Mycelial dynamics during interactions between *Stropharia caerulea* and other cord-forming, saprotrophic basidiomycetes

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Summary

- Macroscopic mycelial interactions between *Stropharia caerulea* and four other cord-forming wood decomposer basidiomycetes (*Phanerochaete velutina*, *Phallus impudicus*, *Hypholoma fasciculare* and *Agrocybe gibberosa*) on nonsterile soil were quantified using nondestructive image analysis.
- Interaction development and outcome was species dependent. Once *S. caerulea* mycelium became fully confronted by nonself mycelia, extension usually ceased and mycelia formed (defensive) aerial ridges and/or (invasive) cords. *P. velutina* rapidly replaced *S. caerulea*, but with significantly ($P < 0.05$) reduced biomass and mass fractal dimension ($D_{BM}$). *S. caerulea* regressed, biomass, $D_{BM}$ and surface fractal dimension ($D_{BS}$) became significantly ($P < 0.05$) reduced.
- *S. caerulea* produced temporary defensive ridges against *P. impudicus*, the latter extending through and replacing *S. caerulea* mycelium, but with significantly ($P < 0.05$) reduced biomass. *S. caerulea* and *H. fasciculare* deadlocked, the latter producing dense (high $D_{BM}$) noninvasive lateral mycelial fans resulting in persistent mycelial fronts. *S. caerulea* and *A. gibberosa* initially deadlocked, the latter completely encircling *S. caerulea*. In response, *S. caerulea* produced defensive mycelial ridges at the interaction margin, and fans extended over *A. gibberosa*.
- These results are discussed in relation to mycelial foraging strategies, mycelial morphogenesis and determinants of interaction outcome.

Key words: image analysis, fractals, mycelial morphology, *Stropharia caerulea*, *Phanerochaete velutina*, *Phallus impudicus*, *Hypholoma fasciculare*, *Agrocybe gibberosa*.

Introduction

The large size, persistence and migratory nature of mycelial-cord systems at the woodland soil–litter interface (Thompson, 1984; Boddy, 1993, 1999) make interaction with other fungi inevitable during the lifetime of a mycelium. Although mycelial systems will interdigitate significantly in the soil (Rayner et al., 1987), resources act as foci for mycelial effort (Dowson et al., 1988a,b), providing arenas for both diffuse mycelial interactions at resources and between more aggregated mycelia extending between resources. The efficiency of migratory mycelial foraging and capture of discrete resources is now well documented (Dowson et al., 1986, 1988a,b, 1989; Bolton, 1993), and mycelial systems have also been shown to be responsive to litter inputs behind foraging fronts (Wells et al., 1997). To retain access to resources, a mycelium must be capable of defending its territory and to gain new ones through replacement of resident fungi. In general, cord-forming decomposer fungi, as secondary colonisers, are more combative and replace noncord-forming primary colonisers (Chapela et al., 1988; Holmer & Stenlid, 1997). Fungal interaction studies have largely been made on artificial media (Boddy, 2000), the type of which may alter the nature of interactions (Whipp, 1987). Interpretation therefore must be made cautiously as these approaches may not reflect the heterogeneity of the natural environment (Rayner & Webber, 1984; Boddy, 2000). However, recent detailed studies on agar
have revealed some of the roles of extracellular metabolites in inducing complex morphogenetic mycelial patterns, similar to those produced during interspecific mycelial interactions (Griffith et al., 1994a,b,c).

The interaction studies that have been performed on soil, have primarily examined extension rates, loss or capture of resources and provided qualitative descriptions (Dowson et al., 1988c; Robinson et al., 1993; Holmer & Stenlid, 1993, 1996, 1997). We hypothesize that interactions will not only affect extension rates of the fungi, but also mycelial biomass and biomass distribution (pattern/space-filling). Morphological changes of cord-forming fungi can be quantified non-destructively using image analysis and fractal geometry as a mycelial descriptor (Donnelly et al., 1995; Boddy et al., 1999; Boddy, 1999, 2000). This approach has been used recently to quantify responses of noninteracting mycelia to a diverse range of environmental conditions when grown in isolation (Donnelly et al., 1995; Donnelly & Boddy, 1997a,b, 1998; Boddy, 1999) and is equally applicable to quantifying changes resulting from confrontations between mycelial systems. This paper describes and quantifies the mycelial dynamics of Stropharia caerulea (Krieck), in interaction with a range of cord-forming basidiomycetes found in woodlands and disturbed habitats, and which exhibit a range of foraging strategies and combative ability (Boddy, 1999, 2000). It is hypothesized that mycelia of those fungi that are considered short-range foragers will be more responsive to the presence of extra-resource mycelium of another fungus than those considered to be long-range foragers.

Materials and Methods

Fungal isolates

Origin, source and site of fungal species used are shown in Table 1. Cultures were maintained on 2% (w/v) malt agar (MA; 20 g Munton & Fison spray malt, 15 g Laboratory M no. 2 agar 1\(^{-1}\) distilled water) and incubated in the dark at 20°C. Preparation of inocula

Wide-necked conical flasks (2 l) containing 500 ml 2% MA were inoculated separately with 9 cm Petri plate agar cultures of either S. caerulea (2 flasks), Phallus impudicus Pers., Agrocybe gibberosa (Fl.) Fay, Hypholoma fasciculare (Huds. Fr.) Kumm or Phanerochaete velutina DC.(Pers.) Parmasto. Timings were designed to synchronize flask colonization between fungi with different growth rates. Wood blocks (4 cm\(^3\)) were cut from a freshly felled beech (Fagus sylvatica L.) tree and frozen. When required, these were defrosted, soaked in distilled water overnight, steam autoclaved at 121°C for 30 min and re-autoclaved after 24 h. Inocula were prepared by adding 35 wood blocks to each colonized flask culture. After incubation at 20°C in darkness for 42 d, colonized wood blocks were scraped free of adhering mycelium and agar.

Preparation of soil interaction trays

Loam topsoil was collected from a mixed deciduous woodland in Coed Beddick Inclosure, Tintern, UK (Nat. G. R. SO528018), sieved to < 4 mm mesh and then air dried in trays (Stewart Plastics, Croydon, UK) at room temperature. Soil matric potential was adjusted to ~0.02 MPa (Fawcett & Collis-George, 1967) with distilled water, and wetted soil (200 g) was compacted in square plastic bioassay trays (24 × 24 cm, Nunc Gibco, Paisley, UK).

Interactions were investigated by pairing inoculum blocks 7 cm apart in a soil tray, in the following combinations: S. caerulea vs either self, A. gibberosa, H. fasciculare, Phal. impudicus or Phan. velutina. In controls S. caerulea inocula were paired with either nutritionally inert plastic bottle caps or autoclaved uncolonized beech wood blocks (4 cm\(^3\)). Three replicates of all combinations were made.

Photography and image capture

Trays were photographed using an Olympus OM 10 camera (Olympus Optical Co. Ltd., London, UK) body fitted with a 50-mm OM-system macro lens and Ilford FF4 (125 ASA) 35 mm black and white film (Ilford Imaging UK Ltd., Cheshire, UK), as described by Donnelly & Boddy (1997a). Usually, two photographs were taken before contact between mycelia, two during the week following contact, and then at longer intervals as mycelial changes decreased, recording being stopped 125 d after inoculation. Film negatives were developed and images analysed for radial extent, biomass and fractal dimensions (see below) of extra-resource mycelia as described

<table>
<thead>
<tr>
<th>Species</th>
<th>Source material</th>
<th>Habitat</th>
<th>Location</th>
<th>National Grid Ref.</th>
</tr>
</thead>
<tbody>
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<td>Garden compost</td>
<td>Tintern</td>
<td>SO528018</td>
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<td>Hypholoma fasciculare</td>
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<td>Phallus impudicus</td>
<td>Mycelial cord</td>
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<td>Sallow Valets inclosure</td>
<td>SO611145</td>
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<tr>
<td>Phanerochaete velutina</td>
<td>UWC Culture collection</td>
<td>–</td>
<td>Forest of Dean</td>
<td>–</td>
</tr>
<tr>
<td>Stropharia caerulea</td>
<td>Fruit body</td>
<td>Birch stand/Nettlebed</td>
<td>Ex. Dowson et al. (1988b)</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1 Cord-forming fungi, source and location
in detail by Donnelly et al. (1999). Briefly, film negatives were projected by slide projector onto a white screen. Light images collected by a 1 inch Vidicon tube video camera (model 1800, National Panasonic, Osaka, Japan), were captured on a framestore (Synapsee, Synoptics Ltd, Cambridge, UK) and image processed (Donnelly et al., 1999) using SEMPER 6 plus, version 6.3 (Synoptics Ltd, Cambridge, UK) on an IBM compatible PC (System 300, Dell Computers Ltd, Bracknell, UK).

The SEMPER program contains a ‘windowing’ option, whereby desired regions of an image may be extracted for separate processing (Donnelly et al., 1999). This feature was used to analyse interacting mycelial systems of each species and to separate morphologically distinct regions within a single mycelium.

Quantification of mycelial growth and distribution

Measurements of radial extent were calibrated by drawing pixel lines against known lengths. For each image, extension was determined from 3 to 8 radial measurements from the inoculum to the extending mycelial margin. Biomass calibrations were made, as described by Donnelly & Boddy (1997a), by quantifying pixel areas of extra-resource mycelia for 20 systems of S. caerulea and Phan. velutina and 10 systems of all other species, which were then harvested, oven dried and weighed (mg). Linear regression of maximum radial extent and biomass with time were used to estimate mycelial extension rate (mm d\(^{-1}\)) and biomass production rate (mg d\(^{-1}\)).

Fractal geometry, a measure of space filling, was used to quantify mycelial distribution as described by Donnelly et al. (1995, 1999) and briefly as follows. Fractal structures are composed of smaller copies of the whole structure, that is they have self-similarity, but are dimensionally irregular. A fractal structure formed in two dimensions will have a dimension between 1 and 2; in contrast, regular (nonfractal) two dimensional structures have dimensions of either 1 (a line) or 2 (a plane).

Fractal dimensions of extra-resource mycelia for each process image were determined using the box-count technique by overlaying on a series of grids containing boxes of varying side length (s) from 3 to 61 pixels. The number of boxes intersecting the image were counted for each box size (Donnelly et al., 1995, 1999). Fractal images obey the power law relation over a range of length scales:

\[
N(s) = c s^{-D_B}
\]

(\(N(s)\), the total number of boxes of side length s that intersect the image; \(D_B\), the box count fractal dimension; and c, a constant. \(D_B\) is derived from the negative gradient of the regression line of log \(N(s)\) against log s as follows:

\[
\log N(s) = \log c + (-D_B) \log s
\]

Two box count fractal dimensions were determined; the surface fractal dimension (\(D_{BS}\)) and the mass fractal dimension (\(D_{BM}\)).

\(D_{BS}\) quantifies the distribution of the mycelial perimeter and internal edges, which if regular will approximate a line (\(D_{BS} = 1\)), and with greater space filling \(1 < D_{BS} < 2\). \(D_{BM}\) describes the plane filling of the entire mycelial system, \(1 < D_{BM} < 2\) in 2 dimensions, tending towards 2 where large parts of the system are solid. In such plane filled systems, the fractal property of the system may only be at the margin, forming a surface fractal. Where the system is composed of more discrete structures, \(D_{BS}\) and \(D_{BM}\) may reach equivalence (\(1 < D_{BS} = D_{BM} < 2\)) forming a mass fractal structure (Obert et al., 1990; Donnelly et al., 1995; Boddy et al., 1999; Boddy, 1999).

Estimation of decay rate

Initial inoculum decay state was estimated for each species as relative density (RD, g cm\(^{-3}\)) of a sample of five colonized blocks. Final RD was determined for all inocula at harvest from which the rate of decay (g cm\(^{-3}\) d\(^{-1}\)) was determined.

Determination of replacement within inocula

Where a mycelium appeared to colonize an opposing inoculum wood block, replacement was confirmed by splitting the inoculum block in two and removing small (2 mm\(^2\)) wood chips which were plated on MA and incubated as described above. Identity of fungi isolated from inocula was determined from cultural morphology and on the basis of somatic compatibility by pairing against original strains.

Statistical analysis

Randomised block one-way ANOVAs followed by Schéffe \(a posteriori\) tests (Schéffe, 1959) were used to detect treatment effects on mycelial biomass and fractal dimension. Differences in mycelial extension and biomass production rates were detected by comparison of slopes of regression.

Results

Noninteractive mycelial development

Most mycelial systems extended radially on inert-baited soil trays until trays were fully colonized, after which time mycelial systems thinned, for example S. caerulea (Fig. 1a,c), except H. fasciculare which spontaneously regressed after 35 d. Mycelial systems with wood bait controls also developed radially and on wood bait colonization most species developed thickened and persistent cords interconnecting baits and inocula (Fig. 1b,d). On contact with both inert and wood baits S. caerulea mycelial margins became more fanned by 35 d (Fig. 1e,f). On colonization of wood baited controls, S. caerulea also produced patches of localized de-differentiated hyphae along mycelial cord lengths (Fig. 1d).
In inert controls, *A. gibberosa* produced regular fans creating a ‘scalloped’ margin (Fig. 2a). Wood bait controls were contacted by 28 d and tangential anastomoses were evident up to 180° around wood baits by 49 d, by which time trays were filled. By 124 d, *A. gibberosa* mycelium had thinned with major radial cords remaining on inert baited trays and to a lesser degree on wood baited systems.

*H. fasciculare* contacted baits by 28 d, and outgrowth from both colonized wood baits and margins previously ‘stalled’ on contact with wood, occurred after 49 d (Fig. 3a). *H. fasciculare* filled trays by 63 d, and mycelia thinned markedly after 96 d, although mycelial resource interconnectives remained thickened.

*Phal. impudicus* contacted baits by 28 d, produced anastomoses local to wood baits by 49 d (Fig. 4a) and filled inert baited and wood baited trays by 63 d and 70 d, respectively.

*Phan. velutina* contacted baits by 20 d, and filled inert baited trays by 28 d. On wood baited trays, *Phan. velutina* cords interconnected resources thickened by 28 d, and mycelia filled trays by 49 d. Mycelial systems of *Phan. velutina* had thinned markedly by 93 d, and appeared to regress except around and between colonized resources.

There was significant (*P* ≤ 0.05) interspecific variation in extension rates of mycelial systems developing on inert baited soil trays (Table 2), species developing as cord aggregates extending faster than more diffuse mycelial systems.

For most species maximum biomass was produced on control trays (Fig. 5a,d,g,j), characteristically peaking between 30 and 50 d. Biomass production rates ranked *Phan. velutina* > *Phal. impudicus* > *H. fasciculare* > *A. gibberosa* > *S. caerulea* (Table 3). Differences in mycelial morphology were reflected in fractal values, species developing initially as plane filled mycelia with regular margins having $D_{BM}$ close to 2 and $D_{BS}$ close to 1, for example *H. fasciculare* (Figs 3a and 5e,f). Well-aggregated mycelia had almost equal mass and surface $D$-values, for example *Phal. impudicus* (Fig. 4 and Fig. 5h,i). Changes in mycelial distribution were also evident, declining $D_{BM}$ values after c. 28 d (Fig. 5e,f,l) indicated thinning of mycelial systems.

Self interactions

Self-paired *S. caerulea* systems grew radially until opposing mycelia made contact after 14 d or until tray sides were
reached (Fig. 6a–c). Mycelial extension rate was similar to controls (Table 2, P > 0.05) until fusion replaced extension after 28 d. Both tip to tip and tip to side fusion occurred between cords (Fig. 6d) and a fusion line was discernible across the interaction front after 28 d (Fig. 6e). Biomass peaked between 35 and 63 d after inoculation following complete contact between systems; biomass from inocula colonized in one flask was significantly (P £ 0.05) greater than from the other (Fig. 7b). Fractal dimensions peaked at 21 d, declining until harvest (Fig. 8a,b). Post fusion, physiological unity was indicated by synchronous biomass and morphological changes (Figs 7b and 8c,d) such as thickening of major cords and regression of minor ‘interstitial’ cords.

Nonself interactions
S. caerulea mycelia extended at rates similar (P > 0.05) to controls until contact was made with opposing mycelia (Table 2). Once S. caerulea became fully confronted, extension ceased in most interactions and mycelia switched to defensive or invasive modes.

Phan. velutina extended into areas occupied by S. caerulea by 21 d without significantly slowing (P > 0.05; Table 2), although Phan. velutina mycelium overgrowing S. caerulea became orange–dark brown in colour and exuded droplets of liquid (Fig. 9a,b). After initial contact with Phan. velutina, S. caerulea began to regress and mycelial biomass was reduced at a rate of 0.04 ± 0.01 mg d\(^{-1}\) (Table 3). Several Phan. velutina cords reached S. caerulea inocula by 28 d, and began colonizing as diffuse mycelia (Fig. 10a). Lateral Phan. velutina cords grew around S. caerulea extra-resource mycelium, enclosing the latter by 35 d (Fig. 10b). S. caerulea was replaced by 49 d, and inocula were well colonized by Phan. velutina which had thickened resource connected cords (Fig. 10c,d). The maximum extra-resource biomass attained by Phan. velutina during replacement of S. caerulea was significantly (P < 0.05) less than in systems with inert-baited controls (Fig. 5j). Mycelial distribution clearly altered during interaction between S. caerulea and Phan. velutina. Both D_BM and D_RX of S. caerulea were significantly (P £ 0.05) reduced from the time of contact with Phan. velutina until being replaced by 49 d (Fig. 8g,h). By contrast, only D_RX of Phan. velutina was significantly (P £ 0.05) reduced compared to inert controls (Fig. 5i).

S. caerulea and Phal. impudicus mycelia made contact by 21 d. S. caerulea producing mycelial ridges up to 5 mm wide, which appeared most dense around invasive Phal. impudicus.
cords (Figs 4b and 9c). Phal. impudicus extended through S. caerulea mycelium at a rate similar to controls (Table 2, P > 0.05) and mycelium reached the soil tray edges by 63 d (Fig. 4d). The morphology of Phal. impudicus did not alter (as quantified by fractal dimension; P > 0.05) whilst invading S. caerulea (Fig. 5h,i) although some Phal. impudicus cords were halted at ridges of S. caerulea mycelium and became superseded by more invasive cords. The number of major radial cords in interactive regions was c. 35% less (P £ 0.01, data not shown) than noninteraction controls, and biomass was significantly (P £ 0.05) reduced compared to controls (Fig. 5g). Total mycelial biomass production by S. caerulea was also significantly (P £ 0.05) reduced compared to controls (Fig. 5g). H. fasciculare produced dense discoloration in localized regions directly in contact with S. caerulea mycelial cords (Fig. 9e,f). Marginal mycelia of H. fasciculare became less uniform in interaction zones compared with noninteractive margins (Fig. 9e). By 35 d. H. fasciculare produced dense (DBM = 1.83 – 0.03) yellow pigmented lateral mycelial fans (Fig. 3c) which emerged after initial contact with S. caerulea in two systems and rapidly colonized open soil regions (Fig. 3d). Lateral mycelia of both species continued to extend at 90° to initial confrontation, filling open soil areas by 49 d. Slowly extending (Table 2) mycelial fronts formed diagonally between both species, persisting even as mycelia thinned markedly by 124 d (Fig. 3e).

Fig. 3 (a) Regrowth of Hypholoma fasciculare following colonization of a beech wood control, after 49 d. Note regrowth from 'stalled' margins on emergence from wood bait (arrowed). (b–e) Interaction between Stropharia caerulea (top right) and H. fasciculare. (b) Contact between mycelial systems after 28 d. Note mycelial displacement of H. fasciculare (arrowed). (c) Dense fan production by H. fasciculare after 35 d (arrowed). (d, e) Development of regions of quiescent 'no man's land' between mycelial systems (arrowed) after 63 d and 124 d, respectively, with considerable mycelial thinning by the latter. (f) Mutual replacement of H. fasciculare and S. caerulea in a different soil system to b–e, after 96 d. Directions of replacement are arrowed. Scale bars, 2 cm.
interaction zone, and in two systems, extending around *S. caerulea* and *Phal. impudicus*. (b) Localized aerial tufts of *S. caerulea* mycelium (arrowed) around *Phal. impudicus* cords forming after 28 d.
(c) Temporary ridge formation (arrowed) by *S. caerulea* (top right) after 28 d. (d–f) Decline of ridge as *Phal. impudicus* grows through *S. caerulea* after 63 d (d), 96 d (e) and 124 d (f). Scale bars, 2 cm (except (b) scale bar, 1 cm).

**Table 2** Extension rates (mm ± SEM) of mycelial systems on nonsterile soil with either an inert control bait, uncolonized beech wood bait or interacting another mycelial system. Figures in the same column with different letters are significantly different (*P* £ 0.05; **P** £ 0.01). Interspecies significant differences in inert controls are indicated by a different number

<table>
<thead>
<tr>
<th>Species on this row in interaction with:</th>
<th><em>Stropharia caerulea</em></th>
<th><em>Agrocybe gibberosa</em></th>
<th><em>Hypholoma fasciculare</em></th>
<th><em>Phallus impudicus</em></th>
<th><em>Phanerochaete velutina</em></th>
</tr>
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<tr>
<td>Inert control</td>
<td>1.8 ± 0.2a,1</td>
<td>3.7 ± 0.1a,2</td>
<td>1.6 ± 0.3a,1</td>
<td>2.6 ± 0.1a,3</td>
<td>6.1 ± 1.1d,4</td>
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<td>Wood control</td>
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<td>2.4 ± 0.6a</td>
<td>3.0 ± 0.2b</td>
<td>1.9 ± 0.1b</td>
<td>7.2 ± 1.1d</td>
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<td>–</td>
<td>2.5 ± 0.2a</td>
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<tr>
<td>Post wood contact</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.6 ± 0.2b</td>
<td>–</td>
</tr>
<tr>
<td><em>S. caerulea</em></td>
<td>2.4 ± 0.1a</td>
<td>3.0 ± 0.3a</td>
<td>1.6 ± 0.3a</td>
<td>2.8 ± 0.1a</td>
<td>5.3 ± 0.5d</td>
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<td><em>P. impudicus</em></td>
<td>2.4 ± 0.1a</td>
<td>3.8 ± 0.6b</td>
<td>1.0 ± 0.1c</td>
<td>0.3 ± 0.01d**</td>
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<td><em>P. velutina</em></td>
<td>0.03 ± 0.01d**</td>
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Interaction zones, and in two systems, extending around *S. caerulea* at rates similar to controls (Table 2, *P* £ 0.05). *A. gibberosa* completely encircled *S. caerulea* mycelium by 63 d (Fig. 2c). *S. caerulea* extended at control rates (Table 2) in two systems until enclosed by *A. gibberosa*, the former producing mycelial ridges at the interaction margin by 20 d (Fig. 2a). Since significant overall reduction in biomass by *S. caerulea* (Fig. 7c), dense (*DMM* = 1.89 ± 0.04) localized fans of *S. caerulea* mycelium were produced in two systems, extending over *A. gibberosa* (Fig. 2d). The production of bridging fans by
S. caerulea coincided with a 40% reduction in hyphal cover in the remaining S. caerulea mycelium, which persisted as thickened cords (Fig. 2d). However, in one rapidly extending S. caerulea system (Table 2) the reverse occurred by 63 d, where S. caerulea produced a wide (1.6–0.4 cm) aerial ridge at the interaction front which reduced the extent of A. gibberosa (Fig. 2e). S. caerulea cords emerged extending over A. gibberosa mycelium by 93 d until harvest (Fig. 2f).

Effect of interactions on inoculum decay
There was no significant (P > 0.05) effect of interspecific mycelial interactions upon inoculum decay rates for S. caerulea, although decay rates ranged from 1.0 to 1.8 mg cm\(^{-3}\) d\(^{-1}\). Similarly there was no significant (P > 0.05) difference in inoculum decay rates between controls and interaction systems of other cord-formers (0.7–1.6 mg d\(^{-1}\)).

Fig. 5 Mycelial development of (a–c) Agrocybe gibberosa (d–f) Hypholoma fasciculare (g–i) Phallus impudicus and (j–l) Phanerochaete velutina. (a, d, g, j) Mycelial biomass; (b, e, h, k) surface fractal dimension and (c, f, i, l) mass fractal dimension in inert control (squares), wood control (triangles) and interactive with Stropharia caerulea (circles). Biomass error bars ± SEM. Fractal error bars omitted for clarity, but < 5% of mean. M, time of mycelial contact in interactions; B, mycelial contact with beech wood block control. Significant differences between interaction treatment and both controls indicated by *P ≤ 0.05, **P ≤ 0.01 and one control by †P ≤ 0.05, ††P ≤ 0.01.
The foraging patterns of mycelia developing in control systems with additional inert or wood resources were similar to those in previous studies (Boddy, 1999). Phal. impudicus and Phan. velutina, with their rapidly extending mass fractal structures, characterized by well-defined, rapidly extending cords throughout the system might be considered as longer range foragers (equivalent to ‘guerrilla’ foraging plants) (Boddy, 1999). They are unsuccessful at capitalizing on relatively homogeneously supplied nutrients, but successful at discovering large, sparsely distributed resources – fallen branches, trunks and stumps, with little colonization of leaf litter. A. gibberosa, which is also mass fractal but produces low biomass from wood inocula (with a low final \( D_{BM} \) – 1.4), presumably exhibits similar foraging strategies, but tends to utilize graminaceous material (Robinson et al., 1993).

**Table 3** Biomass production rates (mm d\(^{-1}\) ± SEM) of mycelial systems on nonsterile soil with either an inert control bait, uncolonized beech wood bait or another mycelial system of *S. caerulea*. Figures in the same column with asterisks are significantly different (*\( P \leq 0.05 \), **\( P \leq 0.01 \), ***\( P \leq 0.001 \)) from controls; figures in the same row with different letters are significantly different (\( P \leq 0.05 \))

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<th>Phanerochaete velutina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inert control</td>
<td>0.34 ± 0.05*</td>
<td>0.95 ± 0.14*</td>
<td>1.74 ± 0.32**</td>
<td>1.53 ± 0.12**</td>
<td>2.25 ± 0.52b</td>
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<td>Wood control</td>
<td>0.19 ± 0.04</td>
<td>0.71 ± 0.11</td>
<td>1.7 ± 0.34</td>
<td>1.03 ± 0.07</td>
<td>2.47 ± 0.3</td>
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<tr>
<td><em>S. caerulea</em></td>
<td>0.30 ± 0.15</td>
<td>0.48 ± 0.09**</td>
<td>1.35 ± 0.9</td>
<td>0.73 ± 0.1***</td>
<td>1.45 ± 0.3</td>
</tr>
<tr>
<td>A. gibberosa</td>
<td>0.07 ± 0.05</td>
<td></td>
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<tr>
<td>H. fasciculare</td>
<td>0.10 ± 0.04</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>P. impudicus</em></td>
<td>0.09 ± 0.06**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. velutina</em></td>
<td>0.04 ± 0.01***</td>
<td></td>
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</tbody>
</table>

Fig. 6 *Stropharia caerulea* self mycelial interactions after (a) 21 d; (b) 63 d and (c) 124 d; note thickening of first fused cords and thinning of both mycelial systems by 124 d. (d) Tip to tip (TT) and tip to side (TS) cord fusions after 28 d. (e) Fusion line (arrowed) formed between systems after 21 d. Scale bars, 2 cm.

**Discussion**

The foraging patterns of mycelia developing in control systems with additional inert or wood resources were similar to those in previous studies (Boddy, 1999). Phal. impudicus and Phan. velutina, with their rapidly extending mass fractal structures, characterized by well-defined, rapidly extending cords throughout the system might be considered as longer range foragers (equivalent to ‘guerrilla’ foraging plants) (Boddy, 1999). They are unsuccessful at capitalizing on relatively homogeneously supplied nutrients, but successful at discovering large, sparsely distributed resources – fallen branches, trunks and stumps, with little colonization of leaf litter. A. gibberosa, which is also mass fractal but produces low biomass from wood inocula (with a low final \( D_{BM} \) – 1.4), presumably exhibits similar foraging strategies, but tends to utilize graminaceous material (Robinson et al., 1993). By contrast, the surface fractal structures, produced by
H. fasciculare, characterized by abundant mycelial biomass, diffuse, slowly extending search fronts with a more clearly defined margin, might be considered as an adaptation to short-range foraging, searching areas intensively and likely to be successful in discovering abundant, relatively homogeneously supplied resources (Boddy, 1999). Indeed, though a common decomposer of larger wood resources, H. fasciculare frequently colonizes leaves and small twigs, cupules, etc. in the field (Dowson et al., 1988b). S. caerulea, with its slowly extending but responsive mycelium, is commonly found on nutrient rich sites, colonizing Urtica rhizomes and other small woody and nonwoody litter.

To some extent correlated with these differences in foraging behaviour, these species exhibited differences in responses to encounter with extra-resource mycelium. Thus the long range foragers Phan. velutina and Phal. impudicus successfully replaced S. caerulea, being much less sensitive to the latter than were other combatants. The well-defined, well-insulated (sensu Rayner, 1991) cords of Phan. velutina even extended into S. caerulea territory without slowing. Likewise, Phal. impudicus extended at similar rates to controls, though some cords were halted by the mycelial ridges produced by S. caerulea, and were then superseded by more invasive cords. This relative insensitivity parallels that shown when encountering new resources (Dowson et al., 1986, 1988a); whereas many cord-forming saprotrophic basidiomycetes reallocate biomass on encountering new resources Phan. velutina and Phal.

**Fig. 7** Stropharia caerulea biomass in (a) inert control (open squares), wood control (open triangles) or (b) mycelia interacting with self, wood blocks originally colonized in different flasks (closed squares) (closed triangles), mycelia interacting with (c) Agrocybe gibberosa (closed circles), Hypholoma fasciculare (closed squares) (d) Phallus impudicus (closed diamonds) or Phanerochaete velutina (open circles). Error bars ± SEM. Asterisks indicate significant differences (*P ≤ 0.05) of interacting mycelia from noninteractive controls. Daggers indicate significant differences between mycelia developing from wood blocks originally colonized in different flasks (†P ≤ 0.05, †††P ≤ 0.001).

**Fig. 8** Surface fractal dimensions (a, c, e, g) and mass fractal dimensions (b, d, f, h) of mycelial systems of Stropharia caerulea in (a, b) inert control (open squares), wood control (open triangles) or (c, d) mycelia interacting with self, wood blocks originally colonized in different flasks (closed squares) (closed triangles); (e, f) Agrocybe gibberosa (closed squares); Hypholoma fasciculare (closed circles); (g, h) Phallus impudicus (closed diamonds); Phanerochaete velutina (open circles). Error bars omitted for clarity, but ± 5% of mean. Asterisks indicate significant differences (*P ≤ 0.05) of interacting mycelia from noninteractive controls.
*Phal. impudicus* only do so when resources are encountered that are considerably larger than that already occupied. By contrast, to these long-range foragers, *H. fasciculare* and *A. gibberosa* deadlocked or were sometimes partially or completely replaced, and their mycelial morphology changed. Though, as in previous studies (cf. Dowson et al., 1988c), mycelial responses of the long range foragers during interactions were less obvious than for other species, all mycelia did respond to the presence of antagonists after mycelial contact (no changes were evident before contact). With both *Phan. velutina* and *Phal. impudicus* extra-resource biomass was reduced, and with the former $D_{BM}$ (but not $D_{BS}$) declined, while in the latter the number of major cords in interaction regions was approx. 35% less than in controls. Furthermore, the colour of *Phan. velutina* mycelium changed in zones where *S. caerulea* was being replaced, resembling colour changes during interactions in agar culture resulting from oxidation of phenolics by phenoloxidase and peroxidase (White & Boddy, 1992a,b). With *Phal. impudicus* localized changes in pigment occurred occasionally where cords crossed *S. caerulea* mycelium, and lysis was restricted to small (< 1 cm) segments of cords; by analogy with ‘hyphal interference’ this has previously been termed ‘mycelial interference’ (Dowson et al., 1988c). Pigment production may reflect sealing off of hyphal boundaries by polymerization of water resistant ‘hydrophobins’ within the hyphal walls, providing protection from attack (Griffith et al., 1994a,b,c). Biomass production may be inversely related to phenoloxidase production (Johansson

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**Fig. 9** (a) Colour shift in mycelium of *Phanerochaete velutina* over-growing *Stropharia caerulea* after 14 d. (b) Close up of *Phan. velutina* mycelium showing intense colour reaction over most dense *S. caerulea* mycelial regions. (c) Discrete mycelial cords of *Phal. impudicus* growing through diffuse mycelia of *S. caerulea* after 28 d. (d) Localized lysis of *Phal. impudicus* cord (arrowed) overlying *S. caerulea* cords after 93 d. (e, f) Localized pigmentation of *Hypholoma fasciculare* mycelium around *S. caerulea* cord tips. Scale bars (a–c, e, f), 2 cm; (d), 0.4 mm.
et al., 1999), which might partially explain decrease in the former during interactions.

The emergence of distinct mycelial morphologies where mycelial fronts of different species meet is a well documented effect of interactions in agar culture (Rayner et al., 1995; Boddy, 2000), and is clearly seen here on soil. The reduction of \( D_{BM} \) may be due to emergence of aerial ridge mycelia at interactive margins, most notably seen with \( S. caerulea \). A standing ridge proved effective in preventing penetration by diffuse foraging mycelia of \( H. fasciculare \) (though the latter did break through in places as dense fans) and the numerous small cords of \( A. gibberosa \). However, the much more rhizomorphic cords of \( Phal. impudicus \) breached this defence and the aerial ridge disappeared when cords of the latter reached the \( S. caerulea \) inoculum wood block, possibly indicating loss of supply to the \( S. caerulea \) mycelial margin from the resource base. Induction of hyphal aggregation to form cords when diffuse mycelia encounter other organisms is well known (Robinson et al., 1992; Rayner et al., 1995; Boddy, 2000), and highlights the increased combative ability following aggregation. Localized increase in \( D_{BM} \) of emergent mycelial fans or ridges and concomitant decrease in \( D_{BM} \) of the rest of the mycelium, is
indicative of supply to the emergent phase by the mycelial network, facilitating mycelial overgrowth of hostile areas. Reduced $D_{BM}$ of the rest of the mycelium may, however, render the colony more susceptible to invasion if the interfacial region is broached (Rayner et al., 1995). There is qualitative (White & Boddy, 1992a,b) and quantitative (Griffith et al., 1994a,b,c) evidence that emergent phases at interaction fronts are correlated with changes in phenoloxidase activity and induction of secondary metabolism leading to pigment production. Pigment production was seen when Phan. velutina and Phal. impudicus cords crossed S. caerulea mycelium (as discussed above), and H. fasciculare also produced increased pigmentation around regions of contact with S. caerulea, and also on production of dense emergent mycelial fans. However, phenoloxidases are not expressed by all species at the same time during interspecific interactions (White & Boddy, 1992a).

Discussion so far has largely concerned interactions between extra-resource mycelia. However, interactions also take place within resources when extra-resource mycelium has crossed or replaced that of the antagonist and reached a resource. Interactions are then between extra-resource mycelium and intrasource mycelium, and subsequently interactions will take place within the resource. The outcomes of interactions within wood are not necessarily the same as those between extra-resource mycelia (Dowson et al., 1988c), though this probably depends to some extent on whether intrasource mycelium is being fed from within the resource alone or is still being supported by a large extra-resource mycelial system. When opponents occupy only relatively small resources, as in the present study, a combattant that has reached its opponent’s resource base will undoubtedly be able to draw on its established mycelium. A ‘two-pronged’ attack – of both extra-resource mycelium and at the resource base – was particularly effective for Phan. velutina here, replacement of S. caerulea occurring more rapidly when mycelium of the former reached the resource base of the latter.

Only one strain of each species was used in this study, so extrapolations must be made cautiously. However, not only might outcome of interactions vary depending on strain, outcomes can vary depending on where they are occurring (see above) and can be influenced by microenvironment and the presence of other organisms (Boddy, 2000). Further, outcomes of interactions involving the same genetic individual are not always the same, even under apparently identical conditions. In 20 replicate encounters of Corticium versicolor vs Peniophora lycii in agar culture, three different interaction patterns were discerned: in two of these C. versicolor replaced P. lycii, and P. lycii replaced C. versicolor in the other (Rayner et al., 1995). Such differences were also evident in the present study: in one replicate S. caerulea was able to encroach into the territory occupied by Phal. impudicus extra-resource mycelium after 96 d, rather than itself being replaced. In two systems fans of S. caerulea mycelium extended over A. gibberoua; in the other system there was deadlock. S. caerulea deadlocked with H. fasciculare but in one system there was replacement of the one fungus in one region and replacement of the other fungus in another region. Clearly, there is often a delicate balance, with internal and external factors sometimes causing a shift in the balance. Variability in responses is dramatically illustrated by comparing responses of S. caerulea to mycelium of different species and changes with time. Thus, for example, aerial ridges were formed against the invasion front of Phal. impudicus which subsequently disappeared when the latter broke through and reached the inoculum base; contact with the foraging front of H. fasciculare resulted in a flattened morphology of S. caerulea followed by aerial ridge formation; against A. gibberoua defensive ridges and sometimes fans and mycelial cords were produced; but against rapid and aggressive replacement by Phan. velutina there were no dramatic changes in morphology. The underlying triggers and associated biochemical changes remain to be elucidated.

In summary, this paper confirms and considerably extends results of previous studies on outcomes of interactions between saprotrophic basidiomycetes in model soil systems (Dowson et al., 1988c; Robinson et al., 1993; Holmer & Stenlid, 1996, 1997). Image analysis has proved extremely valuable in allowing detailed examination of specific regions, and together with fractal geometry has enabled quantification of changes in mycelial morphology and distribution during interactions. Extra-resource mycelia biomass and morphology (both visual and as quantified by fractal dimension) sometimes changed dramatically during interactions in soil, short-range foragers tending to respond more than long-range foragers. Though the outcome of mycelial confrontations in soil can determine whether or not one fungus or another will prevail, some species circumvented the extra-resource mycelial challenge by overgrowing the opponents mycelium and challenging mycelium within the resource directly. It would be predicted that similar behaviour is likely to occur on the forest floor, though field studies are still urgently awaited.

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