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Synthetic sheep urine alters fungal community structure in an upland grassland soil

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ABSTRACT

Agricultural improvement (fertilisation, liming, intensification of grazing) of acidic upland pastures results in loss of indigenous flora and notable changes in microbial community structure. Such practices have recently raised concerns regarding the possible impacts on natural ecosystem biodiversity and functioning. The effects of synthetic sheep urine (SSU) and plant species on fungal community structure in upland grassland microcosms were investigated. Plant species typical of agriculturally unimproved (*Agrostis capillaris*) and improved (*Lolium perenne*) pastures were treated with low, medium or high concentrations of SSU, with harvests carried out 10 d and 50 d after SSU application. Root biomass was negatively affected by SSU addition whereas shoot biomass did not display any significant change. Fungal richness (number of operational taxonomic units) was negatively correlated with SSU concentration ($p < 0.001$), and also with time ($p < 0.001$).

Multi-dimensional scaling plots revealed significant changes in fungal community composition, depending on concentration of SSU and plant species type, while canonical correspondence analysis also emphasised the importance of interacting environmental variables. In addition, SIMPER analyses supported the finding that shifts in fungal community composition under different SSU and plant treatments had occurred. Overall, while SSU appeared to be influential in determining fungal community structure, community changes were largely driven by interacting environmental factors. This study contributes to our understanding of the potential implications of intensified farming, in particular increased pressure from grazing animals, on fungal community structure in semi-natural grassland systems.

Introduction

In Ireland and the UK, the predominant floristic formation on acidic upland grassland soils is a species-rich but low-yielding plant community, dominated by *Agrostis capillaris*, with several other grass and broadleaved species at lower abundances (Rodwell 1992). Often, these semi-natural grasslands are considerably improved by fertilisation, grazing and/or reseeding (Blackstock *et al.*, 1999), which typically causes a shift to more productive pastures dominated by *Lolium perenne*. This results in diminished botanical diversity, which may impact on belowground biodiversity (Green 1990; Grime 1998) and have associated effects on a range of microbial-driven biogeochemical and decompositional processes (Stoate *et al.* 2001). While loss of floristic diversity is a notable consequence of improvement of upland pastures, many other factors are involved in intensification, including increased pressure on land by grazing animals. In grazed pastures, factors that have

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been proposed as drivers of soil microbial community change include soil pH and nutrient availability (Clegg *et al.* 2003; Kennedy *et al.* 2004, 2005), floristic composition (Grayston *et al.* 1998, 2001, 2004), tillage-induced changes in soil physical structure (Ibekwe *et al.* 2002), and the effects of grazing (Bardgett *et al.* 1997; Rossignol *et al.* 2006), although it has been difficult to ascribe any single factor as predominating. Such pastures rarely receive fertiliser, and nitrogen supply is frequently reliant upon inputs *via* animal returns (Haynes & Williams 1993; Williams *et al.* 1999).

In recent years, several studies have focused on the effects of sheep urine deposition on belowground soil processes and microbial community structure, particularly focusing on bacterial communities (Mahmood & Prosser 2006; Nunan *et al.* 2006; Ritz *et al.* 2004; Rooney *et al.* 2006). In upland grasslands, fungi are known to regulate many central ecosystem processes including nutrient acquisition and cycling, mineralisation of organic matter and decomposition, while mycorrhizal fungi are symbiotically associated with most grassland species (Smith & Read 1997), but effects of sheep urine deposition on fungal community structure have not yet been studied.

Urine deposition in grazed pastures is spatially variable and results in increased heterogeneity in soil chemistry, particularly due to increased soil pH directly below urine patches and the sudden influx of N to the soil (Williams *et al.* 1999, 2000). Urine returns by grazing animals can result in addition of large quantities of urine-N (400–1 200 kg N ha^{-1}) to soils (Jarvis & Pain 1990; Fraser *et al.* 1994), contributing to the high level of spatial variation characteristic in the microbiology of grazed upland pastures (Ritz *et al.* 2004). The main nitrogenous component of urine is urea, which is rapidly transformed by hydrolyzation to NH_4^+ (Thomas 1988), the fate of which is largely determined by microbial processes such as nitrification and mineralisation (Williams *et al.* 1999, 2003). In a field experiment examining the effects of urine deposition in Scottish grasslands, Williams *et al.* (1999, 2000, 2003) showed that urine deposition substantially altered soil microbial communities in terms of bacterial and fungal counts, basal respiration rates and community level physiological profiles, although these studies were not consolidated by molecular community studies. More recently, sheep urine deposition has been found to be a major determinant of bacterial community structure in grazed systems (Nunan *et al.* 2006; Mahmood & Prosser 2006; Rooney *et al.* 2006), based largely on molecular microbial fingerprinting. The work reported herein presents the first study that demonstrates the effects of sheep urine application on fungal community structure, testing the hypothesis that urine deposition influences fungal community structural change in upland grassland soils and that this process may be influenced by the grass species present.

Materials and methods

Soil

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

Microcosms

Microcosms were prepared by weighing 80 g (oven dry mass) soil into black PVC pots (40 mm diameter, 110 mm height), which had been pierced to allow free drainage of water. Pots were planted with 20–25 surface sterilized (2 % sodium hypochlorite for 5 min) seeds (Emorsgate Seeds, Kings Lynn, UK) of either *A. capillaris* or *L. perenne*. A set of control pots was left unplanted (bare soil). Forty-eight pots of each of the two plant species and bare soil were prepared, and microcosms were incubated in a greenhouse in a randomized block design for 75 d from seed germination, from August 4 to October 17, 2004. Water content (water potential data not available) was maintained at 35 % (of overall pot weight) by addition of distilled water as necessary. Plants remained untrimmed throughout the experiment, and exhibited healthy growth throughout the initial 25 d of the experiment, prior to treatment. A synthetic sheep's urine solution was prepared according to Clough *et al.* (1996) and applied at three concentrations, equivalent to 200, 500 and 800 kg N ha^{-1}, representing low, medium and high concentrations, respectively. Urea was mixed with the inorganic salts solution immediately before application. After 25 d, both planted and unplanted pots were treated as follows: (1) no addition of SSU (zero SSU); (2) addition of SSU equivalent to 200 kg N ha^{-1} (low SSU); (3) addition of SSU equivalent to 500 kg N ha^{-1} (medium SSU); (4) addition of SSU equivalent to 800 kg N ha^{-1} (high SSU). Microcosms were destructively sampled after 35 d (10 d after SSU treatment); and after 75 d (50 d after SSU treatment). All soil within microcosm pots was separated from plant matter and was considered rhizosphere soil. Soil was sieved to $<$4 mm and stored at -20 °C for molecular analysis. Plant root and shoots were separated, weighed and dried at 70 °C for 5 d.

Total soil DNA extraction and purification

Total soil DNA was extracted as described by Brodie *et al.* (2002). Briefly, 0.5 g of soil was added to tubes containing glass and zirconia beads, to which CTAB (hexadecyltrimethylammonium bromide) extraction buffer was added. DNA was eluted in a final volume of 50 μl and was suitable for PCR amplification.

Fungal community fingerprinting using ARISA analysis

The primer set ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS-4 (TCCTCCGCTTATTGATATGC) was used to amplify the fungal intergenic spacer region (ITS) (Gardes & Bruns 1993; White *et al.* 1990). The fungal ITS region contains the two internal transcribed spacers and the 5.8S rRNA gene (ITS1-5.8S-ITS2), and amplified sequences contained this region plus 22 bp from the forward primer and an unknown section of the 28S rRNA gene. The forward primer was labelled with Beckman Coulter fluorescent dye D4 (Proligo). PCR reactions were carried out according to Kennedy *et al.* (2004). Thermocycling conditions were as follows: a hot start at 94 °C for 2 min (1 cycle), after which 2.5 U Taq DNA polymerase were added;