Fungal Ecology 33 (2018) 92–103

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Vegetation and edaphic factors influence rapid establishment of distinct fungal communities on former coal-spoil sites

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ARTICLE INFO

Article history:
Received 6 September 2017
Received in revised form
11 January 2018
Accepted 9 February 2018
Available online 24 March 2018

Corresponding Editor: Petr Baldrian

Keywords:
Next generation sequencing
Waxcap
Soil ecology
Mycorrhiza
Ergosterol
Immigration

ABSTRACT

We investigated re-establishment of fungal communities on eight former colliery sites in South Wales following revegetation 22–27 y earlier. Regraded bare coal-spoil was seeded to sheep-grazed grasslands, with saplings planted into coal-spoil for woodlands. Metabarcoding (28S rRNA, D1 region) of soil fungal populations showed that woodland and grassland habitats were clearly divergent but edaphic variables only weakly affected fungal community structure. Root-associated basidiomycetes dominated all habitats, with ectomycorrhizal fungi more abundant in woodlands and Clavariaceae/Hygrophoraceae ('CHEG' fungi) in grasslands. The composition of coal-spoil grassland communities resembled that of a typical upland grassland site, suggesting that propagule immigration was not a limiting factor. However, fungal biomass (ergosterol) was 3-fold lower, reflecting high bulk density and poor structure. Re-establishment of fungal communities in coal-spoil soils represents an important barometer of restoration success. From a fungal conservation perspective, such sites represent important refugia for waxcap fungi subject to habitat loss from agricultural intensification.

1. Introduction

Fungi play a central role in soil as decomposers of organic matter, plant mutualists and pathogens; hence they have a significant impact on plant growth and carbon sequestration (Paul, 2014). The effect on plant growth of mycorrhizal fungi depends on the nutrient status of soils (Jonsson et al., 2001) and their impact can range along a continuum from parasitism to mutualism (Jones and Smith, 2004) although evidence indicates a positive interaction in grasslands (Van Der Heijden et al., 2006). Saprotrophic fungi are important for the mineralisation of organic matter, the nutrients released being made available via mycorrhizal fungi to plants (Lindahl et al., 2002). Therefore, if fungi from the saprotrophic and symbiotrophic guilds do not establish well on newly vegetated land, plant growth will be impacted, affecting litter decomposition and carbon sequestration in soil (Clemmensen et al., 2015). The extent to which fungal populations establish in new habitats and the structure of these populations are, therefore, important questions when considering successional systems or restoration of habitats.

Coal-spoils represent a legacy from the industrial revolution in the UK and during the 1980s several projects looked at regeneration of their soil and habitats. Spoils typically have very limited organic matter and are depauperate of microbial populations, very different from natural communities. For example, Elhottova et al. (2006) found fungi to be present in the lacustrine clays of a post-mining site, primarily microfungi such as *Penicillium* and *Aspergillus*, but these assemblages were very different from soil within semi-natural woodland or grassland. Pedogenesis can occur *de novo* on coal-spoils in a manner not dissimilar to that in post-glacial or post-volcanic areas. In this respect, the restoration may be similar to a primary successional system for soil biota and so provide an indication of how fungal communities develop in new habitats.

Factors driving microbial community development are poorly understood. Soil pH has been shown to correlate with bacterial composition but the correlation with fungal community

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https://doi.org/10.1016/j.funeco.2018.02.002

composition appears much weaker (Lauber et al., 2008; Rousk et al., 2010). A factor important in the development of soil communities on former coal-spoil is soil structure, since the heterogeneous structure of well-developed soil provides a variety of niches for soil fungi and other organisms (Mummey et al., 2002). Formation of soil starts with the weathering of the parent material (Frouz et al., 2011) followed by biological action such as earthworm activity, root exudation and fungal secretions (Bronick and Lal, 2005). Soil organic carbon (SOC) content has been used as a predictor of microbial biomass (Fierer et al., 2009), but little is known of the influence of these factors on fungal community structure.

Plant community composition impacts fungal community composition via the quantity and quality of the organic inputs, which affects the saprotrophic community structure; and also through the types of root symbioses that are formed (Read and Perez-Moreno, 2003). In the northern hemisphere, succession to woodland leads to the establishment of large populations of ectomycorrhizal fungi (EcM) (Tedersoo et al., 2010), with a consequent decrease in both the abundance and species richness of arbuscular mycorrhizal fungi (AMF) (Johnson et al., 1991b).

A diverse group of macrofungi, known as 'CHEG' fungi (Clavariaceae, Hygrophoraceae, Entolomataceae and Geoglossaceae) are abundant in undisturbed nutrient-poor grasslands (Griffith et al., 2013). Although their ecology is not well understood, many lines of evidence now suggest that they are biotrophic and probably mycorrhizal (Griffith et al., 2002; Seitzman et al., 2011; Halbwachs et al., 2013). Restored grasslands at coal-spoil sites represent potentially suitable habitats for these fungi, since no fertilisers are applied beyond the initial establishment phase and short swards are maintained by sheep grazing.

Here fungal populations are considered at coal-spoil tips in South Wales that were restored to grassland and woodland 25 y previously. The restoration of disturbed land to a self-sustaining grassland or woodland, requires an effective soil fungal community (Sun et al., 2017). Restorations of the spoil tips were undertaken by direct seeding of grass and clover or planting of tree saplings directly into regraded coal shale without any addition of soil. The coal-spoil had previously been 'stored' in large dumps for several decades and contained very little organic matter with limited populations of active microbes (Johnson et al., 1991a). The coal-spoil was re-profiled before restoration so any vegetation and below ground communities that had established naturally would also have been buried.

Metabarcoding of DNA extracted from soil was used to determine the composition of fungal communities hypothesising that vegetation cover (grassland vs. woodland) would be a key determinant of the structure of their communities. It was also hypothesised that the structure of communities of root-associated fungi (e.g. mycorrhizas) would be more affected by vegetation, than those of saprotrophic fungi. The latter would be expected to be influenced more by soil parameters. We also sought to determine whether fungal communities in restored soils would differ from those in more natural undisturbed soils, due to the early stage of soil development, and the resultant differences in the physicochemical characteristics of the soil.

2. Materials and methods

2.1. Study sites and sampling

The eight sites were chosen based on the presence of both woodland and grassland habitats established during restoration. Grassland areas had originally been seeded with *Lolium perenne*, *Festuca* spp. and clovers (*Trifolium* spp.), and had received some initial inorganic fertiliser, although the amounts are unknown. Commonly planted trees in woodland areas included *Alnus glutinosa, Betula pubescens, Corylus avellana, Salix* spp., *Populus* spp., *Larix decidua* and *Sorbus aucuparia*, with some climax species such as *Quercus petraea* (Supplementary data S14). At one site (Cwm Daren) a pure stand of *L. decidua* was also present and this was included as a separate sample to provide a comparison with the mixed woodland at the same site. One younger site (Deep Navigation; 12 y since restoration) was included in our study; woodland and grassland vegetation was also not noticeably less mature than at other sites (Supplementary data S1).

All soil sampling was undertaken in October 2013. At each site and land use combination, five 3×3 m quadrats were randomly located across the area. Nine cores were taken from each quadrat using a 9 mm diameter soil auger to a depth of 10 cm, with all cores pooled into one sample per quadrat (ca. 150 g/sample; total of 80 samples). In woodland quadrats, the uppermost organic (O) layer was removed prior to soil coring. Edge effects from undisturbed land were avoided by sampling at least 20 m from any boundaries. Samples were stored cold and frozen at −80 °C within 12 h of sampling. Ten intact soil cores (5.6 cm diameter, 6 cm deep) were taken from each site 1 cm below the surface of the soil for bulk density measurement.

Cores were also taken (October 2013) from a semi-natural, grazed grassland (Brignant Longterm Experiment; lat/long: 52.3652, −3.8297; 367 m asl.) on Manod soil series. The Brignant experiment was established in 1994 to monitor the effects of contrasting management (haycutting/grazing/liming) on floristic diversity but is used here since its climatic conditions, altitude and soil type are very similar to those in upland grasslands found in areas adjacent to the coal-spoil sites. The Brignant experiment comprises seven triplicated treatments. Fertiliser (60 kg N. ha^{-1} and 30 kg P. ha^{-1}, applied in May) was added on only one treatment to mimic standard management of upland sheep-grazed meadows. Three summer-grazing treatments across three replicates were selected as a comparison for the restored sites and these were sampled at a similar coring density to the coal-spoil grasslands. Cores were combined for each replicate treatment, nine samples in total. No suitable reference woodland of equivalent age and composition could be found for comparison with woodland areas, since remnant woodland around the spoil tips were old (>100 y) oak woodland or mature forested monocultures.

2.2. Soil processing and measurements

Samples for DNA analyses were frozen at −80 °C and freeze-dried. After freeze-drying, soil was passed first through a 4 mm sieve, the stones removed and weighed, and then through a 500 μm sieve. The sieved soil was thoroughly mixed. DNA was extracted from 200 mg of the dried soil sample using PowerSoil DNA extraction kit (MO BIO Laboratories, Inc. Carlsbad USA).

Soil organic matter (OM) content was measured through the loss on ignition method (LOI) (Ball, 1964). The restored soils contained coal particles that would inflate the organic matter content if ignited. To determine the ignition point of the coals from the restored sites, particles of coal were ground and ignited at a range of temperatures from 300 to 400 °C for 18 h and then weighed to determine at what temperature weight loss occurred. The highest temperature without weight loss was 320 °C so this was chosen as the temperature for ignition. Freeze-dried soil (ca. 2 g) was ignited for 16 h and the weight loss was recorded. Igniting at this temperature however does not burn all the plant derived OM. Igniting natural grassland soils at 320–400 °C indicated that on average 90% of weight is lost at 320 °C when soils were burnt overnight, so this was used as the conversion factor to determine actual OM content.

Total soil nitrogen and carbon were measured (Vario MACRO