Pedobiologia 61 (2017) 9–21

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Overlap in substrate utilisation and spatial exclusion in some microfungi which act as early cellulose colonisers in a Mediterranean environment

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ARTICLE INFO

Article history:
Received 20 September 2016
Accepted 15 January 2017

Keywords:
Litter
Soil
Cellulose
Metabolic functions
Soil ecology
Aboveground-belowground interactions

ABSTRACT

A trapping system was set up to isolate culturable cellulolytic fungi at the soil-leaf litter interface in an area of Mediterranean maquis located in southern Italy. Seven cellulolytic taxa were isolated and cultured to represent the pioneers in the primordial phase of cellulose colonisation. The functional diversity of fungal isolates was analysed using a phenotype microarray technique to generate a profile of their functional traits. The extent of the overlap in substrate utilisation by the various species was subsequently determined.

Some of the species that acted as key cellulose decomposers are considered globally distributed air-borne contaminants. Most of the cellulolytic species that were isolated during the experiment are also well known for their competitive abilities. Our study showed that at an early stage of cellulose colonisation a few fungal species with potential overlap in the overall metabolic function were able to establish themselves. The species that prevailed at the onset of cellulose colonisation did not co-occur in the field sites. A sort of "founder effect" could be hypothesised for microfungi when colonising sterile cellulose. This would suggest that scale (i.e. the spatial component) exerts a strong influence on the causative mechanisms that link genetic fungal diversity to specific ecosystem functions.

1. Introduction

Fungi are broadly distributed across all terrestrial ecosystems (O'Brien et al., 2005; Peay et al., 2010; Taylor et al., 2014) and strong biogeographic links between distant continents show that the dispersal of some species over long distances is relatively efficient in comparison to that of macro-organisms (Tedersoo et al., 2014). In theory, some taxa are ubiquitous, but only a small number of them can exert a huge influence on particular environments (Peay et al., 2010; Taylor et al., 2014). These species are thought to act as keystone species, meaning organisms that play a unique and crucial role in the way an ecosystem functions (Mills et al., 1993). The ecological significance of some keystone species could lie in the fact that they behave as pioneers on particular substrates (such as lignin, cellulose, collagen, etc.) (Robinson et al., 2005). Micro-habitats and substrates are of considerable significance in determining fungal species' distribution (Persiani et al., 2008) and, according to some authors, local differences in distribution are greater than continental differences (Peay et al., 2010). Other important variables such as soil temperature and pH can have a strong influence on the mechanisms of substrate colonisation and decomposition as well as on fungal species distribution (Neher et al., 2003; Manici et al., 2014).

Therefore, in order to understand the patchiness of fungal distribution and competition phenomena, the scale at which environmental variables can affect the ecological functions of fungi should be carefully considered. Cellulose is the most abundant organic compound in the biosphere and the largest source of metabolic carbon on land. The chemical stability of cellulose, at neutral pH and environmental temperatures and in the absence of

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http://dx.doi.org/10.1016/j.pedobi.2017.01.004
0031-4056/

cellulolytic organisms, is very high. In theory, cellulose can remain completely unchanged for millions of years. However, bacterial and fungal activity can break it down very rapidly. In particular, the enzymatic activity of filamentous fungi has been shown to be responsible for most of the carbon turnover in cellulosic debris (Wicklow, 1981; Hättenschwiler et al., 2005; Wilson, 2011). Even if a considerable number of fungi have been described as possessing the ability to attack cellulose, as yet little is known about the functional abilities towards other carbon sources and nitrogen containing compounds of the cellulolytic fungi that colonise cellulose under natural conditions (Wilson, 2011). Recently, a study on cellulolytic communities in soil, based wholly on a culture-independent technique, demonstrated that only a part of an established fungal community is actively involved in decomposition at any given time, whereas even taxa representing a negligible proportion of such a community may contribute significantly to the decomposition process (Voříšková and Baldrian, 2013). Nguyen et al. (2015) stressed that integration of molecular taxonomy with fungal ecology and functionality is still lacking.

The aim of this study was to evaluate niche overlap among microfungi that act as early cellulose colonisers in a Mediterranean environment. Here, the term "niche overlap" means the overlap of resource use by different species (Elton, 1927) or the ensemble of the "functional attributes of a species and its corresponding trophic position". Competition theory predicts that community structure is shaped by resource partitioning between co-occurring species, and that niche overlap is a key factor in studies examining community structure and species coexistence (Geange et al., 2011). Thus, the coexistence of fungi on a substrate is thought to be regulated by nutritional resource partitioning (Wilson and Lindow, 1994).

During this research project, we investigated whether spatial overlap among species was related to fungal *in vitro* metabolic profiles. In addition, we investigated whether or not the species that first occupy cellulosic substrate possess similar functional abilities towards carbon sources other than cellulose, and whether their distribution follows a spatial structure. To clarify these matters, a system for trapping cellulolytic fungi was set up and used to isolate and cultivate pioneer cellulose-degrading fungal species. The use of cellulose layers to serve as a very simplified model of leaf litter colonisation was a deliberate strategy employed to test the specific interaction of fungi in the previously described context. The experimental set-up included a sampling scheme capable of delivering information on the spatial scale at which fungi occur and interact.

2. Materials and methods

2.1. Natural site

The study was carried out in a coastal area located in southern Italy (Castel Volturno, Caserta, IT) within a natural reserve of evergreen Mediterranean shrub land (40°57′N–13°33′E). The area is characterised by alluvial deposit dunes at an elevation of 6–9 m above sea level. The soil is composed of loose siliceous-calcareous marine sand and volcanic pyroclastic material. The local climate is Mediterranean with a mean annual temperature of 18.6 °C and average annual rainfall of 680 mm. Four sites hosting similar plant cover and characterised by the same slope were selected (site Nos. 2, 4, 9, 11). The leaf litter at the sites was mainly composed of *Cystus incanus* L., *Myrthus communis* L., *Quercus ilex* L., *Rhamnus alaternus* L., *Asparagus acutifolius* L., *Phillyrea angustifolia* L. and *Pistacia lentiscus* L.

2.2. Cotton cellulose bags

A trapping system for collection of cellulolytic fungi at the soil-leaf litter interface was devised and set up in the field (Fig. 1). Layers of square filter paper (10 × 10 cm) made of pure cellulose (Whatman filter paper CHR1, cotton linter Cat. No. 3001 917) were placed one on top of the other to form pads which were inserted in bags made of glass fibre mesh (Maurer, "insect screen" Ferritalia Distr., Padova, IT, cod. 80329H120). The fabric's mesh

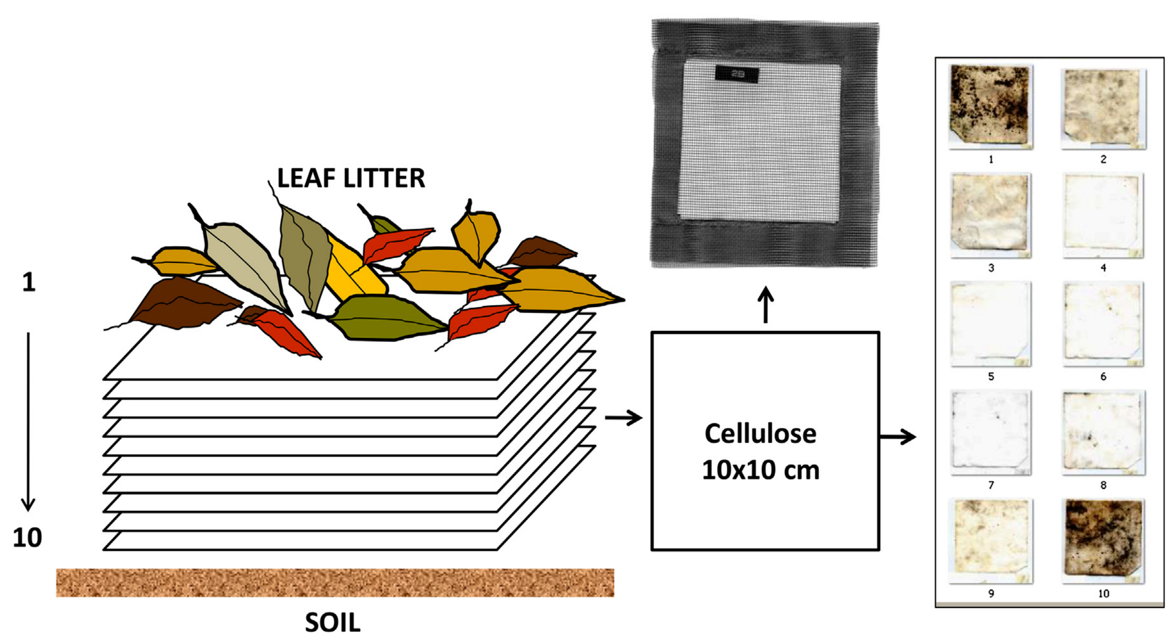

Fig. 1. Trap system for cellulolytic fungi to be used at the soil-leaf litter interface. Ten square filter paper layers of pure cellulose were overlapped to obtain a pad, and inserted in sewed bags made of a net of glass fibres. All the bags were fixed on top of the soil by plastic pegs. The position of the ten layers of cellulose respect to soil and leaf litter (layer n.1 on litter side, layer n.10 on soil side) was maintained in order to be able to associate fungal presence to a specific trend of colonisation (from soil side or from litter side).