CHAPTER 15

Saprotrophic Basidiomycetes in Grasslands: Distribution and Function

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Contents

Abstract

Natural and semi-natural grasslands dominate many terrestrial ecosystems, with succession prevented by herbivore grazing, low rainfall and fire. Inputs to grassland soils are typically low in lignin, often comminuted and in the form of dung with below-ground inputs from roots being important. The several hundred basidiomycete species which are preferentially found in grassland can be placed into four functional groupings: litter decomposers, dung fungi, terricolous species and root endophytes. However, detection of these in the absence of basidiocarps has hampered their study, an exception being the fairy ring-forming species. It is clear that basidiomycetes contribute to lignocellulose degradation in grassland soil and litter, though it is likely that ascomycetes play a relatively greater part in this process than in woodland systems. Changes in agricultural management have led to the loss of many semi-natural grasslands in Europe and there are concerns about losses of several grassland taxa, such as *Hygrocybe* and *Entoloma* spp.

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1. INTRODUCTION

Fungi are key agents of nutrient cycling and thus of central importance to any understanding of carbon sequestration and nutrient cycling processes in all terrestrial ecosystems. However, mycologists have historically tended to have a sylvan bias and most fungal ecologists (as evidenced by several chapters in this book) have focused on woodland systems, resulting in wide knowledge of wood-decay fungi (Rayner and Boddy, 1988) and ectomycorrhizal taxa (Smith and Read, 1997). Similarly most fungal forays are held in woodland habitats where a diverse array of resources and host plants contribute to much higher levels of fungal diversity than are found in other habitats such as grasslands.

Of the 3,600 macrofungi found in the Netherlands, 80% are prevalent in woodlands (Arnolds and de Vries, 1989). Of the 20% of taxa generally found in non-wooded habitats, 10% (ca. 360 species) showed a preference for grasslands. Grassland basidiomycetes have received greater attention in recent decades, initially in Scandinavia (Rald, 1985; Arnolds, 1992a), and more recently in the UK (Rotheroe *et al*., 1996) and other parts of Europe (Adamcik and Kautmanova, 2005). This increase in attention was spurred by the precipitous loss of semi-natural grassland habitats ('traditional' lowland haymeadows) due to modern mechanized agriculture, mainly through ploughing of permanent pastures and application of synthetic fertilizers. Thus, the study of the ecology of grassland basidiomycetes has largely been driven by conservation concerns (Chapter 17), although it is clear that elucidation of the role played by basidiomycetes and other fungi in grassland nutrient cycling is important for understanding the dynamics of carbon sequestration in the context of global climate change.

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2. WHAT IS GRASSLAND?

The most intensively studied grasslands are those of Northern Europe and North America, and this review will focus mainly on these habitats. However, it is apposite to provide an overview of the global diversity of grassland systems and how they differ from the other main habitat types. Globally, grassland habitats cover ca. 20% of the terrestrial land area (Swift *et al*., 1979; Parton *et al*., 1995), occurring mainly where low or seasonal rainfall (250–1,500 mm $year^{-1}$) has prevented the establishment of woodland, due to the actions of grazing mammals, drought and fire (Ford *et al*., 2004). Distinctive grassland ecosystems occur at a range of latitudes, for example, in the tropics (e.g. East African savanna, *Los Llanos* in Colombia/Venezuela) and in temperate climes (steppes, prairies and pampas). At higher altitudes, montane grasslands occur (e.g. in Andean Páramo and alpine meadows), often merging into tundra and heathland. It is very likely that human activity, through livestock farming, fire-setting and logging for fuel, has extended these grassland areas at the expense of woodlands. Such anthropogenic plagioclimax grasslands have in the past two centuries increased in distribution, due to the migration of Europeans and their agricultural practices, for instance, in New Zealand and North America.

The semi-natural grasslands which dominate many parts of Northern Europe (e.g. covering >50% of the UK land area (DEFRA, 2005)) are generally believed to be the result of millennia of anthropogenic deforestation. Palynological evidence indicates that most of Northern Europe was under continuous forest cover until ca. 4,000 BP and that there has been progressive deforestation. However, it has been suggested (Vera, 2000; Bakker *et al*., 2004) that in pre-human (quaternary) times, Northern Europe comprised significant areas of grassland (large 'forest glades'), with larger grazing mammals playing a key role in the maintenance of habitat heterogeneity. These ideas remain controversial (Kirby, 2003; Mitchell, 2005), not least because very little grass pollen is detected in cores dating from quaternary times, with the main area of disagreement relating to the extent of these pre-historic grasslands (wood pasture with grassy glades, e.g. the New Forest in England or much larger open areas). Long-held views of the distinction between grasslands and woodlands may require reappraisal, with cycling of plant cover over century timescales (grassland → scrub → woodland → parkland → grassland) being a potential successional scenario. From a soil perspective, such thinking is intriguing since it raises the possibility that grasslands and woodlands are less different than is usually perceived. Mycologically this is not a huge surprise since several macrofungal taxa, which are predominantly found in grasslands in Europe (e.g. *Hygrocybe* spp.), are typically associated with woodland habitats in most other parts of the world (Cantrell and Lodge, 2000; Griffith *et al*., 2004).

For understanding the ecology of decomposer basidiomycetes in grassland systems, it is important to consider how grasslands differ from other ecosystems, notably woodland. First, soil respiration in grasslands (by decomposers and plant roots in approximately equal measure) tends to be ~20% higher than in comparable woodlands (Raich and Tufekcioglu, 2000), mainly because the temperature of grassland soils fluctuates more widely and is higher in summer, due to greater insolation (Morecroft *et al*., 1998). In Kansas woodland summer soil temperatures are 5 °C lower than in adjacent grassland, with soil carbon flux 38% lower as a consequence (Smith and Johnson, 2004). Seasonal droughts cause fluctuations in soil moisture and root penetration to depths of several metres (Baker *et al*., 2007). Following death *in situ* this leads to significant accumulation of SOM at depth (Reijs *et al*., 2003).

The main theatre of fungal activity in grasslands is at or beneath the soil surface. Low and fluctuating moisture can limit microbial processes, with surface litter often being particularly inhospitable. For basidiomycetes, dry periods (especially in prairie-type grasslands) constrain fruiting, potentially disguising the existence/abundance of macrofungi in these habitats. It has been suggested that grassland species exhibit adaptations to reduce transpiration from basidiocarps (e.g. the slimy caps of *Hygrocybe* spp.; Friedrich, 1940). Organic matter inputs into grassland soils differ in several fundamental ways from woodland, influencing the prevalence of different types of decomposer organisms:

(1) Litter inputs into grassland soils are of smaller unit size with a greater surface area for microbial attack, with much lower amounts of secondarily thickened resource units (branches twigs, etc.).

(2) Investment in secondary metabolite production, including lignins, is also lower in grasses than in other plants, so the fungitoxic extractives formed in woody tissues are absent.
(3) Mammalian herbivores consume 43–73% of above-ground net primary production (NPP) in grasslands (compared to <10% in woodlands; Swift *et al*., 1979) and consequently a large proportion of plant litter (ca. 50% of ingested C) enters the soil system in highly comminuted and partially digested form as dung. Although herbivore activity increases NPP (Stark and Grellmann, 2002), mineralization of vegetation in the digestive tracts of grazers and in dung reduces microbial biomass in grassland soil by up to 30% (Sankaran and Augustine, 2004). Variations in grazing intensity also influence surface litter accumulation, with litter accumulation leading to increased occurrence of fire, and thereby reduced N retention (Holdo *et al*., 2007).
(4) A high proportion of plant biomass in grasslands (60–70%; Swift *et al*., 1979) is below ground, especially under higher grazing pressure (Augustine and Frank, 2001).
(5) Regular defoliation by grazers leads to a high turnover of root tissues (a process still not well understood), so a greater proportion of plant biomass enters the soil system from roots (Turner *et al*., 1993).

Grasslands in areas of high human population are among the most disturbed habitats, being susceptible to destruction by ploughing and also abandonment (removal of grazing). Such transitions are usually linked to political/social or economic changes, for instance, the redistribution of land following the French revolution (Dutoit *et al*., 2004) leading to ploughing up of grasslands, or conversely the abandonment of arable farming (Highland Clearances in Scotland and the Great Depression in the US). It is likely that similar cycles have occurred in earlier periods of history, but even recent shifts can be difficult to discern (e.g. ridge and furrow evidence of historic ploughing), although with the exception of some upland and wooded areas it is quite likely that most North European grasslands have been cultivated at some point in the past. Shifts in populations of higher plants on grasslands in response to such changes have been well studied, but comparable investigations of higher fungi have been much more limited. There are, however, some historical descriptions of fungi which provide some useful clues, for instance, the association of some basidiomycetes with old pastures (Davies, 1813).

Natural grassland systems are maintained by grazing, and removal of herbivores usually leads to gradual afforestation. Amenity grasslands such as lawns and road verges are maintained by mowing. The nutrient cycles in such grasslands are dependent on management strategy. Removal of clippings removes nutrients from the system, a process which broadly mimics grazing (with N often added as fertilizer). Where clippings are returned, there is a thick litter layer and changes in plant diversity ensue due to nutrient enrichment. Supplementary feeding of stock in grasslands also represents a comparable form of nutrient addition, only partially offset by grazing activity.

3. FUNCTIONAL GROUPS OF GRASSLAND FUNGI

There has been a tendency in fungal ecology to assign species of known function to particular groupings and to use the term saprotrophic as a 'dustbin' group for the remainder. With the exception of the rust and smut fungi (which have no contact with dead organic matter), all basidiomycetes have some saprotrophic ability and for many involved in mutualistic associations with plants, their ability to release nutrients from organic matter (Read and Perez-Moreno, 2003) is a crucial part of the mutualism. Furthermore, the situation is confused by the occurrence of species which inhabit recently dead plant tissues having first colonized the living host (latent endophytism). Examples of such establishment strategies in woodland systems include *Oudemansiella mucida* on beech (Rayner and Boddy, 1988) and some members of the genus *Crinipellis* (Griffith and Hedger, 1994). For these examples there is circumstantial evidence that biotrophic infection by basidiospores occurs even though the dominant phase of the life cycle is saprotrophic.

The niche occupied by basidiomycetes is usually ascribed to the resources upon which they fruit but fruiting on dead tissues does not exclude some biotrophic/endophytic capability. The degree to which such a life strategy is necrotrophic is also difficult to establish. For instance, some fairy ring-forming basidiomycetes (see below) are occasionally termed 'weakly pathogenic'. Many asymptomatic endophytic fungi are known, though basidiomycetes have tended to be overlooked due to their slow growth on agar media. The potential diversity of basidiomycete endophytes in grasses was highlighted in bamboo by Zhang *et al*. (1997) and a similar situation was also found in cocoa leaves (Arnold *et al*., 2003). Thus, many predominantly saprotrophic basidiomycetes may have life cycles that are more complex than previously suspected. Hibbett *et al*. (2000) have estimated that ca. 50% of saprotrophic homobasidiomycetes (including many agarics) may have evolved from ectomycorrhizal ancestors. As such, several species may belong to more than one of the groups defined below.

3.1 Litter Decomposers

Primary above-ground inputs into grasslands (depending on the grazing regime) are in the form of plant litter, often forming a 'thatch' layer on the soil surface. Culture-based studies of grassland litter have tended to focus on ascomycetes (Hudson, 1968) but some basidiomycetes, usually forming small basidiocarps, are also abundant (e.g. *Mycena* spp. on grass litter, *Galerina* spp. on mosses), with others such as *Crinipellis stipitaria*, a possible latent invader, associated with more xerophytic grass tussocks but never soil (Warcup, 1951a; Parker-Rhodes, 1952). There is significant fungal translocation of N from soil to surface litter (Frey *et al*., 2000), and there are likely to be fungi which colonize and decompose litter but only fruit on soil. Microcosm studies using grass litter have demonstrated the effectiveness of *Mycena* spp. in lignin decomposition but also that decay rates are reduced when species compete (Deacon *et al*., 2006). In temperate grasslands, litter is rapidly incorporated into soil, largely through earthworm activity.

However, in African savanna termites, notably *Macrotermes michaelseni*, consume a high proportion of grass litter (Dangerfield and Schuurman, 2000). These eusocial insects cultivate lignolytic basidiomycete mutualists belonging to the genus *Termitomyces* in conspicuous nests, providing the fungus combs with partially digested faecal material and consuming the resulting hyphae (Chapter 9).

3.2 Dung Decomposers

In grazed grasslands dung from herbivorous mammals is a major input to the soil and is initially decomposed by distinctive communities of fungi and invertebrates. Dung fungi play a key role in the catabolism of the lignocellulose and the microbial polymers (from intestinal bacteria, protozoa and fungi), although leaching/dispersal by rainfall and invertebrate activity leads to the rapid incorporation of dung into soil (Dickinson and Craig, 1990). Relative to plant litter or soil organic matter, dung is a high quality resource (C:N ratio ranging from 20 to 40 depending on host and diet; Richardson, 2001; Reijs *et al*., 2003). Enhanced resource quality, partial digestion of plant polymers (with the exception of lignin) by gut microbes and increased access to microbial exoenzymes (due to comminution) lead to rapid decomposition (Nagy and Harrower, 1980).

Basidiomycetes and other fungi adapted to growth on dung tend to have pigmented spores, permitting them to withstand ingestion and digestion by herbivores (*enterophilic*), so they are already present in the faeces on excretion (Harper and Webster, 1964; Webster, 1970). It was originally thought that the fruiting of dung fungi exhibited a succession, but with regard to biomass and activity, it is more likely that the various groups of dung fungi all develop in parallel but achieving critical biomass for fruiting at different times (Webster, 1970), and culminating, for example, in the formation of basidiocarps (mostly *Coprinus* spp.) after 10–50 days (Richardson, 2001). Significant decomposition of lignin occurs in dung (Waksman *et al*., 1939), and the activity of basidiomycetes is correlated with this process (Wicklow *et al*., 1980b). Dung also comprises a proportion of debris from intestinal microbes, and some grassland basidiomycetes can decompose bacterial cell wall polymers effectively (Fermor, 1988). Dung from different herbivore species exhibits different patterns of fungal colonization (Ebersohn and Eicker, 1997). However, it is unclear whether this is due to variations in the unit resource size, differences in fungal inoculum present or differences in the resource quality of the dung (Wicklow *et al*., 1980a).

Dung invertebrates generally inhibit fungal activity (Lussenhop and Wicklow, 1985), through nutrient competition, grazing by larvae on hyphae and physical disruption of the resource (McGranaghan *et al*., 1999). However, invertebrates are susceptible to freezing in winter, possibly explaining the increased abundance of fruit bodies in winter (Richardson, 2001). Application of anthelminthics, some with selective antifungal activity (Edgington *et al*., 1971), also inhibits invertebrates (Hutton and Giller, 2003; Warren and Paul, 2006). There can be considerable competition between microbial colonizers (Harper and Webster, 1964; Safar and Cooke, 1988), dung microcosms inoculated with combinations of fungi showing slower decomposition than when singly inoculated (Wicklow and

Yocom, 1981). Several dung fungi, notably *Coprinus* spp. (Ikediugwu and Webster, 1970), are able to disrupt the hyphae of competing species and there are several examples of production of inhibitory metabolites.

Coprinus spp. fruit abundantly in laboratory microcosms (Webster, 1970), whereas in nature there is a greater diversity of basidiomycetes, for example, species of *Conocybe*, *Panaeolus*, *Psathyrella*, *Psilocybe* and *Stropharia*. The highly fluctuating moisture conditions of the grassland environment (possibly providing triggers for primordium formation; Chapter 5) and interaction with underlying soil, absent from microcosms, may explain this difference. Wicklow and Moore (1974) did not find any significant colonization by soil microbes, suggesting that competition from the enterophilic dung fungi prevented subsequent colonization by soil fungi. However, several species, termed subcoprophilous, are more often associated with dunged fields rather than dung itself (Lisiewska, 1992) and these species generally have melanized spores (e.g. *Panaeolina foenisecii*, *Psilocybe semilanceata*), potentially able to tolerate gut passage.

3.3 Terricolous/Lignicolous Decomposers and Fairy Rings

For most terricolous (i.e. fruiting on soil) basidiomycetes, ecological information is largely reliant on spatiotemporal analysis of fruiting, though some studies on mycelia, notably those of Warcup (see below), have provided valuable insights. Vertical stratification of grassland soils is usually less than in woodland due to invertebrate activity and it is not known whether mycorrhizal fungi dominate the deeper soil horizons, as is the case in woodland (Lindahl *et al*., 2007).

The most obvious manifestations of basidiomycete activity in grasslands are fairy rings, which are more visible in close-cropped and homogeneous vegetation than in other habitats where they also occur (Dowson *et al*., 1989; Chapter 5). Radial expansion rates of fairy rings range from 8 cm year^{-1} for *Marasmius oreades* (Smith, 1980) to over 100 cm year^{-1} for *Lepista sordida* (Terashima *et al*., 2004). Maximal ring diameters of 100–300 m (Shantz and Piemeisel, 1917; Kreisel and Ritter, 1985) have been reported with estimated ages of up to 200–700 years (Shantz and Piemeisel, 1917; Burnett and Evans, 1966; Kreisel and Ritter, 1985). Fairy rings are classified according to whether vegetation is killed at the ring margin (type 1), grows more vigorously (type 2) or is unaffected (type 3) (Shantz and Piemeisel, 1917). More than 50 species of grassland basidiomycetes have been reported to form type 1 or 2 fairy rings, mostly belonging to the genera *Marasmius*, *Lepista*, *Agaricus*, *Clitocybe*, *Lycoperdon* and *Calvatia* (Couch, 1995), with others, such as *Hygrocybe* and *Panaeolus* spp., forming type 3 rings occasionally (Figure 1). Several studies have demonstrated the genetic integrity of fairy rings, by investigation of mating type factor distribution (Burnett and Evans, 1966), molecular markers (Abesha *et al*., 2003) or mycelial pairings to determine somatic compatibility (K. Roderick, unpublished). There has been speculation, but no experimentation as to whether unmated mycelia (homokaryotic primary mycelia) can form rings without fruiting (Parker-Rhodes, 1955).

Fairy rings or arcs are formed by the annular growth of a mycelial system with apparent dieback of mycelium internal to the growth front. It has often been

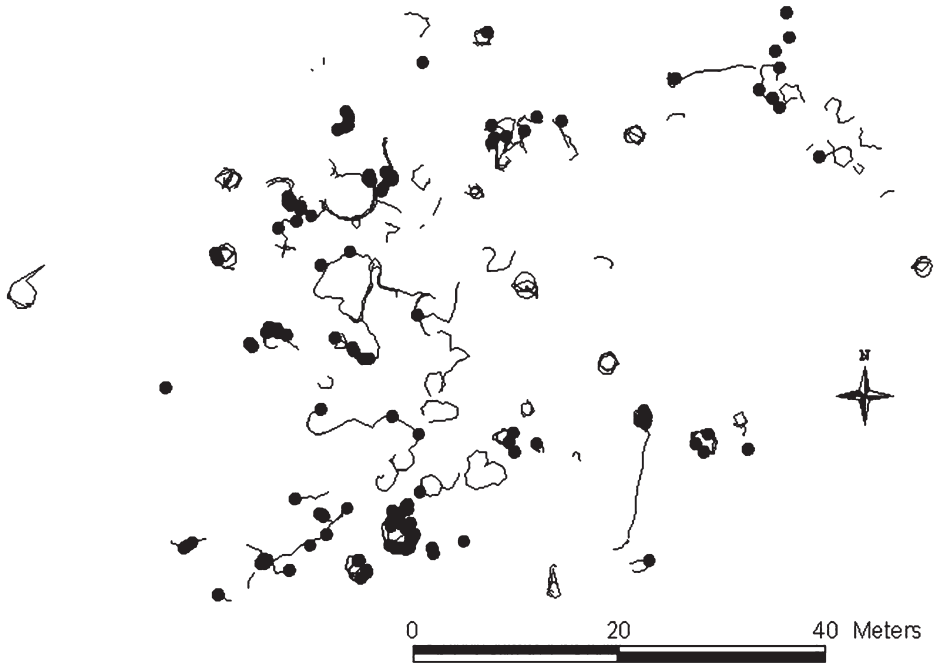

Figure 1 Differential GPS Mapping of *Agaricus campestris* Fairy Rings on the UW Aberystwyth Campus (SN595818), Showing Altered *Lolium/Festuca* Vegetation (Lines) and Basidiocarps (Dots), during the Summer of 2004. Note that Many Rings did not Produce Basidiocarps and the Heterogeneous Distribution of the Rings.

noted that such growth is simply an emergent property of localized nutrient depletion/toxin accumulation behind the growth front. Soil organic matter and nitrogen are depleted within the rings of several species (Lawes *et al*., 1883; Edwards, 1984, 1988; Kaiser, 1998). It is likely that nutrient redistribution via dung would increase nutrient levels within larger rings but there would still be an annular region of depleted nutrient internal to the mycelial front. The study of Dowson *et al.* (1989) is the only study to our knowledge to have explored the reasons for continued outward expansion of a ring, demonstrating that polarity of growth of *Lepista nebularis* was maintained after translocation of ring fragments, though why this should be the case remains unclear (Chapter 1). Their observation that mycelia disappeared when their orientation was reversed to face mycelia from their original ring is consistent with observations that when adjacent rings intersect the underlying mycelium degenerates (Parker-Rhodes, 1955).

Excavation of soil at the margin of type 1 and 2 rings reveals dense mycelial growth visible to the naked eye, whereas for species forming type 3 rings (e.g. *Hygrocybe* spp.) even microscopic observation of soil beneath the basidiocarps does not reveal an abundance of clamped mycelia (Warcup, 1959; G.W. Griffith and G.L. Easton, unpublished). The areas of mycelial abundance in type 1 and 2 rings coincide with areas of more luxuriant or killed/'scorched' vegetation,

Figure 2 Type 3 Fairy Ring of *Hygrocybe pratensis* (Ystumtuen, Aberystwyth; SN731799). (Inset) An ISSR Fingerprint Gel Showing the Genetic Identity of Basidiocarps from the Same Ring and Difference Compared with Basidiocarps from an Adjacent Ring (10 m Away). One Sample (*, Arrow) Contained Additional Bands due to the Presence of an Endophyte (*Paecilomyces marquandii*).

sometimes in concentric rings (Edwards, 1984; Terashima *et al*., 2004). Appearance of vegetation symptoms is highly seasonal and linked to soil moisture conditions, with rings (both basidiocarps and vegetation effects; Figure 2) often visible only in certain years (Shantz and Piemeisel, 1917), and possibly linked to growth or reproductive phases of the fungal life cycle (Fisher, 1977). This has led to some confusion regarding the classification (type 1 or 2) of species (Halisky and Peterson, 1970). Soil respiration and nutrient content are elevated beneath zones of luxuriant vegetation associated with *Agaricus arvensis* (Edwards, 1984), suggesting that enhanced decomposition of SOM is responsible for increased plant nutrient availability. In the same rings, symptoms of K deficiency were observed in associated grasses, suggesting that fungal tissues concentrated nutrients (with basidiocarps containing 6% N, 3% K and 1% P by dry weight), at the expense of adjacent plants. Edwards (1984, 1988) estimated that basidiocarps contained ca. 25% of all the K present in areas of dense fungal growth. The ability of grassland basidiomycetes to concentrate K and related elements in fruit bodies has subsequently received attention in the context of radiocaesium (^{137}Cs) accumulation following the Chernobyl reactor explosion (Dighton *et al*., 1991; Anderson *et al*., 1997).

The high mycelial density in annular areas of rings causes changes in the hydrological properties of the soil (Warcup, 1959; Terashima and Fujiie, 2005). Increased soil hydrophobicity is linked to hyphal secretions, possibly hydrophobins, which coat soil particles. On managed turfgrasses the resulting 'dry patch' symptoms can be alleviated by use of surfactants and fungicides (York and Canaway, 2000). Under suitable climatic conditions (rings are usually most visible in dry summers), these localized changes in soil hydrology can alter growth of vegetation, potentially masking the beneficial effects of elevated soil nutrients described above. However, in rings of some type 1 species, secretion of toxins (such as cyanide) has been implicated (Blenis *et al*., 2004), while other species (e.g. *M. oreades*, *Vascellum curtsii* and *Bovista dermoxantha*) have a necrotrophic ability following colonization of healthy root and leaf tissues (Filer, 1965; Terashima *et al*., 2004). Several type 1 species exhibit host specificity with regard to symptom production, with Terashima and Fujiie (2005) reporting a ring of *L. sordida* causing type 2 symptoms on *Zoysia japonica*, but disappearing on reaching an area vegetated by *Lolium perenne*.

3.4 Root Endophytes/Pathogens

In addition to the facultative necrotrophic abilities of fairy ring fungi, other agarics, e.g. *P. semilanceata*, are able to colonize healthy cortical tissues of grasses but without clear evidence of any deleterious symptoms in the host (Keay and Brown, 1990). Similarly colonization of grass roots has been observed under field or microcosm conditions by species such as *Melanoleuca grammopodia* and *Conocybe dunensis* (McKay, 1968). In both there was some evidence of host specificity, with *P. semilanceata* exhibiting a preference for *Agrostis tenuis* and *Poa annua* over *L. perenne*, and infection rates by basidiomycete (clamped) hyphae being much higher for *Ammophila arenaria* than other sand dune grasses. *Thanatephorus cucumeris* (anamorph *Rhizoctonia solani*) is commonly isolated from grassland and arable soil (Garrett, 1951; Warcup and Talbot, 1962) and is a capable cellulolytic saprotroph. It is also an economically important necrotrophic pathogen in grassland, causing various diseases (e.g. 'brown patch', root rot and aerial blight) in turfgrasses and other grassland plants (Couch, 1995). However, *T. cucumeris* and related species in the Ceratobasidiaceae are detected in healthy roots (Jumpponen and Johnson, 2005), and are also able to form mycorrhizal symbioses with orchids, and *Carex* spp. (Haselwandter and Read, 1982; Roberts, 1999).

Presence of basidiomycetes is occasionally revealed by culture-based examination of healthy roots from grasslands but at low frequency (Warcup, 1959; Wilberforce *et al*., 2003). However, use of fungal-specific PCR primers has recently shown a great diversity of basidiomycetes in healthy root tissues. Wilberforce (2003) found that basidiomycetes comprised 15% of clones from an oligotrophic temperate grassland in the UK, while Jumpponen and Johnson (2005) found ca. 30% of clones in a library derived from tallgrass prairie roots to be basidiomycete in origin. However, like many aspects of root biology, decomposition of these organs is poorly understood and further work is required to elucidate the function of many of these endophytes. The recent discovery, by Harrington and

Mitchell (2002), of ectomycorrhiza-like structures formed by *Cortinarius cinnamomeus* on the roots of *Carex flacca* and *C. pilulifera* in calcareous grassland, consistent with earlier observation of the association of *Tricholoma melaleucum* with *Carex glauca* (Wilkins and Patrick, 1939), illustrates that mycorrhizal associations involving agarics and non-woody hosts may be more common in temperate habitats than previously thought. Distinctive assemblages of ectomycorrhizal fungi do occur with shrubs in grasslands (e.g. *Helianthemum nummularium*), but association of agarics with non-woody hosts is usually restricted to Arctic-alpine habitats (Gardes and Dahlberg, 1996). Thus, the assignment of mycorrhizal status can be problematic especially in the absence of evidence of distinctive morphological structures.

4. DETECTION OF GRASSLAND FUNGI

The question of what role is played by particular species or groups in relation to ecosystem function is fundamental to microbial ecology. The technological and conceptual challenge required by any attempt to answer this has led to an obsession with methods. For unit-restricted taxa (see Chapter 1) such as many dung fungi, it would appear to be a relatively simple question, though current data are based almost exclusively on basidiocarp presence. However, most fungal activity in grasslands takes place in the soil, the physicochemical complexity and small scale heterogeneity of which make it difficult to map the location of hyphae (Feeney *et al*., 2006). For terricolous basidiomycetes in particular, this presents a challenge, since their distribution can be addressed at a range of spatial scales from soil crumb to field level (from a few micrometres to many metres). For most species (excepting fairy ring-forming fungi) such detailed spatial information is largely absent, and without this information it is difficult to elucidate what resources are being decomposed by particular species.

Standard dilution plating seldom recovers basidiomycete colonies, mainly because they are slow-growing but also because their hyphae are tightly associated with soil particles (Warcup, 1951b; Thorn *et al*., 1996). However, Warcup (1959) was able to isolate several taxa from pasture soil and roots by plating soil crumbs or micromanipulating individual hyphae. Among the diverse basidiomycetes isolated by these methods were several resupinate taxa, including *Peniophora* and *Athelia* spp. (Warcup and Talbot, 1962), which only rarely fruit (on the underside of soil clods or in worm tunnels; Eriksson, 1949). Direct counts of fungal hyphae by microscopy have been informative with regard to fungal standing crop, showing the increase in fungal biomass in a grassland chronosequence following arable cultivation (van der Wal *et al*., 2006). Quantification of basidiomycete mycelium, identifiable to some degree by the presence of clamp connections, has been achieved in woodland systems (Frankland, 1982; Robinson *et al*., 2005) but not to our knowledge in grasslands. Current biochemical approaches (e.g. ergosterol, phospholipid fatty acids (PLFA), etc.), while informative about overall fungal activity/biomass, are hitherto unable to dissect out the basidiomycete component. We refer the reader to the excellent review on the

merits of these approaches by Robinson *et al.* (2005). The activity of saprotrophic basidiomycetes has also been investigated by study of lignolytic enzymes from grassland soils (Gramss, 1997). AU :2

Although lacking in specific biomarkers or reliable isolation methods, the study of basidiomycetes is distinctly advantaged by the fact that many species form macroscopic fruit bodies. Indeed, with the exception of a limited number of well-studied species, inferences about the ecology of basidiomycetes are largely derived from the spatiotemporal distribution of these reproductive structures. However, fruiting patterns of grassland fungi present if anything a greater challenge than those of woodland taxa since environmental conditions in grasslands are generally less conducive to basidiocarp formation and persistence, especially the often low and fluctuating levels of atmospheric humidity. In drier grasslands especially, fruiting data are very sparse (e.g. North American mycologists seldom conduct grassland forays; Leon Shernoff, personal communication), but recent data from molecular studies suggest that many of the species present fruit only very rarely (Lynch and Thorn, 2006).

Most data of basidiocarp occurrence are collected informally and non-quantitatively in fungus forays and thus are not easily interpretable in any ecological context. Gilbert's (1875) study of basidiomycetes in response to various agricultural treatments at Park Grass Rothamsted is probably the first systematic survey of grassland fungi, finding that rings of *M. oreades* were most abundant on plots treated with lime superphosphate (either alone or in combination with sodium and magnesium sulphates) and mostly absent from plots treated with N (ammonium or manured) or K. A broadly similar pattern was found for *Hygrocybe* spp., which were present in greatest diversity on untreated plots. Wilkins and Patrick (1939, 1940) were the first to apply a more quantitative approach, recording basidiocarp numbers in fixed quadrats (ca. 700 m^2) visited repeatedly over 2 years. When assessing basidiomycete diversity in different habitat types, they found ca. 20% of the 620 species encountered were present in grassland compared to ca. 60% in deciduous woodland but only 38 spp. exclusive to grassland (e.g. *Hygrocybe*, *Lycoperdon* and *Panaeolus* spp.) and fewer species being found on clay soils compared to chalk or sand. The most common species at the 20 grassland sites was *H. virginea*, present on all soil types at '80–100% constancy'. After 70 years of agricultural intensification, it would be interesting to examine whether these fruiting patterns have changed at these sites. Arnolds (1989) found that diversity of grassland fungi was much greater in fields where there had been no addition of synthetic fertilizer, a finding confirmed by more recent surveying of permanent quadrats at a range of replicated grassland field experiments (Griffith *et al*., 2002, 2004). This is consistent with a decrease in the ratio of fungal:bacterial biomass (based on PLFA profiles) following fertilization (Bardgett *et al*., 1999).

The vagaries of basidiocarp production have been noted many times and several studies have illustrated discrepancies between patterns of fruiting and mycelial abundance below ground (Horton and Bruns, 2001). Even basidiocarp surveys repeated over several years may provide an incomplete picture of below-ground diversity (see Chapter 5; Parker-Rhodes, 1951), although information can

be gathered for large areas in a very time- and cost-efficient manner. The potential pitfalls of basidiocarp surveys of grasslands are lucidly described by Arnolds (1992b) and Watling (1995), including consideration of differential longevity of basidiocarps, fruiting periodicity, annual fluctuations and succession.

DNA-based approaches have transformed our understanding of microbial ecology, for instance, with regard to ectomycorrhizal fungi in woodlands (Horton and Bruns, 2001; Lindahl *et al*., 2007; Chapter 10). The most useful data currently available are from sequencing of clone libraries based on PCR amplification with fungal-specific primers. These provide a useful snapshot of the species present, often revealing the presence of unexpected taxa (compared to basidiocarp data). Use of taxon-specific primers has revealed that basidiomycetes are two- to three-fold less abundant (relative to total fungal abundance) in prairie grassland soil than woodland (Fierer *et al*., 2005; O'Brien *et al*., 2005). The most detailed study to date (Lynch and Thorn, 2006) identified almost 300 basidiomycete species in adjacent pasture and arable plots, with up to 9 species in some 10 g soil samples. These comprised 45 species of clavarioid fungi (20% of the total), as well as other taxa (e.g. *Hygrocybe* and *Entoloma* spp.) typically observed in oligotrophic grassland in Europe. Thus, the diversity revealed by genetic analysis greatly exceeded both the limited range of basidiocarps found at the site http://lter.kbs.msu.edu/ and the 51 morphospecies isolated on selective media (Thorn *et al*., 1996). A similar disparity between molecular data, culture-based approaches and basidiocarp surveys was also observed in Welsh grasslands (Hunt *et al*., 2004).

Cloning and sequencing is costly when scaled up and more rapid fingerprinting approaches, such as terminal restriction fragment length polymorphism (T-RFLP) or fungal automated ribosomal intergenic spacer analysis (FARISA), can robustly reveal treatment effects, for example, along grassland fertilization gradients (Brodie *et al*., 2003; Kennedy *et al*., 2006). More powerful still is a dual approach allowing peaks in T-RFLP profiles to be identified from sequence data. However, the possibility of bias (due to primer specificity or differential efficiency of DNA extraction) can skew data (Anderson *et al*., 2003; Avis *et al*., 2006). A potential problem with genetic approaches relates to effective sampling, given the often very heterogeneous distribution of grassland basidiomycetes (Figure 2). One hectare of grassland contains ca. 1,000 t of topsoil (crudely assuming 10 cm soil depth and bulk density of 1 g cm^{-3}) and it is very difficult to devise an effective sampling strategy to ensure representative coverage (when DNA extraction methods are limited to 1–10 g soil) without a very large budget. Technological advances, possibly soil fungus microarray chips (Sessitsch *et al*., 2006) or metagenomics, will increase efficiency of genetic approaches but basidiocarp surveys will remain a valuable complement of grassland research.

5. CONTRIBUTION OF SAPROTROPHIC BASIDIOMYCETES TO NUTRIENT CYCLING AND SOIL STRUCTURE

The main input into grassland decomposition systems is lignocellulose. As described by Baldrian (Chapter 2), saprotrophic basidiomycetes are able to

secrete batteries of extracellular enzymes but our knowledge of lignocellulose decay in soil and the organisms involved is less detailed than for larger woody resources. While there have been detailed studies of decomposition in woodland systems (Frankland, 1982; Steffen *et al*., 2000, 2002), the only comparable studies in grasslands have focused on fairy rings (see above).

It is the decomposition of lignin that is generally accepted to be the rate-limiting stage in carbon and nutrient cycling in terrestrial ecosystems. In addition to containing less lignin, the composition of grass lignin contains 10–20% phenolic units, a higher proportion than in wood (Lapierre *et al*., 1989). This may allow easier catabolism by laccase and manganese peroxidase that directly degrade only phenolic units (Camarero et al., 1994). Grass lignins are also more extensively cross-linked with polysaccharides cell wall polymers (via *p*-coumaryl subunits to hemicelluloses) than are wood lignins (Iiyama *et al*., 1990; Lam *et al*., 1992). These factors make grass lignins more readily degradable (Lapierre *et al*., 1989). As is the case for woodland litter/soil, most lignolytic basidiomycetes in grasslands belong mainly to the Agaricales, though as noted above Aphyllophorales are also present. There is evidence that the role of ascomycete fungi in lignin degradation may be relatively more important (Kluczek-Turpeinen *et al*., 2003; Deacon *et al*., 2006), with several soil-inhabiting species having been shown to be able to mineralize grass lignin more rapidly than wood lignin (Rodriguez *et al*., 1996).

Lignins in soil are a major source material for the formation of humic compounds. There is a correlation between the lignin content of organic inputs and the amount of humus formed (Hammel, 1997; Heal *et al*., 1997) but it is difficult to assess the degree to which plant lignins are transformed through humification. The enzymes involved in ligninolysis can also mediate formation and degradation of humic compounds (Gramss *et al*., 1999; Scheel *et al*., 1999; Steffen *et al*., 2002). These phenoloxidases can mediate covalent binding of aromatic compounds and it is suggested that humic compounds are the partially oxidized products of phenoloxidase activity in soil (quinones condensed with peptides, amino sugars and aromatics; Gramss *et al*., 1999). While the energetic benefits of degrading complex aromatic polymers are considered to be marginal (Steffen *et al*., 2002), humic compounds (unlike lignin) contain N (much soil N is present in this form), so for basidiomycetes in oligotrophic grassland such sources may be important. However, the mobilization of the recalcitrant organic N pool in soil is a poorly understood process (O'Connor, 1983).

Through mucilage secretion and mycelial entanglement of soil particles, fungi are considered to be important in the formation of water-stable aggregates by binding microaggregates (50–250 μm) into macroaggregates (> 250 μm) (Tisdall and Oades, 1982). The role of the glycoprotein glomalin, secreted by AM fungi, in this process is well established (Rillig and Mummey, 2006) but basidiomycetes including *R. solani* also contribute to aggregate stabilization (Tisdall *et al*., 1997). An unidentified grassland basidiomycete, closely related to *Peniophora*, has also been shown to secrete large quantities of a polysaccharide with significant soil-binding properties (Caesar-TonThat and Cochran, 2000; Caesar-TonThat *et al*., 2001). Antibodies raised against cell walls of this fungus reacted strongly with

larger (>2 mm) soil aggregates from dry grassland soils and to a lesser extent in adjacent arable soils. A less desirable effect of basidiomycetes on soil texture is due to the water repellent properties of their hyphae (White *et al*., 2000), thought to be associated with the secretion of hydrophobin proteins (Rillig and Mummey, 2006).

6. EFFECTS OF GRASSLAND MANAGEMENT AND CLIMATE CHANGE

In intensive modern farms, grassland areas are ploughed and reseeded (usually with *L. perenne* in Europe) on a 5–10 year cycle, and their soils in consequence bear more similarity to arable fields than permanent grasslands. Additionally, the past 50 years have seen the widespread use of synthetic fertilizers to improve grassland productivity. Thus, disturbance and eutrophication have led to the demise of most macrofungal fruiting in these habitats, although it has yet to be demonstrated that the mycelia are also absent. Losses of fungal diversity generally mirror declines in plant and invertebrate diversity, and in the case of these better studied groups changes in grassland management can also lead to loss of diversity (Rook and Tallowin, 2003). Shifts from haymaking to silage production or from cattle and sheep to sheep only grazing have also altered patterns of abundance of higher plants and insects. For soil dwelling fungi such changes might be anticipated to have a lesser effect, although changes in patterns of root death and photosynthate translocation will affect the nutrition of soil microbes (Turner *et al*., 1993). Conversely, microclimatic conditions for basidiocarp formation are altered by sward height variation, and macrofungal fruiting in rank grassland is much reduced compared to adjacent grazed areas (Griffith *et al*., 2006). However, as appears to be the case in many prairie grasslands where vegetation is much longer than the 3–15 cm sward height typical of European pastures, the health of the underlying mycelium may be little affected by aboveground vegetation height. Mown grasslands, especially historic lawns, represent important refugia for grassland fungi. While these habitats are often spared fertilizer application, the failure to remove clippings can cause eutrophication and loss of diversity, especially in areas of higher nitrogen deposition (i.e. most of Europe).

Fungi are seldom considered in issues of land use but there is a growing body of evidence that sites with diverse fungal communities do not necessarily host diverse plant communities. This is consistent with the idea that soil nutrient conditions are far more important than sward management. While many sites with diverse grassland fungal communities receive some legal protection (SSSI, etc.), fungal diversity is seldom mentioned in the notification statements (Chapter 8). Since site visits by nature conservation staff generally occur in the summer, there is little information about macrofungal diversity. Recent UK legislation (EIA (Agriculture) Regulations, 2001) controls change of use of agricultural land (e.g. ploughing of pasture), but since biodiversity assessments are generally conducted in the summer, low plant diversity can lead to destruction of valuable fungal sites.

With prospective changes in agricultural support, the re-establishment of semi-natural habitats is gaining attention. Dispersal of fungi is not perceived to be a significant factor limiting recolonization but reductions in soil nutrient status, coupled with a latent period between colony establishment and fruiting, can lead to delays in reappearance. Our work at various restoration sites, consistent with other studies (Lange, 1991), suggests that fruiting of the more common member of the more prized grassland taxa (*Hygrocybe*, *Entoloma* spp., etc.) may occur within a decade of cessation of nutrient addition. We note, however, that some of the most diverse sites for grassland fungi were subject to significant disturbance in recent centuries (e.g. post-industrial sites such as iron works, canal/reservoir embankments).

Since grasslands contain 12% of the world's SOM (33 kg m^{-2} in temperate grasslands; Conant *et al.*, 2001), factors that affect the activity of saprotrophic basidiomycetes in grasslands can impact on atmospheric CO_2 levels and consequent climate change (Freibauer *et al.*, 2004). Global warming and changing rainfall patterns combined with changes in agricultural subsidies are likely to lead to changes in climax vegetation types (Raich and Tufekcioglu, 2000), with scrub invasion and afforestation of grasslands generally resulting in increased soil C pools (Smith and Johnson, 2004). However, there are examples where the opposite has occurred. Planting of exotic pines in Andean *paramo* grasslands has caused loss of SOM, apparently due to the saprotrophic activity (soil C mineralization) of the usually ectomycorrhizal symbiont, *Suillus luteus* (Chapela *et al.*, 2001). There is already evidence of changes in phenology of basidiocarp production in UK grasslands since the 1970s with grassland species showing contrasting patterns to woodland saprotrophs (Gange *et al.*, 2007; Chapter 5).

Many parts of the world now experience high levels of aerial deposition of 'fixed' N (from intensive agriculture and vehicle emissions), a consequence of anthropogenic fixation of nitrogen (Haber–Bosch process), which has increased 10-fold since pre-industrial times (Fowler *et al.*, 2004), and now exceeds natural fixation by bacteria (Galloway *et al.*, 1995). Even modest nitrogen deposition (5–10 kg N ha^{-1} $year^{-1}$) reduces diversity of ectomycorrhizal agarics in boreal forests (Lilleskov *et al.*, 2002), probably due to alteration of soil nitrogen cycles (especially mobilization of organic nitrogen), which are very likely also to affect saprotrophic species. Although critical N loads for grasslands are higher than for woodlands, loss of plant diversity in UK grasslands (receiving 6–50 kg N ha^{-1} $year^{-1}$) is correlated with nitrogen deposition (Stevens *et al.*, 2004; Chapter 17). Projected N deposition in 2050 for the world's 34 biodiversity hotspots suggests that half of these, including grassland systems such as the Brazilian *cerrado*, will be subjected to >15 kg N ha^{-1} $year^{-1}$ (Phoenix *et al.*, 2006).

7. CONCLUSION

Almost 60 years have elapsed since Chesters (1949) postulated that the basidiomycetes were ''the missing link in soil mycology''. Our colleagues focusing on woodland ecosystems have made great advances in elucidating the role of

these fungi in plant nutrition and decomposition processes. While the specialized catabolic functions performed by lignolytic basidiomycetes are relatively less important and partly mediated by ascomycete fungi, several lines of evidence suggest that grassland basidiomycetes may play a more important role in plant nutrition than previously suspected. With respect to fungal conservation, grasslands outside Europe merit more detailed study, given the unexpectedly high diversity revealed by molecular investigations. There is some urgency to this last point. As evidenced in Europe by the past 50 years of agricultural intensification, future uncertainty with respect to climate change and agricultural practices places remaining semi-natural grasslands at high risk of destruction.

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