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Impact of lime, nitrogen and plant species on fungal community structure in grassland microcosms

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Summary

A microcosm-based approach was used to study impacts of plant and chemical factors on the fungal community structure of an upland acidic grassland soil. Seven plant species typical of both unimproved and fertilised grasslands were either left unamended or treated with lime, nitrogen, or lime plus nitrogen. Fungal community structure was assessed by a molecular approach, fungal ARISA, while fungal biomass was estimated by measuring soil ergosterol content. Addition of nitrogen (with or without lime) had the largest effect, decreasing soil pH, fungal biomass, and fungal ribotype number, but there was little corresponding change in fungal community structure. Although different plant species were associated with some changes in fungal biomass, this did not result in significant differences in fungal community structure between plant species. Addition of lime alone caused no changes in fungal biomass, ribotype number or community structure. Overall, fungal community structure appeared to be more significantly affected through interactions between plant species and chemical treatments, as opposed to being directly affected by changes in individual improvement factors. These results were in contrast to those found for the bacterial communities of the same soils (Kennedy *et al*., 2004), which changed substantially in response to chemical (lime and nitrogen) additions.

Introduction

Grasslands are a major landscape form and vegetation type in Ireland and the UK (Green, 1990), with species-rich, acidic grasslands dominated by *Agrostis capillaris* common in upland areas (Blackstock *et al*., 1999). These are frequently converted by liming and fertilisation to species-poor mesotrophic plant communities, dominated by *Lolium perenne* and *Trifolium repens* (Rodwell, 1992). This process, known as intensification or improvement, causes changes in soil microbial populations, with fertiliser and lime applications typically resulting in increased bacterial numbers and decreased fungal biomass (Lovell *et al*., 1995; Bardgett *et al*., 1999a; Bardgett *et al*., 1999b; Brodie *et al*., 2002, 2003). Bacterial community structure has also been shown to change in response to intensification (Brodie *et al*., 2002), however molecular fingerprinting of fungi has revealed little difference in fungal diversity after improvement (Brodie *et al*., 2003). Fungi account for the bulk of microbial biomass in upland acidic grasslands, and play major roles in nutrient cycling processes such as proteolysis, phosphorus mobilisation and decomposition either as free-living saprotrophs or as mycorrhizae (Wainwright, 1988; Read and Perez-Moreno, 2003).

There is no clear view at present of those factors which determine belowground microbial diversity. Several possible factors driving shifts in microbial community structure during intensification have been postulated, including changes in variables such as pH (Frostegård *et al*., 1993; Bååth, 1996; Blagodatskaya and Anderson, 1998; Bååth and Anderson, 2003; Steenwerth *et al*., 2003), nitrogen (Steer and Harris, 2000; Clegg *et al*., 2003;

Gray *et al*., 2003; Girvan *et al*., 2004), soil physico-chemical characteristics (physical properties, tilling, pH, carbon, nitrogen, phosphorus) (Groffman *et al*., 1996, Latour *et al*., 1996; Marschner *et al*., 2001; Schutter *et al*., 2001; Girvan *et al*., 2003) and plant community structure (Grayston *et al*., 1998; Miethling *et al*., 2000; Grayston *et al*., 2001; Marschner *et al*., 2001; Smalla *et al*., 2001). These studies largely relate to either bacterial or total microbial communities. In a companion paper to this study, we showed, based upon microcosm experiments, that the addition of lime, nitrogen, or lime plus nitrogen had a much greater impact on bacterial community structure in acidic upland grassland soils than the effect of grassland plant species (Kennedy *et al*., 2004).

Little is known concerning the drivers that determine soil fungal populations during environmental change. Lime has been shown to influence fungal community structure in upland grasslands (Bardgett *et al*., 1996), while nitrogen addition increased fungal biomass in a forest spruce soil (Smolander *et al*., 1994) and changed the composition of fungi isolated from pine forest and haymeadow soils (Arnebrant *et al*., 1990; Donninson *et al*., 2000b). The recent development of effective molecular profiling approaches, which fingerprint fungal community structure by exploiting the variable ITS genetic region, have allowed fuller characterisation of soil fungal populations (Ranjard *et al*., 2001; Lord *et al*., 2002; Anderson *et al*., 2003). In a study of fungal community structure over a vegetational gradient between an acidic upland and an improved mesotrophic grassland, Brodie *et al*. (2003) showed that fungal diversity did not appreciably change over the gradient, although fungal

biomass declined towards the plant species-poor mesotrophic grassland. However, as this was a field-based study, it was not possible to separate biotic and abiotic influences as causative factors. The objective of this paper was to isolate individual plant species and chemical treatment regimes in order to test the effects of abiotic and plant-related factors on fungal community structure using a monoculture microcosm approach.

Results

The pH of microcosm soil from each plant species was assessed at harvest (Table 1). ANOVA revealed that most data variation could be attributed to the addition of lime (p<0.001) and nitrogen (p<0.001), and their interaction (p<0.001). Responses to the chemical treatments were fairly consistent across all plant species, with lime addition (with or without nitrogen) resulting in an average increase of 0.6 pH units over untreated soil, while nitrogen addition alone acidified the soil by an average of 0.4 pH units compared to untreated soil. Plant species was also found to have a smaller but significant effect (p=0.002) on pH, which could be attributed to *A. capillaris* having a significant acidifying effect on the soil.

The ergosterol content of soil was used as an estimate of fungal biomass. ANOVA indicated most of the variation within the data could be attributed to plant species (p<0.001) and nitrogen (p=0.002). The plant species means listed in Table 1 revealed that soil from *F. rubra* had the lowest fungal biomass and soil from *A. capillaris* the highest. Along with soil from *F. rubra*, soils exhibiting low fungal biomass as compared to bare soil included those from

the mesotrophic species *L. perenne* and *T. repens*, while soils with higher biomass were from *N. stricta*, an upland acidic grassland species, and *H. lanatus*, a species found in grasslands of intermediate fertility. The effect of nitrogen could be seen in means for chemical treatments, with nitrogen addition resulting in a reduction in fungal biomass, while addition of lime plus nitrogen resulted in a larger reduction.

Fungal automated ribosomal intergenic spacer analysis (FARISA) was used to generate a ribotype profile for each sample, consisting of the fragments present and their relative abundances. A total of 782 fragments was detected after analysis of all soils, with each separate fragment indicating a unique ITS region length and counted as one fungal ribotype. ANOVA of fungal ribotype numbers (Table 1) revealed that the only parameter having a significant effect was nitrogen (p=0.004), with nitrogen addition (with or without lime) decreasing ribotype number. Plant species had no effect on fungal ribotype number.

Analysis of FARISA profiles by a randomization test (data not shown) indicated very little change in fungal community structure in response to chemical treatments or plant species. When comparisons between FARISA profiles of unamended and chemically-treated soils from each plant species were made, only one significant difference was found, which occurred in *F. ovina* after addition of nitrogen. There was no significant change in soil FARISA profiles for any other plant species in response to addition of lime, nitrogen, or lime plus nitrogen. There were also no significant differences

between the FARISA profiles of any plant species in unamended soils. A few isolated differences were found between different plant species within a chemical treatment. Addition of lime resulted in significant differences between the FARISA profiles of soil from *F. rubra* and soil from *N. stricta*, *L. perenne*, and *T. repens*. No significant differences were found between fungal profiles from plant species after addition of nitrogen or lime plus nitrogen.

Canonical correspondence analysis, a multivariate approach, was used to determine how environmental parameters influenced FARISA profiles, after initial analysis revealed they exhibited a unimodal response to the environmental variables. The results (Table 2 and Figure 1) revealed that a small (cumulative sum of 4.6% for Axes 1 and 2) but significant (p<0.005) percentage of the overall variation in FARISA profiles could be explained by relating environmental factors to fragment data. The cumulative species-environment relation for both axes was 39.7%, indicating these axes accounted for a sizeable percentage of variation in FARISA profiles that could be attributed to environmental factors. Species-environment correlations for both axes were above 0.62, indicating a strong correlation between FARISA profiles and environmental parameters. A plot of canonical correspondence is shown in Figure 1, with environmental factors (plant species, lime, nitrogen, and the interaction between lime and nitrogen) depicted as arrows, while the top twenty (ranked by abundance) FARISA fragments are shown as dots. The length of environmental factor arrows indicates their relative importance in terms of accounting for variation in FARISA profiles. Most environmental parameters were roughly equal to one another, indicating that no one

parameter or set of parameters had a greater impact on fungal community structure than another; however, intra-set correlations (Table 2) indicated that *F. ovina* and nitrogen were most strongly correlated with Axes 1 and 2, respectively, while *N. stricta*, lime and the lime-nitrogen interaction also had high correlations. Interestingly, thirteen of the top twenty FARISA fragments appeared to have some correlation with *N. stricta*. However, these relationships were not strongly supported by ANOVA analysis of the top twenty fragments (Table 3), which revealed significant effects were mostly in the form of interactions, rather than from the environmental parameters (plant species, lime and nitrogen) alone. In addition, the significances found were all at lower significance levels ($p<0.05$ or 0.01); none of the environmental parameters analysed was significant at $p<0.001$. This provides evidence to support the trend seen in randomization test analysis, of plant species, lime and nitrogen not causing substantial change in fungal community structure.

Discussion

Although there were changes in fungal biomass and ribotype number in response to changes in lime, nitrogen, and plant species, fungal community structure did not change substantially, especially when compared to the large changes seen in bacterial community structure in response to these improvement factors (Kennedy *et al*., 2004). Although the addition of lime increased pH, measurements of fungal community structure were relatively unaffected by liming, with little change occurring in fungal biomass, ribotype number, or fungal community structure. Reductions in pH, fungal biomass, and fungal ribotype number were noted after addition of nitrogen. However,

analysis of fungal community profiles indicated that overall there was little change in fungal community structure in response to nitrogen addition. Addition of lime plus nitrogen raised pH to the same level as lime alone, but resulted in reductions in soil fungal biomass and fungal ribotype number as compared to unamended soil. These reductions were larger than those caused by either lime or nitrogen alone, indicating an interaction between these two chemical factors.

The soil fungal community appeared to show little response to different plant species, although there were some significant differences between fungal biomass levels in soil from different grass species. Fungal biomass was highest in *A. capillaris*, which may indicate extensive colonisation of this plant by arbuscular mycorrhizal fungi (Vandenkoornhuyse *et al*., 2002, 2003). However, this difference in fungal biomass was not reflected in molecular analyses of fungal community structure, and plant species had no significant effect on fungal ribotype number.

Although measurements of fungal biomass and ribotype number provide valuable information on fungal presence in soils, they must be interpreted with caution. Community fingerprinting methods such as ARISA produce highly complex data sets. Although ribotype number (calculated as the average number of ribotypes found in several replicates) can be a useful tool, it may be an overly simplistic representation of community structure, and not sensitive to actual changes in populations (Griffiths *et al*., 2004). Profiles based on the presence and relative abundance of ribotypes provide a more comprehensive

view of fungal community structure than simpler measures such as ribotype number. Additionally, DNA-based community fingerprinting approaches can be difficult to interpret because of species differences in rRNA gene copy number (Anderson *et al*., 2004), biases resulting from PCR amplification (von Wintzingerode *et al*., 1997; Polz and Cavanaugh, 1998; Jumpponen, 2003), and the difficulty in standardizing the amount of DNA analysed in each replicate (Crosby and Criddle, 2003). However, as all samples were subject to the same biases, it was still possible to compare between them on a relative basis, especially after standardisation of fragment peak heights into proportions per sample.

Our study analysed FARISA ribotype profiles using a randomization test procedure, which reveals statistically significant differences between individual treatments. Few significant changes in response to changes in plant species or addition of lime and/or nitrogen to soil were found, indicating little change in fungal community structure in response to these amendments. FARISA data were further explored by canonical correspondence analysis, which indicated that most factors (plant species, lime, and nitrogen) explained roughly similar amounts of variation in fungal profiles. These trends were supported by ANOVA results, which indicated that abundances of most fungal fragments were only significantly affected through interactions between plant species and chemical treatments, as opposed to being directly affected by changes in individual factors.

Overall, the improvement factor having the largest impact on the fungal community was nitrogen, with addition of nitrogen (with or without lime) appearing to decrease fungal biomass and fungal ribotype number, without causing large changes in fungal community structure. Nitrogen addition (as ammonium nitrate) influences microbially mediated nitrogen cycling processes such as nitrification and nitrogen fixation, with higher nitrogen concentrations promoting bacterial decomposition and lower concentrations promoting decomposition by fungi (Bardgett *et al*., 1999b). Decreases in fungi after fertilisation have also been noted in other studies (Bardgett *et al*., 1999a; Lowell and Klein, 2001; Brodie *et al*., 2003), and may be due to an inhibitory effect of nitrogen on fungal groups such as decomposers (Fog, 1988). Reductions in soil fungal biomass after addition of lime plus nitrogen have also been noted in field experiments, with intensification (increasing inputs of lime and nitrogen) decreasing fungal biomass in hay meadows (Donninson *et al*., 2000a) and upland acidic grassland soil (Brodie *et al*. 2003), and reducing numbers of most vesicular-arbuscular mycorrhizal fungal species (Sylvia and Neal, 1990; Johnson, 1993). Several studies have found unfertilised soils to have higher fungal:bacterial ratios than fertilised soils (Bardgett *et al*., 1996, 1999a; Bardgett and McAlister, 1999; Donninson *et al*., 2000a; Grayston *et al*., 2001, 2004), which corresponds to the reduction in fungal biomass found in our study after addition of lime and nitrogen. In our study, there were very few significant changes in fungal community structure in response to nitrogen addition, despite reductions in fungal biomass and ribotype number. This may indicate nitrogen caused a decrease in the biomass of the majority of fungi,

without affecting any group in particular, so that the relative abundances of fungal fragments did not change.

Lack of evidence for a plant species rhizosphere effect on soil fungal community structure was perhaps surprising, considering that several studies have found specific associations of arbuscular mycorrhizal fungi with the grass species used in our study (McGonigle and Fitter, 1990; Zhu *et al*., 2000; Genney *et al*., 2001; Vandenkoornhuyse *et al*., 2002, 2003; Johnson *et al*., 2003; Gollotte *et al*., 2004). However, a study by Innes *et al*. (2004) found that the effect of plant species on soil fungal PLFAs differed according to soil fertility, providing evidence that interactions between soil chemical status and plant species (and their resulting effect on fungal communities) are complex and difficult to predict.

The lack of change in soil fungal community structure in response to chemical amendments was in stark contrast to results obtained for bacterial community structure in these microcosm soils, which indicated that addition of lime and nitrogen had a large impact on bacterial TRFLP profiles, while plant species had little effect (Kennedy *et al*., 2004). The fungal community appeared to be more resistant to change in response to chemical addition than the bacterial community, although there was evidence that interactions between chemical additions and plant species indirectly impacted fungal community structure. These trends are consistent with those observed in other studies. Addition of lime to grassland soils was found to change bacterial, but not fungal, PLFA profiles (Treonis *et al*., 2004), while the structure of active fungal communities

changed less in response to addition of nitrogen to wheat field soils than did the structure of active bacterial communities (Girvan *et al*., 2004).

Caution must be used when extrapolating controlled laboratory experiments to field conditions, as important soil microbial community parameters such as biomass have been shown to differ under field and glasshouse conditions (Ibekwe and Kennedy, 1998). Confidence in microcosm results can be increased by comparison with field studies of the same system (Carpenter, 1996). Our study found fungal community structure more resistant to change in response to improvement factors than bacterial community structure, a trend that has also been noted in field studies of the same soil (Brodie *et al*., 2002, 2003). It must be noted that DNA from dead fungal hyphae may persist for a longer time than that from bacterial cells, meaning changes in fungal community structure may take longer to detect than those in bacteria, and may not have been detectable in the 75-day time span of our experiment.

The results reported in this study represent the first data combining a microcosm-based approach with molecular community fingerprinting of soil fungal communities. DNA-based approaches are able to give a fuller breakdown of populations present than other commonly used techniques such as CLPP and PFLA (Hill *et al*., 2000), and support the hypothesis that fungal communities are more resistant to change in response to chemical soil improvements than are bacterial communities. Fungal community structure was seldom significantly affected by individual changes in plant species, lime,

and nitrogen, but instead appeared to shift slowly in response to complex interactions between these improvement factors.

Experimental procedures

Soil

Soil was collected in June 2000 from an area of Nardo-Galion grassland at Longhill, Kilmacanogue, County Wicklow, Ireland (National Grid Reference O 218 124), as described in Kennedy *et al.* (2004).

Microcosms

Microcosms were prepared as described in Kennedy *et al.* (2004). Briefly, 25 days after seed sowing in a glasshouse (single species microcosms of *Nardus stricta*, *Agrostis capillaris*, *Festuca ovina*, *Festuca rubra*, *Holcus lanatus*, *Lolium perenne*, or *Trifolium repens*, together with an unplanted control), both planted and unplanted soils were treated as follows: Treatment 1 – no addition; Treatment 2 – addition of lime equivalent to 5 tons ha^{-1}; Treatment 3 – addition of nitrogen (as ammonium nitrate) equivalent to 150 kg ha^{-1}; Treatment 4 – addition of both lime and nitrogen as in Treatments 2 and 3. Water content was maintained at 30% w/v by addition of distilled water as necessary. Microcosms were destructively sampled on Day 75. Microcosm soil pH was analysed according to the method of Thomas (1996).

Fungal Biomass

Total soil ergosterol was quantified as described previously (Brodie *et al*., 2003). Briefly, 5 g soil was vortexed and sonicated with methanol and potassium hydroxide solution at 0ºC. Subsequently, after incubation at 85ºC for 30 minutes, followed by cooling at 4ºC for 20 minutes, HPLC grade pentane was used to extract ergosterol from the soil mixture. Three pentane extracts were combined and dried under N_2 gas before being redissolved in methanol and filtered through 0.2 µm Teflon filters, then analysed on a Waters Sugar-Analyser I HPLC system. Incorporation of an internal ergosterol standard allowed the extraction efficiency of ergosterol from soil in these experiments to be estimated at 87 ± 4.7%.

Total Soil DNA Extraction and Purification

Total soil DNA was extracted as described in Brodie *et al*. (2002). Briefly, soil (0.5 g) was added to tubes containing glass and zirconia beads, to which CTAB (hexadecyltrimethylammonium bromide) extraction buffer was added. After incubation at 70°C for 10 min, phenol:chloroform:isoamylalcohol (25:24:1) was added and tubes were then shaken in a Hybaid Ribolyser at 5.5 m/s for 30 s. Following bead-beating, tubes were centrifuged and the aqueous layer was removed and extracted twice with chloroform:isoamlyalcohol (24:1). A further purification procedure was performed involving incubation with lysozyme solution (100 mg/ml) for 30 min at 37°C. Tubes were again centrifuged and the aqueous layer removed and further purified using a High Pure PCR Product Clean Up Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to manufacturer's instructions. DNA was eluted in a final

volume of 50 µl and was consistently suitable for PCR amplification without further treatment.

Fungal Community Fingerprinting by FARISA (Fungal Automated Ribosomal Intergenic Spacer Analysis)

The fungal intergenic spacer region containing the two internal transcribed spacers (ITS) and the 5.8S rRNA gene (ITS1-5.8S-ITS2) was amplified using primer set ITS1-F (CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al*., 1990). Amplified sequences contained the two ITS regions and the 5.8S gene plus 22 bp from the forward primer and an unknown section of the 28S rRNA gene. The forward primer ITS1-F was labelled with Beckman Coulter fluorescent dye D4. PCR reactions were performed in 50 µl volumes of 25 µl master mix (containing 50 units/ml *Taq* DNA polymerase in a proprietary reaction buffer (pH 8.5), 400 µM each dNTP, and 3 mM $MgCl_2$) (Promega, WI, USA), 15 pmol each primer, 25 µg BSA, and ~10 ng extracted total soil DNA. Thermocycling conditions were as follows: a hot start at 95ºC for 4 min (1 cycle); 95ºC for 1 min, 53ºC for 30 s, 72ºC for 1 min (35 cycles); 72ºC for 7 min (1 cycle). PCR products were first visualised on a 1.2% agarose gel, then purified using a High Pure PCR product purification kit (Roche Diagnostics GmbH, Penzberg, Germany) and eluted in 50 µl of sterile water heated to 50ºC. The purified PCR products were quantified on a 1.2% agarose gel before mixing aliquots (0.5 – 1 µl) with 38.4 µl of deionised formamide, 0.2 µl of Beckman Coulter size standard 600 (dye D1), and 0.4 µl of custom-made marker containing 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840, 860, 880,

900, 920, 940, 960, 980, 1000, 1050, 1100, 1150 and 1200 bp DNA fragments, all labelled with Beckman Coulter Dye D1 (BioVentures, Murfreesboro, TN, USA).

Intergenic spacer lengths were determined by electrophoresis using a Beckman Coulter (CEQ8000) automated sequencer. Run conditions were 60ºC temperature, 4 kV, and 120 min separation time to allow for separation of the larger fragments. Analysis of spacer profiles was performed using the Beckman Coulter fragment analysis package 8000. A quartic polynomial model rather than the recommended cubic model was used for size standard calibration as this resulted in improved correlation between expected and actual size standard fragment sizes, particularly for fragments in the range 400–1200 bp. Fragments that differed by less than 0.5 bp in different profiles were considered identical.

Statistical Analysis

Results for pH, fungal biomass, ribotype number and individual abundances of the top 20 overall most abundant FARISA fragments were analysed by one-way factorial analysis of variance (ANOVA) using Genstat v6. The significance level was set at $p<0.05$. Means for chemical treatments and plant species were compared using Bonferroni-corrected *t*-tests. To determine if fungal communities were affected by plant species and chemical treatments, peak heights for each fragment were first converted into proportions of the total peak height of all fragments for each replicate. Plant species and treatments were then tested in pairwise comparisons using a variation of the city-block

(Manhattan) randomization test procedure (Kennedy *et al*., 2004), using a critical level of $p<0.05$ to test the null hypothesis that there were no significant changes in fungal community structure in response to plant species or chemical amendments. In addition, Genstat was used to calculate the overall abundance of each individual fragment for all samples. Fragments were then ranked according to overall abundance and these rankings were used to select individual fragments for ANOVA. Fungal ARISA profiles were explored using canonical correspondence analysis (CCA) (Canoco for Windows, v4.02) after initial analysis by detrended correspondence analysis (DCA) revealed that the data exhibited a unimodal, rather than linear, response to the environmental variables (plant species, lime addition, and nitrogen addition). The resulting ordination biplot approximated the weighted average of each species (in this case, relative abundances of ribotypes) with respect to each of the environmental variables, which were represented as arrows. The length of these arrows indicated the relative importance of that environmental factor in explaining variation in fungal profiles, while the angle between arrows indicated the degree to which they were correlated (Jongman *et al*., 1995). A Monte Carlo permutation test based on 199 random permutations was used to test the null hypothesis that fungal profiles were unrelated to environmental variables.

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Figure and Table legends

Table 1. pH, fungal biomass, and fungal ribotype number measurements as affected by chemical treatments and plant species. Means and standard error of differences (SED) are shown (*n*=32 for chemical treatments, *n*=16 for plant species). Same letter denotes no significant difference between values in each category (chemical treatments or plant species). ANOVA *p*-values for plant species shown as **, $p<0.01$; ***, $p<0.001$; NS, not significant. ANOVA results for chemical treatments and interactions are discussed in the text.

Table 2. Results of canonical correspondence analysis (CCA) of fungal community structure as determined from fungal ARISA data. Values are for Axes 1 and 2 plotted in the CCA diagram in Figure 1. The highest intra-set correlations are highlighted in bold.

Table 3. ANOVA results for the top twenty most abundant fungal ARISA fragments, as ranked by average abundance over all samples.

Figure 1. Canonical correspondence analysis (CCA) ordination diagram of fungal ARISA data, with chemical variables and plant species represented as arrows and FARISA fragments represented as dots. FARISA fragments are labelled according to fragment size.

	pH	Fungal Biomass	Fungal Ribotype Number
		(μg ergosterol g^{-1} dry soil)	(mean fragments per replicate)
CHEMICAL TREATMENTS			
Plant Only	4.23b	1.81b	31.3ab
+ Lime	4.84c	1.79b	37.9b
+ Nitrogen	3.80a	1.73ab	27.0ab
+ Lime + N	4.83c	1.59a	21.6a
SED	0.069	0.059	4.92
PLANT SPECIES			
N. stricta	4.52b	1.84bcd	42.8
A. capillaris	4.13a	2.01d	28.2
F. ovina	4.40b	1.71abc	31.6
F. rubra	4.36b	1.43a	24.7
H. lanatus	4.51b	1.91cd	21.8
L. perenne	4.48b	1.58ab	27.6
T. repens	4.51b	1.63ab	25.1
Bare soil	4.50b	n/a	33.7
p-value	**	***	NS
SED	0.098	0.078	6.95

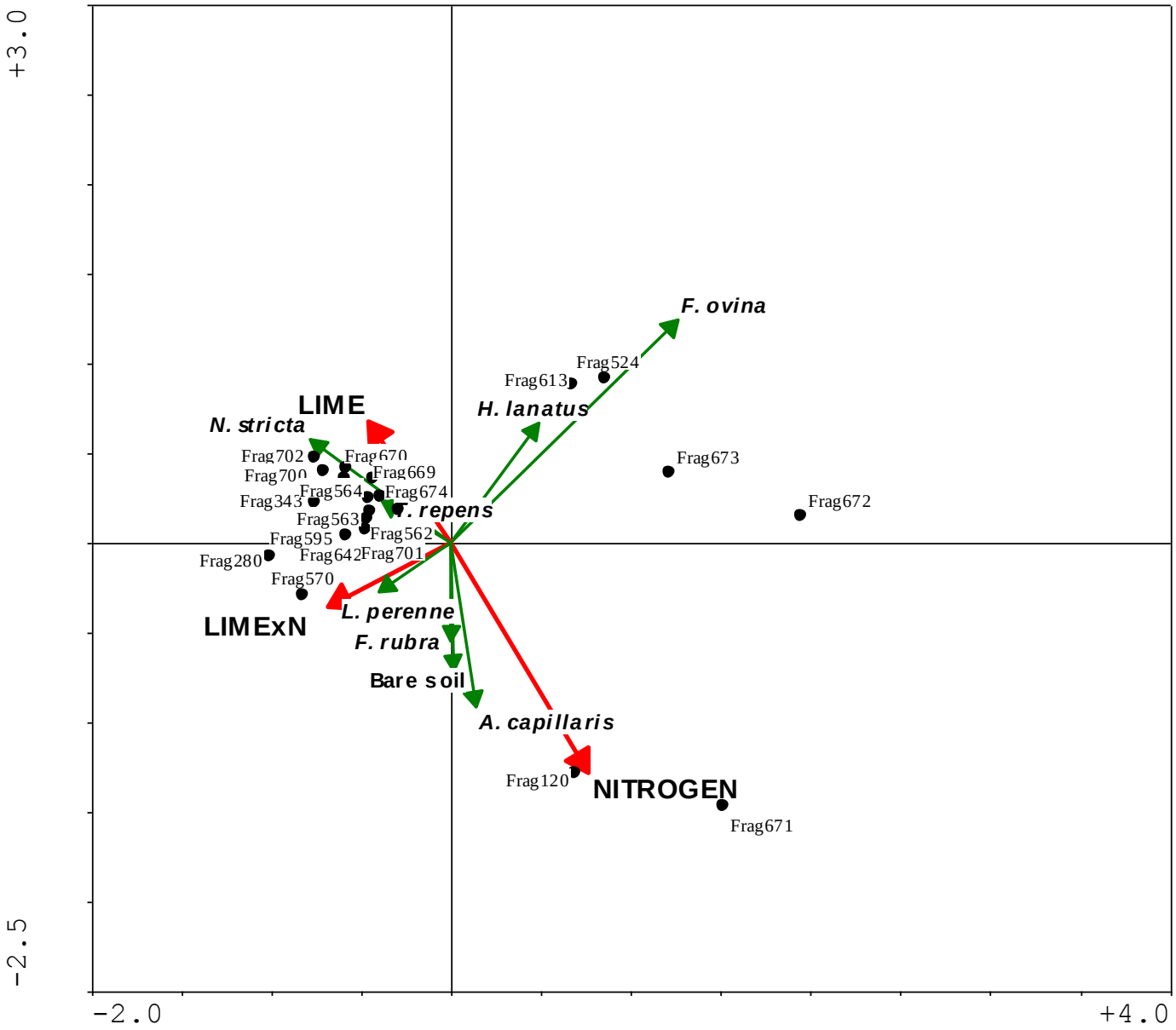
Axis	1	2
Eigenvalue	0.471	0.253
Cumulative percentage variance:		
Of species data	3.0	4.6
Of species-environment relation	25.9	39.7
Species-environment correlations	0.779	0.619
Monte-Carlo significance test:		*For all axes:*
F-ratio	3.545	1.504
p-value	0.005	0.005
Intra-set Correlations (100 x *r*):		
N. stricta	-25	15
A. capillaris	5	-24
F. ovina	**41**	33
F. rubra	-1	-15
H. lanatus	16	17
L. perenne	-13	-7
T. repens	-12	6
Bare soil	1	-19
Lime	-15	18
Nitrogen	25	**-34**
Lime*Nitrogen	-22	-9

	Abundance			*p*-values		
FARISA Fragment	Abundance Rank	% Abundance	Cumulative % Abundance	Plant Species	Lime	Nitrogen
(bp)						
670	1	4.1	4.1	0.054	**0.037**$^{-}$	0.249
564	2	3.8	7.9	0.280*	0.842*	0.302*
671	3	3.7	11.7	0.666	0.610*	0.504*
563	4	3.3	15.0	0.226	0.877*	0.453*
701	5	2.3	17.3	0.243	0.945*	0.502*
669	6	2.2	19.4	0.056	0.703	0.549
700	7	2.1	21.6	0.128*	0.726*	0.113*
672	8	1.7	23.3	0.067	0.661*	0.869*
562	9	1.5	24.8	0.330	0.770	0.397
702	10	1.3	26.1	0.074	0.965*	0.812*
524	11	1.3	27.4	0.091	**0.041**$^{-}$*	**0.004**$^{-}$*****
595	12	1.2	28.7	0.166	**0.042**$^{-}$	**0.034**$^{-}$
596	13	1.0	29.7	0.373	0.274	0.170
565	14	0.9	30.5	0.163*	0.197*	0.094*
611	15	0.9	31.4	0.482	0.567*	0.593*
674	16	0.8	32.2	0.504	0.529	0.433
673	17	0.9	33.0	0.126	**0.008**$^{-}$	0.657
636	18	0.9	33.7	0.331	0.574	0.749
124	19	0.7	34.4	0.308	0.398	0.689
560	20	0.6	35.0	0.271	0.820	0.862

Bold indicates a significant effect (p<0.05)

$^{-}$ Negative effect on fragment abundance

*Significant interaction with each other


+3.0
-2.5
-2.0
+4.0
F. ovina
Frag524
Frag613
LIME
H. lanatus
N. stricta
Frag673
Frag702
Frag670
Frag700
Frag669
Frag672
Frag343
Frag564
Frag674
T. repens
Frag563
Frag562
Frag595
Frag280
Frag642
Frag701
Frag570
L. perenne
LIMExN
F. rubra
Bare soil
A. capillaris
Frag120
NITROGEN
Frag671