate counts, in the pseudomonad populations of upland grasslands may be partly attributed to the botanical composition, through an indirect, rather than the direct impact of management. The experimental plots used in this study were dominated by different grass species, with *Agrostis* spp. and *H. lanatus* L. typically dominating unfertilised plots and *L. perenne* L. dominating the fertilised plots. It is possible that differences in the root exudate profiles and quality of root/shoot organic matter entering the soil may have contributed to the development of different microbial community structures in these soils. The precise mechanisms driving N fertiliser mediated microbial community changes still remain unclear, and may be a result of direct effects, or more subtle indirect effects such as changes in the botanical composition, or a combination of both. Previous studies have reported that low input systems have a greater botanical diversity [31], microbial diversity [4,5,32], and microbial biomass [11] than those systems under more intensive practices. Our results now suggest that the application of N is also changing the structure of microbial communities in soils. Previous phylogenetic studies on the ecology of ammonia oxidising bacteria have reported that clusters appear to be associated with particular environmental factors [21] and that the community structure appears to be associated with different soil management practices [33,34]. The results obtained in this study now add to the growing bank of information regarding agricultural inputs and ecological impacts in soils.

The results for the actinomycetes obtained from both PLFA and PCR–DGGE profiles provide us with some interesting information regarding the ecology of this group of organisms. The abundance of the actinomycete PLFA 10Me18:0 was found to be greatest in the unfertilised soils in this study, and the PCR–DGGE banding profiles indicated both N and drainage related impacts on the community composition. This suggests that grassland management regime is impacting on the diversity of actinomycetes in soils. Previous studies have, however, suggested that the abundance of 10Me18:0 changes under liming [2] and soil depth [35].

A feature of this study was the amount of variation between replicates in PLFA and PCR–DGGE analyses. Spatial variation is synonymous with field based studies and has previously been reported in other phenotypic determinations on microbial communities such as Biolog metabolic profiling [36], fatty acid methyl ester profiling [37] and process based determinations [38]. Evidence for variation in the genetic make-up of microbial populations between replicate field studies is not so widely reported. Using a broad-scale molecular approach, Clegg et al. [5] reported that the variation in the microbial community DNA composition between replicate soil samples could be as great as the variation between treatment effects in upland grasslands, possibly masking treatment related effects. Indeed, a recent study using PCR–DGGE profiling on upland grassland soils suggested that treatment effects may be obscured by the high degree of spatial variation [22]. Again, using a PCR–DGGE profiling approach, other studies have reported little spatial variation along the roots of plants [39] and within grasslands [40]. There is often a great emphasis to adopt field based experiments; however, a balance between controlled laboratory experiments and field data needs to be found. The use of microcosms in controlled environments should not be overlooked as they allow us to resolve which factors are impacting on community structure when spatial variation of field based measurements may mask the effects of such factors.

The results of this study clearly demonstrate the impacts of management practice on the bacterial community composition of grassland soils. Whilst full and real comparisons between soil populations may probably only be achieved through very extensive cloning and sequencing of all components, the utilisation of PCR–DGGE profiling proved to be a powerful tool in assessing community structure differences in soils. Whilst some care should be taken with interpretation of PCR–DGGE results, the discriminatory value of the numerical analysis of the banding patterns can be considered to be real. Future objectives in studying the impacts of management on grassland microbial populations are aimed at attempting to understand the relationships which may exist between microbial community structure and function.

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