

Soil Bacterial and Fungal Community Structure Across a Range of Unimproved and Semi-Improved Upland Grasslands

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Abstract

Changes in soil microbial community structure due to improvement are often attributed to concurrent shifts in floristic community composition. The bacterial and fungal communities of unimproved and semi-improved (as determined by floristic classification) grassland soils were studied at five upland sites on similar geological substrata using both broad-scale (microbial activity and fungal biomass) and molecular [terminal restriction fragment length polymorphism (TRFLP), automated ribosomal intergenic spacer analysis (ARISA)] approaches. It was hypothesized that microbial community structure would be similar in soils from the same grassland type, and that grassland vegetation classifications could thus be used as predictors of microbial community structure. Microbial community measurements varied widely according to both site and grassland type, and trends in the effect of grassland improvement differed between sites. These results were consistent with those from similar studies, and indicated that floristic community composition was not a stable predictor of microbial community structure across sites. This may indicate a lack of correlation between grassland plant composition and soil microbial community structure, or that differences in soil chemistry between sites had larger impacts on soil microbial populations than plant-related effects.

Introduction

Grassland plant communities can often be described in terms of the characteristic species that make up their composition. However, the extent of coupling between

grassland plant composition and soil microbial structure is poorly understood. It is thought that plants can influence microbial populations in soil through rhizodeposition, with changes in the rate and quality of substrate input occurring due to alterations in plant species composition and diversity [40, 50]. Plant species typical of upland grasslands have been shown to exhibit a selective effect on the microbial populations of their rhizospheres, with *Agrostis capillaris*, *Lolium perenne*, and *Trifolium repens* stimulating the growth of *Proteobacteria* [22, 41], whereas specific associations between dominant species of unimproved and improved upland grasslands and mycorrhizal fungi have been discovered [21, 44, 52, 51, 57, 64]. Shifts in overall microbial community structure have also been linked to plant species traits, with these changes usually attributed to differences in substrate deposition of different plants [3, 53, 61].

Exploration of links between plant and microbial community structure in grasslands is desirable because, although vegetation composition is relatively easy to qualify and quantify, soil microbial communities are much more difficult to characterize. Full characterization of soil fungal and bacterial populations requires multiple samplings and the use of expensive and time-consuming analyses, such as DNA-based community fingerprinting [28]. These approaches provide a detailed view of microbial community structure, but require much more time and effort than the vegetational surveys required to classify grasslands. If consistent links could be found between plant composition and soil microbial community structure, grassland vegetational classifications could be used as predictors of associated soil microbial structure.

In recent years, the National Vegetation Classification (NVC) system [49] has been successfully used to characterize plant communities in the UK and Ireland. Although broad classification systems have previously been used for grasslands [46], the NVC allows classifications into much more precise categories. Its applica-

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78 tion to upland grasslands allows differentiation between
79 unimproved grasslands (classified as U4a), and semi-
80 improved grasslands (classified as U4b) [49]. Unim-
81 proved U4a grasslands are dominated by the plant
82 species *Festuca ovina*, *A. capillaris*, and *Galium saxatile*.
83 Semi-improved grasslands, although dominated by the
84 same species as unimproved grasslands, also contain
85 subcommunities of *L. perenne* and *T. repens*, indicating
86 a limited degree of improvement [49].

87 This study attempts to determine if links between
88 plant community composition and microbial commu-
89 nity structure are consistent across a range of geograph-
90 ically separated sites with similar physical and chemical
91 characteristics. The microbial community structure of
92 unimproved (U4a) and semi-improved (U4b) grassland
93 soils was studied at five upland sites on similar bedrock
94 substrata in the Wicklow Mountains, Ireland, using
95 both broad-scale (microbial activity and fungal bio-
96 mass) and molecular [terminal restriction fragment
97 length polymorphism (TRFLP), automated ribosomal
98 intergenic spacer analysis (ARISA)] approaches. The
99 central hypothesis was that microbial community struc-
100 ture would be similar in soils from the same grassland
101 type, and that NVC grassland vegetation classifications
102 could thus be used as predictors of microbial community
103 structure.

104 Methods

105 **Field Sites.** Soil was taken from areas of unimproved
106 (U4a) and semi-improved (U4b) grassland at field sites
107 (Long Hill, Sally Gap, Lough Tay, Kings River, and Annagh
108 Hill) in upland regions of counties Wicklow and
109 Wexford in June 2000 (see Table 1 for grid references).
110 The maximum longitudinal distance between sites was 45

km, and the maximum latitudinal distance between sites 111
was 22 km. All sites were located on granite/quartzite 112
bedrock substrata (as determined by reference to 113
Geological Survey of Ireland map [20]), had similar peaty 114
podzol soil composition (determined in consultation with 115
soil survey bulletin map [17]), and contained areas of 116
unimproved (U4a) and semi-improved (U4b) grassland. 117
Floristic composition was analyzed according to the UK 118
National Vegetation Classification (NVC) system [49]. 119
Briefly, 1 m quadrats were placed randomly five times 120
around the grassland area and plant species composition 121
and estimates of abundance as percent cover were 122
determined visually [49]. 123

Sampling Regime. Three sampling points within 124
each grassland type at each site were randomly selected, 125
and soil samples were taken using a corer to remove 126
three replicate cores (4 cm diameter, 10 cm depth) at 127
each sampling point. As extensive grass roots were visible 128
throughout all cores, all soil was assumed to have been in 129
contact with plant roots and was considered rhizosphere. 130
Soil was sieved to <4 mm to remove plant and root 131
material, and stored at 4°C for less than 7 days for chem- 132
ical analyses, microbial activity, and biomass analysis, and 133
at -20°C for molecular analyses. 134

Soil Physical and Chemical Analysis. Soil samples 135
were analyzed for pH_{water} by an electrometric method 136
[55], using a single junction reference electrode (Orion 137
Instruments, Boston, MA, USA). Total nitrogen was 138
determined by the Kjeldahl method [7], using a Kjeltec 139
system 2000 Digestion apparatus and 2100 Distillation 140
unit (Foss Tecator, Höganäs, Sweden). Soil was digested 141
at 420°C for 1 h with a mixture of potassium sulfate, 142
copper sulfate, and sulfuric acid, before undergoing steam 143

t1.1 **Table 1.** Map grid references [Ordnance Survey of Ireland (OSI) grid coordinates] and chemical composition of soils from U4a and U4b grassland types at each field site ($n = 3$), and the average value for each soil type over all sites ($n = 15$)

t1.2	OSI coordinates	pH		% Nitrogen		Phosphorus (mg P kg ⁻¹)		Potassium (mg K kg ⁻¹)		
		U4a	U4b	U4a	U4b	U4a	U4b	U4a	U4b	
t1.3										
t1.4	Site									
t1.5	Long Hill	O 218 124	3.82	4.57	0.97	0.72	12.8	13.7	241	366
t1.6	Sally Gap	O 116 124	4.23	4.12	0.44	0.47	8.2	12.4	42	43
t1.7	Lough Tay	O 160 087	3.46	3.95	0.68	0.71	11.5	11.6	167	241
t1.8	Kings River	O 008 014	4.36	3.92	0.61	0.72	11.1	12.2	157	173
t1.9	Annagh Hill	T 107 672	3.46	3.68	1.27	1.18	12.7	12.3	283	220
t1.10	Average		3.87	4.05	0.80	0.76	11.3	12.4	178	209
t1.11	SED (Site*Grassland type)		0.159		0.048		0.73		4.9	
t1.12	p Values									
t1.13	Site		***		***		***		***	
t1.14	Grassland type		*		NS		**		***	
t1.15	Site*Grassland type		***		***		**		***	

t1.16 Means and standard error of differences (SED) are shown. ANOVA p values for site, grassland type and their interaction are shown as NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

144 distillation. Nitrogen concentrations were determined after
145 colorimetric titration with 0.1 M hydrochloric acid. Using
146 ammonium sulfate as a standard, the recovery of nitrogen
147 from these soil samples was estimated at $82.8 \pm 2.6\%$.

148 Extractable phosphorus levels in soil samples were
149 determined using the Morgan extraction [45]. Phospho-
150 rus was measured using the colorimetric ammonium
151 molybdate–ascorbic acid method [36]. Absorbance was
152 measured on a Jenway 6300 Spectrophotometer (Essex,
153 UK) and compared to that of standard phosphorus
154 solutions to determine extractable phosphorus concen-
155 tration. Soil potassium concentrations were measured
156 using the ammonium acetate centrifuge method [27].
157 Phosphorus was extracted with ammonium acetate and
158 analyzed by atomic emission spectrometry on a Corning
159 410 flame photometer (High Wycombe, Bucks, UK),
160 with sample readings compared to those of standard
161 potassium solutions to determine potassium content.

162 *Total Microbial Activity.* Total microbial activity
163 was assessed as triphenylformazan dehydrogenase activity
164 and was determined based on a modification of the
165 method of Thalmann [54], as previously described [8].

166 *Fungal Biomass.* Total soil ergosterol was quan-
167 tified as described previously [9]. Briefly, 5 g soil was
168 vortexed and sonicated with methanol and potassium
169 hydroxide solution at 0°C. Subsequently, after incuba-
170 tion at 85°C for 30 min, followed by cooling at 4°C for
171 20 min, high-performance liquid chromatography
172 (HPLC)-grade pentane was used to extract ergosterol
173 from the soil mixture. Three pentane extracts were
174 combined and dried under N₂ gas before being redis-
175 solved in methanol and filtered through 0.2-µm Teflon
176 filters, then analyzed on a Waters Sugar Analyzer I HPLC
177 system (Elstree, Hertfordshire, UK). Incorporation of an
178 internal ergosterol standard allowed the extraction effi-
179 ciency of ergosterol from soil in these experiments to be
180 estimated at $87 \pm 4.7\%$.

181 *Total Soil DNA Extraction and Purification.* Total
182 soil DNA was extracted as previously described [8].
183 Briefly, soil (0.5 g) was added to tubes containing glass and
184 zirconia beads, to which hexadecyltrimethylammonium
185 bromide (CTAB) extraction buffer was added. After
186 incubation at 70°C for 10 min, phenol/chloroform/
187 isoamylalcohol (25:24:1) was added and tubes were
188 then shaken in a Hybaid Ribolyser (Ashford, UK) at
189 5.5 m/s for 30 s. Following bead beating, tubes were
190 centrifuged and the aqueous layer was removed and
191 extracted twice with chloroform/isoamylalcohol (24:1).
192 A further purification procedure was performed involving
193 incubation with lysozyme solution (100 mg/mL) for 30
194 min at 37°C. Tubes were again centrifuged and the
195 aqueous layer removed and further purified using a High

Pure PCR Product Clean Up Kit (Roche Diagnostics 196
GmbH, Penzberg, Germany) according to manufac- 197
turer's instructions. DNA was eluted in 50 µl of the 198
manufacturer's proprietary elution buffer solution and 199
was consistently suitable for polymerase chain reac- 200
tion (PCR) amplification without further treatment. 201

Bacterial Community Fingerprinting by T-RFLP 202
Analysis. Terminal restriction ribotype lengths were 203
determined using a modification of the method of Liu 204
et al. [38]. After extraction and purification of total DNA 205
from soil, the bacterial 16S small subunit rRNA gene was 206
amplified using primer set F27 (5'-AGAGTTTGATCMT 207
GGCTCAG-3') and R1492 (5'-TACGGYTACCTTGT 208
TACGACT-3') [37]. The forward primer F27 was 209
labeled with Beckman Coulter fluorescent dye D4 210
(Invitrogen, Paisley, Scotland, UK). PCR reactions were 211
performed in 50-µl volumes containing 5 µl of 10× Mg- 212
free PCR buffer, 1.25 mM MgCl₂, 15 pmol of each 213
primer, 200 µM of each dNTP, 25 µg BSA, ~10 ng 214
extracted total soil DNA, and 2.5 U *Taq* DNA polymerase 215
(Promega, Southampton, UK). The thermocycling 216
conditions were as follows: a hot start at 94°C for 3 min 217
(1 cycle); 94°C for 1 min, 53°C for 2 min, 72°C for 2 min 218
(26 cycles); 72°C for 7 min. PCR products were first 219
visualized on a 1% agarose gel and purified using a High 220
Pure PCR product purification kit (Roche Diagnostics) 221
according to the manufacturer's instructions. The purified 222
PCR product was quantified on a 1.2% agarose gel then 223
digested enzymatically as follows: approximately 50 ng of 224
PCR product was added to a reaction mixture containing 225
sterile Millipore water, 20 U of restriction endonuclease 226
MspI, and 2 µl of corresponding enzyme buffer. Digests 227
were performed in a final volume of 20 µl and incubated 228
in a water bath at 37°C for 4 h. Digests were desalted and 229
aliquots (1 µl) were mixed with 38.75 µl of deionized 230
formamide and 0.25 µl of Beckman Coulter size standard 231
600 (High Wycombe, Bucks, UK). 232

Fungal Community Fingerprinting by ARISA 233
(Automated Ribosomal Intergenic Spacer Analysis). 234
The fungal genetic region containing the two internal 235
transcribed spacers (ITS) and the 5.8S rRNA gene (ITS1– 236
5.8S–ITS2) was amplified using primer set ITS1-F 237
(CTTGGTCATTTAGAGGAAGTAA) [16] and ITS4 238
(TCCTCCGCTTATTGATATGC) [62]. Amplified 239
sequences contained the two ITS regions and the 5.8S 240
gene plus 22 bp from the forward primer. The forward 241
primer ITS1-F was labeled with Beckman Coulter 242
fluorescent dye D4 (Invitrogen). PCR reactions were 243
performed in 50-µl volumes of 25 µl master mix 244
[containing 50 units/mL *Taq* DNA polymerase in a 245
proprietary reaction buffer (pH 8.5), 400 µM each dNTP, 246
and 3 mM MgCl₂; Promega], 15 pmol each primer, 25 247
µg BSA, and ~10 ng extracted total soil DNA. The 248

249 thermocycling conditions were as follows: a hot start at
 250 95°C for 4 min (1 cycle); 95°C for 1 min, 53°C for 30 s,
 251 72°C for 1 min (35 cycles); 72°C for 7 min (1 cycle). PCR
 252 products were first visualized on a 1.2% agarose gel, then
 253 purified using a High Pure PCR product purification kit
 254 (Roche Diagnostics) and eluted in 50 µl of sterile water
 255 heated to 50°C. The purified PCR products were
 256 quantified on a 1.2% agarose gel before mixing aliquots
 257 (0.5–1 µl, equivalent to ~2 ng DNA) with 38.4 µl of
 258 deionized formamide, 0.2 µl of Beckman Coulter size
 259 standard 600 (dye D1), and 0.4 µl of custom-made
 260 marker (containing ribotypes ranging from 600 to 1000
 261 bp in intervals of 20 bp, and 1000–1200 in intervals of
 262 50 bp, all labeled with Beckman Coulter Dye D1)
 263 (BioVentures, Murfreesboro, TN, USA).

264 **TRFLP and ARISA Ribotype Analysis.** Bacterial
 265 terminal restriction and fungal ARISA ribotype lengths were
 266 determined by electrophoresis using a Beckman Coulter
 267 (CEQ8000) automated sequencer, version 6.0.2 (High
 268 Wycombe, Bucks, UK) (resolution ±1 bp up to 400 bp
 269 ribotype length, ±2 bp thereafter). Run conditions were
 270 60°C separation temperature, 4 kV voltage, and 120 min
 271 separation time to allow for separation of the larger
 272 ribotypes. Analysis of ribotype profiles was performed
 273 using the Beckman Coulter ribotype analysis package 8000,
 274 version 8.0.52, ribotype analysis algorithm version 2.2.1
 275 (High Wycombe, Bucks, UK). A quartic polynomial model,
 276 rather than the recommended cubic model, was used for size
 277 standard calibration as this resulted in improved correlation
 278 between expected and actual size standard ribotype sizes,
 279 particularly for ribotypes in the range 400–1200 bp.
 280 Ribotypes with peak heights less than 1% of the total peak
 281 height for all ribotypes in a sample were regarded as
 282 background noise and excluded from analysis. Ribotypes
 283 that differed by less than 0.5 bp in different profiles were
 284 considered identical. The peak heights of individual ribo-
 285 types were relativized as a percentage of their abundance
 286 within a sample to account for DNA quantity differences
 287 between replicate profiles, resulting in profiles containing
 288 data on ribotypes present and their relative abundances.

289 **Statistical Analysis.** Results for chemical analyses,
 290 microbial activity, fungal biomass, ribotype number, and
 291 individual abundances of the top 20 overall most
 292 abundant bacterial and fungal ribotypes were analyzed
 293 by one-way factorial analysis of variance (ANOVA) using
 294 Genstat v6 (VSN International, Oxford, UK). The signifi-
 295 cance level was set at $p < 0.05$. In addition, Genstat was
 296 used to calculate the overall abundance of each individ-
 297 ual ribotype for all samples. Ribotypes were then ranked
 298 according to overall abundance and these rankings were
 299 used to select individual ribotypes for ANOVA. Bacterial
 300 TRFLP and fungal ARISA profiles were analyzed using
 301 Canoco for Windows, v4.02 (Centre for Biometry,

Wageningen, The Netherlands). Initial analysis by 302
 detrended correspondence analysis (DCA) revealed that 303
 bacterial TRFLP data exhibited a linear response to the 304
 environmental variables (grassland type and site), there- 305
 by indicating redundancy analysis (RDA) as the most 306
 appropriate multivariate approach, whereas fungal ARISA 307
 data exhibited a unimodal response, indicating canonical 308
 correspondence analysis (CCA) was most appropriate. 309
 Initially, RDA or CCA of all ribotypes with all environ- 310
 mental factors, including all of their interactions, was 311
 carried out. This resulted in overly complex analysis results 312
 and diagrams, necessitating a reduction in the amount of 313
 data analyzed. The environmental factors most important 314
 in explaining variation in ribotype profiles were selected by 315
 eliminating those with low canonical coefficients and t - 316
 values [33]. Next, analysis was limited to the top 20 317
 ribotypes, as ranked by abundance. As there was little or no 318
 change in analysis results after limiting the environmental 319
 variables and ribotype set, it was concluded that the 320
 variables and ribotypes accounting for the majority of 321
 variance in the data had been selected. The resulting 322
 ordination biplots approximated the weighted average of 323
 each species (in this case, relative abundances of ribotypes) 324
 with respect to each of the environmental variables, which 325
 were represented as arrows. The length of these arrows 326
 indicated the relative importance of that environmental 327
 factor in explaining variation in ribotype profiles, whereas 328
 the angle between arrows indicated the degree to which 329
 they were correlated [33]. A Monte Carlo permutation test 330
 based on 199 random permutations was used to test the 331
 null hypothesis that ribotype profiles were unrelated to 332
 environmental variables. 333

334 Results

Grassland Floristic Composition. Vegetational analysis 335
 revealed that unimproved U4a grasslands were domi- 336
 nated by *A. capillaris*, *Anthoxanthum odoratum*, and 337
F. ovina, with high frequencies of *Potentilla erecta* and 338
G. saxatile. Semi-improved U4b grasslands, although still 339
 dominated by *A. capillaris*, showed reduced abundances 340
 of *F. ovina* and increased occurrences of *F. rubra*. 341
 Additionally, more mesophytic species such as *Holcus* 342
lanatus and *T. repens* were evident. 343

Soil Chemical Composition. Analysis of soil pH, 344
 nitrogen, phosphorus, and potassium (Table 1) indicated 345
 soil chemical composition varied due to both grassland 346
 type and site. On average, over all sites, U4a grassland 347
 soils had a slightly lower pH, higher nitrogen content, 348
 and lower phosphorus and potassium content than U4b 349
 grassland soils. However, these trends varied by site. 350

Microbial Activity and Fungal Biomass. To 351
 determine broad-scale trends in soil microbial community 352

353 structure, microbial activity and fungal biomass were
 354 determined (Table 2). Microbial activity varied widely
 355 between sites, with soils from Kings River having the
 356 highest values, whereas soils from Annagh Hill and Sally
 357 Gap had the lowest. Grassland type had a smaller but
 358 significant impact, with microbial activity higher in U4b
 359 grassland soils than in U4a grassland soils at every site.
 360 Fungal biomass, measured as ergosterol, also experienced
 361 wide variation due to site, with soils from Long Hill
 362 highest in fungal biomass, while those from Sally Gap
 363 were lowest (Table 2). Grassland type and site interacted
 364 significantly, with soil from U4b grasslands lower in
 365 fungal biomass than those from U4a grassland, at every
 366 site except Kings River.

367 **Bacterial and Fungal Ribotype Number.** Bacterial
 368 TRFLP detected a total of 89 unique terminal fragments
 369 (ribotypes) after analysis of all samples, ranging in size
 370 from 60 to 640 bp. A total of 453 fungal ribotypes was
 371 detected by fungal ARISA, ranging in size from 47 to
 372 1100 bp. Bacterial ribotype number (Table 2) was
 373 affected by a significant interaction between grassland
 374 type and site, with numbers higher in soils from U4b
 375 grasslands than in U4a grassland soil at three sites, but
 376 the reverse true at the other two sites. Fungal ribotype
 377 number (Table 2) was significantly affected only by
 378 grassland type, with fungal ribotype numbers higher in
 379 U4a grassland soil than in U4b soil at every site.

380 **Bacterial Community Structure.** The effects of
 381 grassland type and site on bacterial community structure
 382 were further explored using redundancy analysis (RDA),
 383 with results shown in the ordination plot in Fig. 1. The top
 384 20 most abundant bacterial ribotypes, accounting for 86.4%
 385 of total abundance, were included in the analysis. Axes 1

and 2 together accounted for 30.8% of total variation 386
 within bacterial ribotype profiles, and 72.6% of variation in 387
 profiles that could be attributed to the environmental 388
 variables of grassland type and site. Species–environment 389
 correlations for both axes were quite high (over 0.81), 390
 indicating that changes in bacterial ribotype profiles were 391
 closely correlated with changes in grassland type and site. 392
 Both the first axis on its own, then all axes together, 393
 were analyzed by the Monte Carlo test and found to 394
 explain a significant ($p < 0.05$) amount of variation within 395
 the data. 396

Intrasite correlations revealed that Axis 1 was most 397
 closely correlated with grassland type (U4a and U4b), 398
 indicating that grassland type had the biggest impact on 399
 community structure, while Axis 2 was most closely 400
 correlated with the Sally Gap site. These observations 401
 were supported by noting the length of the arrows for 402
 U4a and U4b grassland types and the Sally Gap site in the 403
 RDA plot in Fig. 1, which appeared longer than arrows 404
 for other sites, indicating that these factors had a large 405
 impact on bacterial community structure. The arrow for 406
 the Kings River site was also long, and values for its 407
 intrasite correlations confirmed that it also had a large 408
 effect on bacterial community profiles. 409

Correlations shown in the RDA plot were supported 410
 by ANOVA results (Table 3); for example, the arrow for 411
 the second most abundant ribotype, TRF 150, appeared 412
 close to the arrow for U4b grassland type on the RDA 413
 plot, and this relationship was confirmed by ANOVA, 414
 which found that the abundance of TRF 150 was 415
 significantly affected by grassland type, and that its 416
 abundance was highest in U4b grassland soil. Grassland 417
 type was found to have a significant effect on many 418
 (50%) of the top 20 most abundant ribotypes. Site also 419
 had a strong effect on abundances of the top 20 420

t2.1 **Table 2. Microbial activity, fungal biomass, and bacterial and fungal ribotype numbers of soils from U4a and U4b grassland types at each field site ($n = 3$), and the average value for each grassland type over all sites ($n = 15$)**

t2.2	Microbial activity ($\mu\text{g TPF g}^{-1}$ dry soil)		Fungal Biomass ($\mu\text{g ergosterol g}^{-1}$ soil)		Bacterial ribotype number (mean TRFs per replicate)		Fungal ribotype number (mean fragments per replicate)		
	U4a	U4b	U4a	U4b	U4a	U4b	U4a	U4b	
t2.3									
t2.4	Site								
t2.5	Long Hill	5	190	3.27	1.43	14	31	38	26
t2.6	Sally Gap	9	52	0.05	0.01	19	31	28	26
t2.7	Lough Tay	31	87	2.01	0.25	6	10	40	18
t2.8	Kings River	388	520	0.44	2.24	17	9	29	25
t2.9	Annagh Hill	2	20	1.92	1.10	27	9	35	16
t2.10	Average	86	174	1.53	1.00	17	18	34	22
t2.11	SED (Site*Grassland type)	69.4		0.842		8.3		12.1	
t2.12	p Values								
t2.13	Site	***		*		*		NS	
t2.14	Grassland type	*		NS		NS		*	
t2.15	Site*Grassland type	NS		*		*		NS	

t2.16 Means and standard error of differences (SED) are shown. ANOVA p values for site, grassland type and their interaction are shown as NS, not significant; * $p < 0.05$; *** $p < 0.001$.

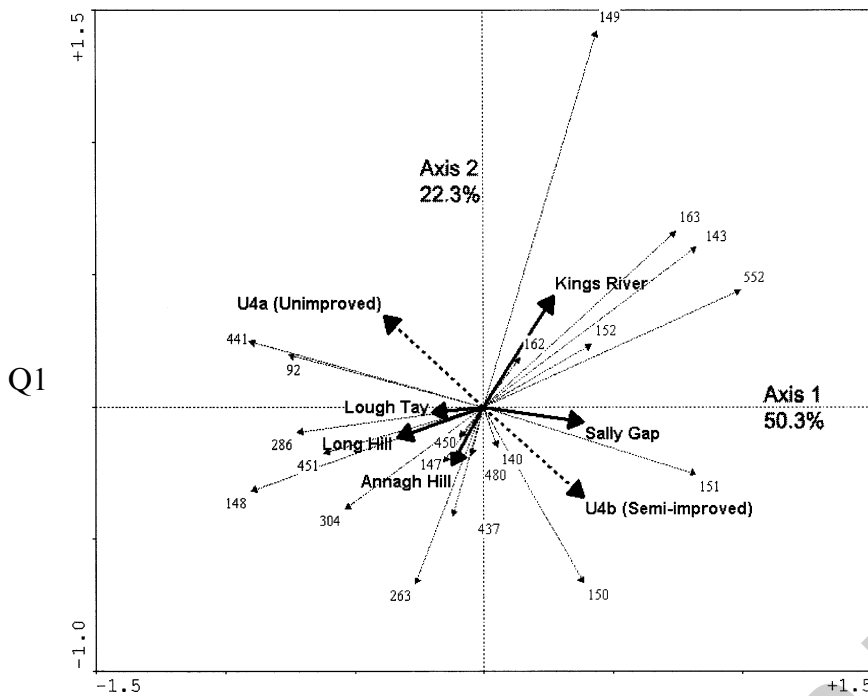


Figure 1. Redundancy analysis (RDA) ordination diagram of bacterial TRFLP data, with grassland type (*dashed lines*) and sites (*bold solid lines*) represented as *large arrows* and bacterial TRF ribotypes represented as *smaller arrows*. TRFs are labeled according to ribotype size (bp). Axis 1 explains 50.3% of the TRFLP–environment variance, whereas Axis 2 explains a further 22.3% of the TRFLP–environment variance.

429 ribotypes, having a significant effect on 30% of them. 430 Ribotypes that were significantly affected by site often had 431 highest abundances at Sally Gap or Kings River, indicating 432 these sites significantly affected the abundances of dom- 433 inant ribotypes. In addition, significant interactions 434 between grassland type and site were also noted.

Fungal Community Structure. Fungal ARISA 435 ribotype profiles were investigated using canonical corre- 436 spondence analysis (CCA) after initial data exploration 437 revealed fungal community profiles responded to 438 environmental variables (grassland type and site) in a 439 unimodal fashion. The top 20 most abundant fungal 440

t3.1 **Table 3.** Abundance rankings and ANOVA results for the top 20 most abundant bacterial TRFLP fragments, as ranked by average abundance over all samples

TRFLP fragment (bp)	Abundance			p Values	
	Rank	% Abundance	Cumulative % abundance	Grassland type	Site
92	1	23.4	23.4	0.084	0.015 (LT)
150	2	12.6	36.0	0.007 (U4b)	0.273
151	3	11.8	47.8	0.074	0.011 (SG)
148	4	7.8	55.6	0.039 (U4a)	0.100
441	5	4.2	59.8	0.004 (U4a) ^a	0.051 ^a
286	6	3.3	63.1	0.043 (U4a)	0.209
143	7	2.8	65.9	0.705	< 0.001 (SG)
552	8	2.6	68.5	0.011 (U4b) ^a	< 0.001 (KR) ^a
152	9	2.3	70.8	0.604	0.341
149	10	2.3	73.1	0.003 (U4a) ^a	< 0.001 (KR) ^a
263	11	2.2	75.3	0.038 (U4a)	0.339(AH)
304	12	1.8	77.1	0.007 (U4a)	0.654
163	13	1.4	78.5	0.035 (U4b)	0.654
480	14	1.4	80.0	0.809	0.916
451	15	1.4	81.3	0.028 (U4a)	0.158
147	16	1.1	82.4	0.265	0.670
437	17	1.1	83.5	0.358	0.499
140	18	1.1	84.5	0.917	0.657
450	19	0.9	85.5	0.058 ^a	0.823 ^a
162	20	0.9	86.4	0.492	0.431

t3.24 Data in bold indicate a significant effect ($p < 0.05$).

Grassland type/site with highest abundance of fragment is indicated next to significant effect (LT: Lough Tay; SG: Sally Gap; KR: Kings River; AH: Annagh Hill).

^aSignificant interaction with each other.

441 ribotypes, accounting for 38.4% of total abundance, were
 442 included in the analysis. Results of CCA (Fig. 2) showed
 443 both Axes 1 and 2 had high eigenvalues (0.556 and
 444 0.303), indicating that grassland type and site accounted
 445 for a large percentage of variation between fungal
 446 profiles. This was confirmed by calculations that
 447 indicated Axes 1 and 2 together accounted for 17.0% of
 448 the total variation within the data, and 63.6% of the
 449 variation that could be attributed to grassland type and
 450 site. Species–environment correlations were also very
 451 high (above 0.76), indicating that changes in fungal
 452 community profiles corresponded strongly with changes
 453 in grassland type and site. Monte Carlo tests of significance
 454 for the first axis alone, and all axes combined, indicated the
 455 analysis accounted for a significant percentage of variation
 456 within fungal ARISA data ($p < 0.05$).

457 The longest arrows in the CCA ordination diagram
 458 (Fig. 2) were for the sites Annagh Hill and Kings River,
 459 indicating these had the greatest impact on community
 460 structure. This was confirmed by intraset correlations,
 461 which revealed that Axis 1 was most strongly correlated
 462 with Annagh Hill, while Axis 2 was associated with Kings
 463 River. Grassland type (U4a and U4b) also had long
 464 arrows and high intraset correlations, suggesting that
 465 grassland type was also an important factor affecting
 466 fungal community structure. A third site, Sally Gap, also
 467 had a long arrow in the CCA plot and high correlation

values, indicating it affected fungal community structure. 468
 In contrast, arrows for the Lough Tay and Long Hill sites 469
 were notably shorter than those for the other sites and 470
 grassland type, signifying that these had a relatively 471
 smaller impact on fungal communities. 472

These observations were supported by ANOVA of the 473
 top 20 most abundant ribotypes (Table 4), which revealed 474
 that grassland type had a significant effect on abundances 475
 of four of the top 20 ribotypes. Interestingly, all of the four 476
 affected had highest abundances in U4b grassland soil. 477
 The effect of site was also evident, with those ribotypes 478
 significantly affected by site having the highest abundances 479
 in Annagh Hill, Kings River, and Sally Gap. In addition, 480
 significant interactions between grassland type and site 481
 affected the abundances of several fungal ribotypes. 482

Discussion

483

The effect of a moderate change in grassland floristic 484
 composition (from unimproved U4a to semi-improved 485
 U4b) on microbial community structure was investigated 486
 at five geographically separate field sites with similar 487
 underlying geological substrata. Both grassland type and 488
 site were found to impact microbial community struc- 489
 ture, but these effects varied and neither influence had a 490
 consistently larger impact than the other. The original 491

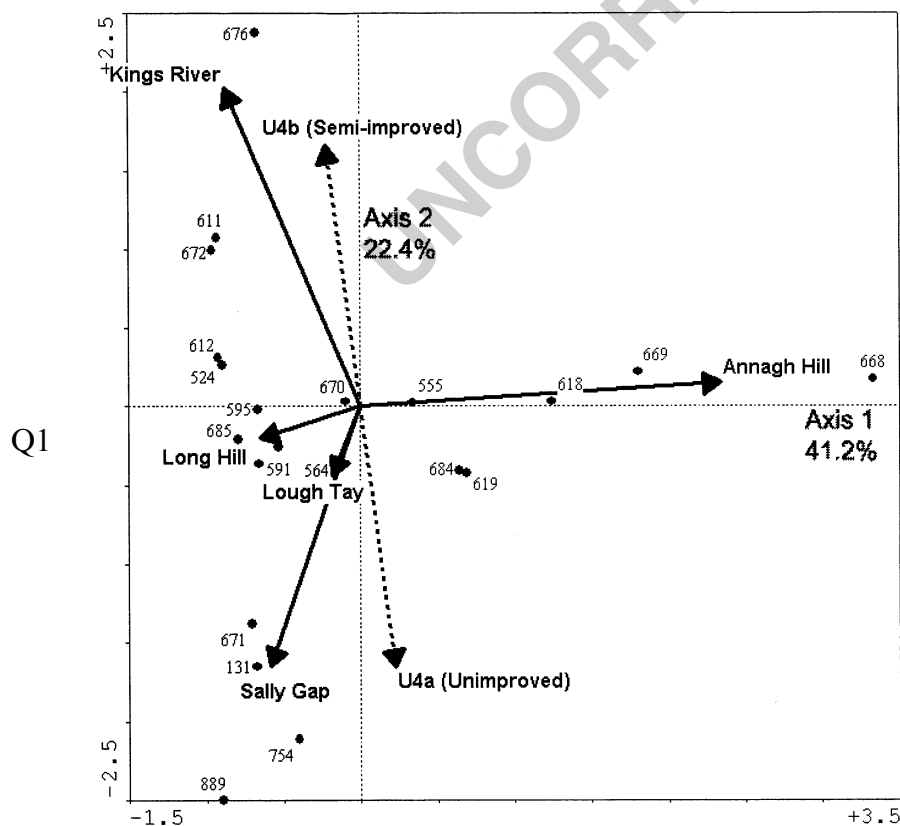


Figure 2. Canonical correspondence analysis (CCA) ordination diagram of fungal ARISA data, with grassland type (dashed lines) and sites (bold solid lines) represented as large arrows and fungal ARISA ribotypes represented as black dots. Ribotypes are labeled according to ribotype size (bp). Axis 1 explains 41.2% of the FARISA–environment variance, whereas Axis 2 explains a further 22.4% of the FARISA–environment variance.

t4.1 **Table 4. Abundance rankings and ANOVA results for the top 20 most abundant fungal ARISA fragments, as ranked by average abundance over all samples**

t4.2		Abundance			p values	
t4.3	ARISA fragment (bp)	Abundance rank	% Abundance	Cumulative % abundance	Grassland type	Site
t4.4	668	1	3.6	3.6	0.754	0.073
t4.5	670	2	3.0	6.6	0.624 ^a	0.196 ^a
t4.6	524	3	2.5	9.1	0.049 (U4b) ^a	0.047 (SG) ^a
t4.7	555	4	2.5	11.6	0.485	0.458
t4.8	684	5	2.4	14.0	0.527	0.326
t4.9	671	6	2.3	16.3	0.859	0.358
t4.10	595	7	2.2	18.5	0.848	0.417
t4.11	672	8	2.1	20.7	0.015 (U4b) ^a	0.001 (KR) ^a
t4.12	611	9	2.0	22.7	0.005 (U4b)	0.025 (KR)
t4.13	612	10	1.9	24.6	0.137	0.131
t4.14	669	11	1.7	26.3	0.264	0.163
t4.15	131	12	1.6	27.9	0.556	0.771
t4.16	564	13	1.4	29.4	0.060	0.601
t4.17	685	14	1.4	30.8	0.033 (U4b)	0.099
t4.18	619	15	1.4	32.2	0.072	0.826
t4.19	618	16	1.3	33.5	0.081	0.049 (AH)
t4.20	889	17	1.3	34.9	0.431	0.329
t4.21	676	18	1.3	36.2	0.193	0.063
t4.22	754	19	1.1	37.3	0.498	0.492
t4.23	591	20	1.1	38.4	0.533	0.061

t4.24 Data in bold indicate a significant effect ($p < 0.05$).

Grassland type/site with highest abundance of fragment is indicated next to significant effect (SG: Sally Gap; KR: Kings River; AH: Annagh Hill).

^aSignificant interaction with each other.

493 question posed in this study was whether a grassland
494 classification system such as the UK National Vegetation
495 Classification (NVC) could be used as a predictor of mi-
496 crobial community structures at geographically separated
497 sites with similar geological origins. Our results indicate
498 that this hypothesis must be rejected, as microbial
499 community measurements varied widely according to
500 site, and trends in the effect of grassland type were
501 inconsistent.

502 Strong variation in soil physicochemical properties
503 between sites was noted, indicating that grassland type
504 did not correspond with soil properties. Changes in pH
505 due to semi-improvement were evident, with U4a grass-
506 lands having a slightly lower pH overall, although this
507 effect varied between sites. Soil nitrogen levels also varied
508 between sites, as has been noted elsewhere in geograph-
509 ically separated sites with similar grassland types [23].
510 Although some site variation was noted in soil phospho-
511 rus and potassium, at most sites U4b grassland soil had
512 higher phosphorus and potassium contents than U4a,
513 possibly due either to movement of these elements from
514 improved areas, or from increased nutrient mobilization
515 due to increased pH [6].

516 Although microbial activity was strongly affected by
517 site, there was a consistent influence of grassland type,
518 with semi-improved U4b soils having higher microbial
519 activity than unimproved U4a soils from the same site.
520 This increase in activity from unimproved to semi-

improved soils was also noted by Brodie *et al.* [8] and 521
Williams *et al.* [63]. The decrease seen in fungal biomass 522
in semi-improved soils at most sites indicates that that 523
there may be more fungi in unimproved soils, which has 524
been a common finding in studies of upland grasslands 525
[1, 2, 4, 9, 23, 24]. 526

Bacterial ribotype numbers were significantly affected 527
by site, but increased from unimproved U4a to semi- 528
improved U4b grassland soil at most sites, mirroring 529
trends in activity. An increase in bacterial ribotype 530
number from unimproved to semi-improved soils was 531
also noted by Brodie *et al.* [8]. Bacterial community 532
structure was explored by multivariate analysis, which 533
indicated that although grassland type had the largest 534
influence, the Sally Gap and Kings River sites also had a 535
strong impact. The large impact of unimproved and 536
semi-improved grassland types on bacterial community 537
structure indicated that semi-improvement resulted in 538
differing bacterial community structures. Other studies 539
have also observed changes in bacterial community 540
structure after semi-improvement [8, 43]. The strong 541
impact of the Kings River and Sally Gap sites may be 542
related to high microbial activity and low soil nutrient 543
levels, respectively. 544

Fungal ribotype number was affected significantly by 545
grassland type, with fungal ribotypes decreasing in semi- 546
improved U4b soil as compared to unimproved U4a soil 547
at all sites, which corresponded with the decrease in 548

549 fungal biomass noticed at most sites. Although site did
550 not significantly affect fungal ribotype number, canonical
551 correspondence analysis (CCA) of fungal ARISA profiles
552 revealed that certain sites (Annagh Hill, Kings River, and
553 Sally Gap) had a large influence on fungal community
554 structure. Grassland type also had an important influence
555 on soil fungal populations, with unimproved U4a and
556 semi-improved U4b soils having differing fungal com-
557 munity structures.

558 Studies of geographically separated upland acidic
559 grasslands have been conducted previously. Clegg *et al.*
560 [12], studying unimproved (U4a), semi-improved (U4b),
561 and improved (MG6) grassland soils at three sites, found
562 that complexity rankings of DNA from different grass-
563 land types varied between sites, based upon percent G +
564 C content. Grayston *et al.* [23, 24], working on similar
565 sites and grasslands, found differences between grassland
566 types at each site, with variation between sites and
567 seasons, using community-level physiological profiling
568 (CLPP), phospholipid fatty acid contents (PLFA), and
569 percent G + C profiling. These studies, in concurrence
570 with the results presented here, indicate a large impact
571 of site on microbial community structure, often obscur-
572 ing differences between grassland types. However, they
573 have relied upon broad-scale analyses such as percent G
574 + C content, PLFA, and CLPP. The results presented in
575 this work mark the first time that DNA-based profiling
576 approaches (TRFLP and ARISA) have been applied
577 together to a geographic survey of upland acidic grass-
578 lands. DNA-based community fingerprinting approaches
579 can be difficult to interpret because of species differences
580 in rRNA gene copy number [13, 15], biases resulting
581 from PCR amplification [34, 47, 56], and the difficulty
582 in standardizing the amount of DNA analyzed in each
583 replicate [14, 39]. However, as all samples were subject to
584 the same biases, it was still possible to compare them on a
585 relative basis, especially after standardization of ribotype
586 peak heights into proportions per sample.

587 There are several possible reasons for the lack of
588 grouping in microbial communities in accordance with
589 grassland type. It may be that changes in floristic
590 composition due to semi-improvement did not signifi-
591 cantly affect microbial community structure, or were too
592 small to have consistent significant effects. There is
593 evidence suggesting that certain plant species impact
594 microbial community structure more than others [32,
595 42]. Other studies on the impact of plant community
596 composition on soil microbial communities [10, 18, 25,
597 26, 29, 31, 59] indicate that changes in functional groups
598 of plants may have stronger impacts than plant diversity
599 or composition *per se*. In our study, floristic composition
600 changed from *Festuca–Agrostis–Galium* domination in
601 unimproved (U4a) grasslands, to *Festuca–Agrostis–*
602 *Galium* with *Holcus–Trifolium* subcommunities in semi-
603 improved (U4b) grasslands. Although *Trifolium* is a

legume, both grassland types were dominated by grasses. 604
Therefore, there was little change in plant functional 605
group composition, which may have contributed to the 606
inconsistency of grassland type effects on microbial 607
community structure. 608

Site can have a considerable impact on microbial 609
community structure [12]. Several studies have found 610
strong links between soil physicochemical properties 611
such as soil texture and type, and microbial community 612
structure [5, 19, 25, 32, 51, 58]. Correlations between 613
soil physicochemistry and microbial community struc- 614
ture have been found both in field and microcosm 615
studies on acidic upland grassland soils [8, 9, 24, 30, 35]. 616
In our study, site affected all soil physicochemical 617
characteristics (pH, percent N, phosphorus, potassium) 618
significantly, and effects of semi-improvement on soil 619
chemistry also varied significantly between sites. These 620
inconsistent trends between sites may mean that minor 621
changes in soil chemistry within a site could be sufficient 622
to support a change in floristic composition, but are 623
not enough to strongly affect soil microbial popula- 624
tions. Often, changes in soil chemical parameters were 625
greater between sites than between grassland types at 626
a site, which may contribute to the impact of site on 627
microbial community structure. For example, the same 628
floristic composition can occur at different sites at a range 629
of pH values (published values for U4a grassland soils 630
range from 3.3 to 6.3, and for U4b grassland soils range 631
from 3.98 to 6.4 [8, 11, 12, 23, 24, 60]); such large 632
differences in pH between sites with the same floristic 633
grassland classification could strongly affect soil micro- 634
bial communities. 635

In practice, the real determinants of microbial com- 636
munity structure between sites and grassland types are 637
likely to be complex interactions between plant composi- 638
tion effects, and individual site characteristics, including 639
soil physicochemical composition. A recent study of an 640
unimproved (U4a) grassland in Scotland reported that 641
CLPP, DGGE, and PLFA profiles were affected by both 642
vegetation class and soil physical and chemical factors, but 643
no single factor or factors stood out [48]; rather, it 644
appeared that these interacted in complex mechanisms 645
to influence soil microbial communities. It is likely that a 646
similar situation exists in relation to the sites and 647
grassland types investigated in our study. 648

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657 **References**

- 658 1. Bardgett, RD, Hobbs, PJ, Frostegård, A (1996) Changes in soil
659 fungal:bacterial biomass ratios following reductions in the intensity
660 of management of an upland grassland. *Biol Fertil Soils* 22: 261–264
- 661 2. Bardgett, RD, Lovell, RD, Hobbs, PJ, Jarvis, SC (1999) Seasonal
662 changes in soil microbial communities along a fertility gradient of
663 temperate grasslands. *Soil Biol Biochem* 31: 1021–1030
- 664 3. Bardgett, RD, Mawdsley, JL, Edwards, S, Hobbs, PJ, Rodwell, JS,
665 Davies, WJ (1999) Plant species and nitrogen effects on soil
666 biological properties of temperate upland grasslands. *Funct Ecol*
667 13: 650–660
- 668 4. Bardgett, RD, McAlister, E (1999) The measurement of soil
669 fungal:bacterial biomass ratios as an indicator of ecosystem self-
670 regulation in temperate meadow grasslands. *Biol Fertil Soils* 29:
671 282–290
- 672 5. Bossio, DA, Scow, KM, Gunapala, N, Graham, KJ (1998)
673 Determinants of soil microbial communities: effects of agricultural
674 management, season, and soil type on phospholipid fatty acid
675 profiles. *Microb Ecol* 36: 1–12
- 676 6. Brady, NC, Weil, RR (2002) *The Nature and Properties of Soils*.
677 Prentice-Hall, Upper Saddle River, NJ, USA, pp 592–637
- 678 7. Bremner, JM (1996) Nitrogen—total. In: Sparks, DL, Page, AL,
679 Helmke, PA, Loepfert, RH, Soltanpour, PN, Tabatabai, MA,
680 Johnston, CT, Sumner, ME (Eds.) *Methods of Soil Analysis: Part*
681 *3, Chemical Methods*. Soil Science Society of America, Madison,
682 WI, pp 1085–1121
- 683 8. Brodie, E, Edwards, S, Clipson, N (2002) Bacterial community
684 dynamics across a floristic gradient in a temperate upland
685 grassland ecosystem. *Microb Ecol* 44: 260–270
- 686 9. Brodie, E, Edwards, S, Clipson, N (2003) Soil fungal community
687 structure in a temperate upland grassland soil. *FEMS Microbiol*
688 *Ecol* 45: 105–114
- 689 10. Buckley, DH, Schmidt, TM (2003) Diversity and dynamics of
690 microbial communities in soils from agro-ecosystems. *Environ*
691 *Microbiol* 5: 441–452
- 692 11. Clegg, CD, Ritz, K, Griffiths, BS (1998) Broad-scale analysis of soil
693 microbial community DNA from upland grasslands. *Antonie van*
694 *Leeuwenhoek* 73: 9–14
- 695 12. Clegg, CD, Ritz, K, Griffiths, BS (2000) %G + C profiling and
696 cross-hybridisation of microbial DNA reveals great variation in
697 below-ground community structure in UK upland grasslands.
698 *Appl Soil Ecol* 14: 125–134
- 699 13. Crosby, LD, Criddle, CS (2003) Understanding bias in microbial
700 community analysis techniques due to *rnn* operon copy number
701 heterogeneity. *BioTechniques* 34: 790–802
- 702 14. Egert, M, Friedrich, MW (2003) Formation of pseudo-terminal
703 restriction ribotypes, a PCR-related bias affecting terminal restric-
704 tion length polymorphism analysis of microbial community
705 structure. *Appl Environ Microbiol* 69: 2555–2562
- 706 15. Fogel, GB, Collins, CR, Li, J, Brunk, CF (1999) Prokaryotic
707 genome size and SSU rDNA copy number: estimation of microbial
708 relative abundance from a mixed population. *Microb Ecol* 38:
709 93–113
- 710 16. Gardes, M, Bruns, TD (1993) ITS primers with enhanced
711 specificity for basidiomycetes—application to the identification
712 of mycorrhizae and rusts. *Mol Ecol* 2: 113–118
- 713 17. Gardiner MJ, Radford T (1980) Soil survey bulletin number 36:
714 Soil associations of Ireland and their land use potential. National
715 Soil Survey of Ireland, An Foras Talúntais (The Agricultural
716 Institute), Dublin, Ireland
- 717 18. Gastine, A, Scherer-Lorenzen, M, Leadley, PW (2003) No
718 consistent effects of plant biodiversity on root biomass, soil biota
719 and soil abiotic conditions in temperate grassland communities.
720 *Appl Soil Ecol* 24: 101–111
19. Gelsomino, A, Keijzer-Wolters, AC, Cacco, G, van Elsas, JD (1999) 721
Assessment of bacterial community structure in soil by polymer- 722
ase chain reaction and denaturing gradient gel electrophoresis. 723
J Microbiol Methods 38: 1–15 724
20. Geological Survey of Ireland (2000) *Geology of Kildare–Wicklow* 725
(Sheet 16) 1:100,000. Geological Survey of Ireland. Ballsbridge, 726
Dublin, Ireland 727
21. Gollotte, A, van Tuinen, D, Atkinson, D (2004) Diversity of 728
arbuscular mycorrhizal fungi colonising roots of the grass species 729
Agrostis capillaris and *Lolium perenne* in a field experiment. 730
Mycorrhiza 14: 111–117 731
22. Grayston, SJ, Wang, S, Campbell, CD, Edwards, AC (1998) 732
Selective influence of plant species on microbial diversity in the 733
rhizosphere. *Soil Biol Biochem* 30: 369–378 734
23. Grayston, SJ, Griffith, GS, Mawdsley, JL, Campbell, CD, Bardgett, 735
RD (2001) Accounting for variability in soil microbial communi- 736
ties of temperate upland grassland ecosystems. *Soil Biol Biochem* 737
33: 533–551 738
24. Grayston, SJ, Campbell, CD, Bardgett, RD, Mawdsley, JL, Clegg, 739
CD, Ritz, K, Griffiths, BS, Rodwell, JS, Edwards, SJ, Davies, WJ, 740
Elston, DJ, Millard, P (2004) Assessing shifts in microbial 741
community structure across a range of grasslands of differing 742
management intensity using CLPP, PLFA and community DNA 743
techniques. *Appl Soil Ecol* 25: 63–84 744
25. Groffman, PM, Eagan, P, Sullivan, WM, Lemunyon, JL (1996) 745
Grass species and soil type effects on microbial biomass and 746
activity. *Plant Soil* 183: 61–67 747
26. Hedlund, K, Santa Regina, I, Van der Putten, WH, Lepš, J, Díaz, T, 748
Korthals, GW, Lavorel, S, Brown, VK, Gormsen, D, Mortimer, SR, 749
Rodríguez Barrueco, C, Roy, J, Smilauer, P, Smilauerová, M, Van 750
Dijk, C (2003) Plant species diversity, plant biomass and responses 751
of the soil community on abandoned land across Europe: 752
idiosyncrasy or above-belowground time lags. *Oikos* 103: 45–58 753
27. Helmke, PA, Sparks, DL (1996) Lithium, sodium, potassium, 754
rubidium, and cesium. In: Sparks, DL, Page, AL, Helmke, PA, 755
Loepfert, RH, Soltanpour, PN, Tabatabai, MA, Johnston, CT, 756
Sumner, ME (Eds.) *Methods of Soil Analysis: Part 3, Chemical* 757
Methods. Soil Science Society of America, Madison, WI, pp 551–574 758
28. Hill, GT, Mitkowski, NA, Aldrich-Wolfe, L, Emele, LR, Jurkonie, 759
DD, Ficke, A, Maldonado-Ramirez, S, Lynch, ST, Nelson, EB 760
(2000) Methods for assessing the composition and diversity of soil 761
microbial communities. *Appl Soil Ecol* 15: 25–36 762
29. Hooper, DU, Vitousek, PM (1997) The effects of plant composi- 763
tion and diversity on ecosystem processes. *Science* 277: 1302–1305 764
30. Innes, L, Hobbs, PJ, Bardgett, RD (2004) The impacts of 765
individual plant species on rhizosphere microbial communities 766
in soils of different fertility. *Biol Fertil Soils* 40: 7–13 767
31. Johnson, D, Booth, RE, Whiteley, AS, Bailey, MJ, Read, DJ, Grime, 768
JP, Leake, JR (2003) Plant community composition affects the 769
biomass, activity and diversity of microorganisms in limestone 770
grassland soil. *Eur J Soil Biol* 54: 671–677 771
32. Johnson, MJ, Lee, KY, Scow, KM (2003) DNA fingerprinting 772
reveals links among agricultural crops, soil properties, and the 773
composition of soil microbial communities. *Geoderma* 114: 774
279–303 775
33. Jongman, RHG, ter Braak, CJF, van Tongeren, OFR (1995) *Data* 776
Analysis in Community and Landscape Ecology. Cambridge 777
University Press, Cambridge, pp 91–173 778
34. Jumpponen, A (2003) Soil fungal community assembly in a 779
primary successional glacier forefront ecosystem as inferred from 780
rDNA sequence analysis. *New Phytol* 158: 569–578 781
35. Kennedy, N, Brodie, E, Connolly, J, Clipson, N (2004) Impact of 782
lime, nitrogen and plant species on bacterial community structure 783
in grassland microcosms. *Environ Microbiol* 6: 1070–1080 784
36. Knudsen, D, Beegle, D (1988) Recommended phosphorus tests. 785

- 786 In: Dahnke WC (Ed.) Recommended Soil Tests Procedures for the
787 North Central Region: Bulletin Number 499 (revised). North
788 Dakota Agricultural Experiment Station. Fargo, ND, pp 12–15
- 789 37. Lane, DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E,
790 Goodfellow M (Eds.) Nucleic Acid Techniques in Bacterial
791 Systematics. John Wiley & Sons, Chichester, pp 115–175
- 792 38. Liu, W-T, Marsh, TL, Cheng, H, Forney, LJ (1997) Characteriza-
793 tion of microbial diversity by determining terminal restriction
794 ribotype length polymorphisms of genes encoding 16S rRNA. Appl
795 Environ Microbiol 63: 4516–4522
- 796 39. Lueders, T, Friedrich, MW (2003) Evaluation of PCR amplification
797 bias by terminal restriction ribotype length polymorphism analysis
798 of small-subunit rRNA and *mcrA* genes by using defined template
799 mixtures of methanogenic pure cultures and soil DNA extracts.
800 Appl Environ Microbiol 69: 320–326
- 801 40. Lynch, JM, Whipps, JM (1990) Substrate flow in the rhizosphere.
802 Plant Soil 129: 1–10
- 803 41. Marilley, L, Aragno, M (1999) Phylogenetic diversity of bacterial
804 communities differing in degree of proximity of *Lolium perenne*
805 and *Trifolium repens* roots. Appl Soil Ecol 13: 127–136
- 806 42. Marschner, P, Yang, CH, Lieberei, R, Crowley, DE (2001) Soil and
807 plant specific effects on bacterial community composition in the
808 rhizosphere. Soil Biol Biochem 33: 1437–1445
- 809 43. McCaig, AE, Glover, LA, Prosser, JI (2001) Numerical analysis of
810 grassland bacterial community structure under different land
811 management regimens by using 16S ribosomal DNA sequence
812 data and denaturing gradient gel electrophoresis banding patterns.
813 Appl Environ Microbiol 67: 4554–4559
- 814 44. McGonigle, TP, Fitter, AH (1990) Ecological specificity of vesicular
815 arbuscular mycorrhizal associations. Mycol Res 94: 120–122
- 816 45. Morgan MF (1941) Chemical soil diagnosis by the universal soil
817 testing system. In: Connecticut Agricultural Experimental Station
818 Bulletin Number 450
- 819 46. O’Sullivan, AM (1982) The lowland grasslands of Ireland.
820 In: White J (Ed.) Studies on Irish Vegetation. Royal Dublin
821 Society, Dublin
- 822 47. Polz, MF, Cavanaugh, CM (1998) Bias in template-to-product
823 ratios in multitemplate PCR. Appl Environ Microbiol 64:
824 3724–3730
- 825 48. Ritz, K, McNicol, JW, Nunan, N, Grayston, S, Millard, P,
826 Atkinson, D, Gollotte, A, Habeshaw, D, Boag, B, Clegg, CD,
827 Griffiths, BS, Wheatley, RE, Glover, LA, McCaig, AE, Prosser, JI
828 (2004) Spatial structure in soil chemical and microbiological
829 properties in an upland grassland. FEMS Microbiol Ecol 49:
830 191–205
- 831 49. Rodwell, JS (1992) British Plant Communities: Grasslands and
832 Montane Communities. Cambridge University Press, Cambridge
- 833 50. Rovira, AD (1969) Plant root exudates. Bot Rev 35: 35–37
- 834 51. Schutter, ME, Sandeno, JM, Dick, RP (2001) Seasonal, soil type,
and alternative management influences on microbial communities 835
of vegetable cropping systems. Biol Fertil Soils 34: 397–410 836
52. Sparling, GP, Tinker, PB (1978) Mycorrhizal infection in pennine 837
grassland. I. Levels of infection in the field. J Appl Ecol 15: 943– 838
950 839
53. Steer, J, Harris, JA (2000) Shifts in the microbial community in 840
rhizosphere and non-rhizosphere soils during the growth of 841
Agrostis stolonifera. Soil Biol Biochem 32: 869–878 842
54. Thalmann, A (1968) Zur methodik ber Bestimmung der dehydro- 843
genaseaktivität im Boden mittels triphenyltetrazoliumchlorid 844
(TTC). Landwirtsch Forsch 21: 249–258 845
55. Thomas, GW (1996) Soil pH and soil acidity. In: Sparks, DL, Page, 846
AL, Helmke, PA, Loeppert, RH, Soltanpour, PN, Tabatabai, MA, 847
Johnston, CT, Sumner, ME (Eds.) Methods of Soil Analysis: Part 848
3, Chemical Methods. Soil Science Society of America, Madison, 849
WI, pp 475–490 850
56. van Wintzingerode, F, Gobel, UB, Stackebrandt, E (1997) 851
Determination of microbial diversity in environmental samples: 852
pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21: 853
213–229 854
57. Vandenkoornhuysse, P, Ridgway, KP, Watson, IJ, Fitter, AH, 855
Young, JPW (2003) Co-existing grass species have distinctive 856
arbuscular mycorrhizal communities. Mol Ecol 12: 3085–3095 857
58. Wardle, DA (1992) A comparative assessment of factors which 858
influence microbial biomass carbon and nitrogen levels in soil. 859
Biol Rev 67: 321–358 860
59. Wardle, DA, Bonner, KI, Nicholson, KS (1997) Biodiversity and 861
plant litter: experimental evidence which does not support the 862
view that enhanced species richness improves ecosystem function. 863
Oikos 79: 247–258 864
60. Webster, G, Embley, TM, Prosser, JI (2002) Grassland manage- 865
ment regimens reduce small-scale heterogeneity and species di- 866
versity of β -proteobacterial ammonia oxidizer populations. Appl 867
Environ Microbiol 68: 20–30 868
61. Westover, KM, Kennedy, AC, Kelley, SE (1997) Patterns of 869
rhizosphere microbial community structure associated with co- 870
occurring plant species. J Ecol 85: 863–873 871
62. White, TJ, Bruns, T, Lee, S, Taylor, J (1990) Amplification and 872
direct sequencing of fungal ribosomal RNA genes for phyloge- 873
netics. In: Innis, MA, Gelfand, DH, Sninsky, JJ, White, TJ (Eds.) 874
PCR Protocols: A Guide to Methods and Applications. Academic 875
Press, New York, pp 315–322 876
63. Williams, BL, Grayston, SJ, Reid, EJ (2000) Influence of synthetic 877
sheep urine on the microbial biomass, activity and community 878
structure in two pastures in the Scottish uplands. Plant Soil 225: 879
175–185 880
64. Zhu, YG, Laidlaw, AS, Christie, P, Hammond, MER (2000) The 881
specificity of arbuscular mycorrhizal fungi in perennial ryegrass– 882
white clover pasture. Agric Ecosyst Environ 77: 211–218 883

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Q1. Figures 1 and 2 are pixelated. Please provide better quality image.

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