

1 **Space and patchiness affects diversity-function relationships in fungal decay communities**

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3 Running title: Diversity-function relationships

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15

16 **Abstract**

17 The space in which organisms live determines health and physicality, shaping the way in which they
18 interact with their peers. Space, therefore, is critically important for species diversity and the function
19 performed by individuals within mixed communities. The biotic and abiotic factors defined by the
20 space that organisms occupy are ecologically significant and the difficulty in quantifying space-defined
21 parameters within complex systems limits the study of ecological processes. Here, we overcome this
22 problem using a tractable system whereby spatial heterogeneity in interacting fungal wood decay
23 communities demonstrates that scale and patchiness of territory directly influence coexistence
24 dynamics. Spatial arrangement in 2- and 3- dimensions resulted in measurable metabolic differences
25 that provide evidence of a clear biological response to changing landscape architecture. This is of vital
26 importance to microbial systems in all ecosystems globally, as our results demonstrate that
27 community function is driven by the effects of spatial dynamics.

28

29

30 **Introduction**

31 Space determines the nature of and scale over which individuals meet and interact. The characteristics
32 of the discrete spatial habitat which an organism occupies affects individual competitive success with
33 a bottom-up effect on population-wide colonisation, speciation and extinction [1]. There is a dynamic
34 link between spatial ecology and competitive success where transitive (where species A outcompetes
35 B, which outcompetes C) communities with a strict competitive hierarchy become intransitive ($A > B$;
36 $B > C$; $C > A$, like the game of rock-paper-scissors) when competing in a spatially more complex
37 system [2], allowing individuals outcompeted under some scenarios to coexist with their
38 competitors [3]. Dimensionality of habitat landscapes influences individual behaviour [4], and
39 stochasticity of species interactions results in changes to the pool of community-produced
40 metabolites [5], altering individual combative ability, community succession and structure between
41 2- and 3-dimensional landscapes [6]. Despite these findings, the mechanisms that influence stability
42 and succession in the context of how communities occupy and exploit space are rarely adequately
43 quantified as most ecological study systems are too complex and largely intractable. A model system
44 is needed that allows such quantification, and understanding of how altered dynamics, coexistence
45 and community-scale biodiversity in the context of space underpins changes in community function.

46 The functional diversity-area relationship, i.e. the correlation between increased habitat size and
47 greater functional diversity, is one theory explaining how space mediates function in biodiverse
48 communities [7]. However, the model does not account for effects of distributions and patch dynamics
49 of species within habitats of varied area, yet these factors cause competitive communities to shift
50 between hierarchical transitive and non-hierarchical intransitive relationship states [2,8].
51 Non-hierarchical intransitivity is an established mechanism of coexistence [9,10] and is thought to be
52 associated with an intrinsically related functional-diversity mechanism [11,12]. Wood decay fungi
53 offer an ideal ecologically relevant model for the study of these processes as they occupy columns of
54 decay forming complex 3-dimensional communities in wood and, through their decomposition

55 activities release carbon and nutrients [13]. They typically form a hierarchical community structure
56 (tertiary/late stage colonists outcompete secondary colonists which in turn outcompete
57 primary/earliest colonists) [14] and competitive interactions between species can be easily observed
58 [15]. In addition to compounds that primarily function in the exploitation and decomposition of
59 lignocellulose [16,17], these fungi produce a plethora of potentially antagonistic compounds which
60 function in changing territory, and differ in quantity and identity during interspecific competition
61 [6,18,19,20].

62 Here, we used a tractable system of wood decay fungi to quantify the impact of space on the
63 mechanisms of coexistence and community composition, in the context of its occupation and
64 exploitation. We compared the combative abilities of fungi in linear 2-dimensional systems and
65 species richer 3-dimensional systems. The study system allowed more detailed analysis of community
66 dynamics between 3-dimensional systems where fungi were dispersed in evenly spaced patches and
67 3-dimensional systems where the weakest member of the community occupied the same volume but
68 as a larger adjacent patch size. Previously, our data revealed an emergent property where
69 intransitivity promoted biodiversity in more spatially diverse 3-dimensional systems where territory
70 was less fragmented [2]. Here, we assess the underlying mechanisms causing community stability and
71 coexistence dynamics to change. We do this by measuring the metabolic response of an individual to
72 changing coexistence dynamics across spatial scales. Our large-scale untargeted metabolomics and
73 other chemical methods analysed a comprehensive network of intracellular, extracellular and
74 gas-phase metabolic products produced during community interactions. We hypothesised that
75 stochasticity of species would influence functional biochemical processes, and that changes to
76 metabolites involved in pathways for resource utilisation and antagonism would alter coexistence
77 dynamics and community composition. Spatially heterogeneous systems containing non-hierarchical
78 communities promote biodiversity [2,21], and here we deepen our understanding of this concept with
79 the novel finding that space occupied alters metabolic function and coexistence, therefore moderating
80 the diversity-function relationship.

81

82 **Methods**

83 *Experimental design and sampling*

84 We constructed pair-wise interactions of 2 cm³ *Fagus sylvatica* (beech) blocks that had been
85 pre-colonised for 12 weeks by placing on agar (5 gL⁻¹ malt extract, 15 gL⁻¹ agar; Lab M, UK) cultures
86 of *Hypholoma fasciculare*, *Trametes versicolor* and *Vuilleminia comedens* wood decay fungi at 20 °C
87 (as in [2]). Interactions were performed in all combinations including, self-pairings (n = 10). We also
88 constructed 3x3x3 27-block cubes containing 9 blocks of each species. 27-block cubes were arranged
89 with all three species dispersed and no two blocks of the same species in contact (n = 10), and also so
90 that all 9-blocks of the weakest competitor, *V. comedens*, occupied an adjacent volume while the
91 other two fungi were dispersed (n = 10) (Fig. 1). Plus, entire 27-block assemblages containing each
92 fungus solely were made [2]. Blocks in pair-wise interactions were arranged with cut vessel ends
93 touching, and in 27-block cubes blocks were joined such that some cut vessel ends were touching but
94 others were not, but all vessels were parallel (Fig. 1). Fungal species and specific strains were selected
95 based on their successional order and expected combative hierarchy in the natural environment:
96 *H. fasciculare* > *T. versicolor* > *V. comedens* [22,23,24] (Supplementary Table 1). Interacting
97 combinations of wood blocks were incubated individually at 20 °C in 70 ml and 500 ml polypropylene
98 pots for pair-wise and 27-block cubes respectively, and were laid upon a layer of perlite (20 ml and
99 85 ml) containing 2 ml and 12 ml of water respectively, as detailed in [2]. After 1 d and 28 d volatile
100 organic compound (VOC) production was measured (described below), and we deconstructed n = 5
101 systems. Each individual block was split along the grain into quarters, and three of the quarters from
102 each block were foil wrapped, flash frozen in liquid nitrogen and stored at -80 °C. These were
103 subsequently analysed for enzyme activity and metabolomics (see below). For the remaining quarter,
104 two chips (~2 mm³) were taken from an inside face and inoculated onto 2 % malt agar, incubated at
105 20 °C, then emerging mycelia identified morphologically, to determine which species occupied the

106 wood (Fig. 2). We removed the chip excised face from the quarter by splitting with a chisel, and
107 determined final density as dry weight (80 °C for in excess of 72 h) per fresh volume (cm³) (blocks
108 sampled at 28 d only), and the rate of decay estimated by comparison with density of blocks scarified
109 (n = 10) at the end of the pre-colonisation period.

110

111 *Overview of metabolite analysis*

112 To quantify changes to metabolic function (antagonistic chemicals and compounds for habitat
113 exploitation/wood decomposition) associated with different spatial dynamics in response to changing
114 community composition, we extracted and measured: the profile of VOCs from the headspace of
115 interactions, the activity of 12 targeted enzymes, chosen because they are directly involved in
116 interspecific competition [6,19]. We also conducted ultra-high performance liquid chromatography-
117 mass spectrometry (UHPLC-MS) metabolomics analysis. All analyses were conducted after 1 and 28 d
118 interactions in blocks originally colonised by *T. versicolor* (n = 5), and in 27-block interactions
119 ‘pseudo’-replicates representing different spatial locations within 3-dimensional cube arrays were
120 analysed (all n = 5). We chose to target *T. versicolor* since the fungus has been well characterised in
121 the past [16,19,25], and because it neither dominated systems (as did *H. fasciculare*) nor was it driven
122 to near extinction (as was *V. comedens*). There were no significant differences (ANOVA: p > 0.05) in
123 enzyme activity or small metabolite levels between pseudoreplicates of *T. versicolor* in 3-dimensional
124 cubes, so we pooled activities of all pseudoreplicates for each system (therefore, n = 15).

125

126 *VOC extraction and data-pre-processing*

127 We collected VOCs from the headspace of interactions after 1 d and 28 d (n = 3) by inserting pots
128 individually and lidless into a multi-purpose roasting bag (46 × 56 cm; Lakeland, UK), which was sealed
129 for 30 min to allow VOCs to equilibrate in the headspace. Then, 500 ml headspace gas was collected

130 onto thermodesorption (TD) tubes (Tenax TA & Sulficarb, Markes International Ltd.) using an EasyVOC
131 manual pump (Markes International Ltd., UK).

132 VOCs were desorbed using a TD100 thermodesorption system (Markes International Ltd., UK) with
133 the following settings: tube desorption 10 min at 280 °C, at a trap flow of 40 ml min⁻¹; trap desorption
134 and transfer 40 °C s⁻¹ to 300 °C, with a split flow of 20 ml min⁻¹ into gas chromatograph (GC; 7890A;
135 Agilent Technologies Inc., USA). VOCs were separated over 60 m, 0.32 mm I.D., 0.5 µm Rx5ms (Restek,
136 UK) with 2 ml min⁻¹ helium as carrier gas under constant flow conditions using the following
137 temperature program: 35 °C for 5 min, 5 °C min⁻¹ to 100 °C, hold 5 min. Mass spectra were recorded
138 from *m/z* 30 to 350 on a time-of-flight mass spectrometer (BenchTOF-dx, Markes International Ltd.,
139 UK). C8-C20 alkane standard (0.5 µl, Supelco) was loaded onto a blank thermodesorption tube as a
140 retention standard and quality control (QC).

141

142 *Enzyme assays*

143 For enzyme assays, we freeze dried the *T. versicolor* frozen blocks for 48 h (Edwards Modulyo, UK),
144 then ground them to sawdust using a spice and coffee grinder (Wahl James Martin, UK). 0.5 g of
145 sawdust was added to 5 ml of 50 mM sodium acetate buffer and shaken overnight at 4 °C. For pair-
146 wise interactions, *T. versicolor* blocks from all interactions and one block from its respective
147 self-pairing were used (n = 5). In 27-block cubes, each fungus occupied 3 different spatial positions in
148 both of the mixed-species assemblages, and *T. versicolor* occupied 4 spatial positions within the
149 assemblage which it fully occupied. For each spatial position for *T. versicolor* blocks within 27-block
150 systems (excluding the central-cube position which *T. versicolor* occupied when it was the sole
151 occupant, as this was not represented in the mixed-species assemblages), 5 replicates were used for
152 assays (full details in Supplementary Table 2).

153 The activities of the following terminal hydrolases were measured using 4-methylumbelliferol
154 (MUF)-based substrates: β -glucosidase (EC 3.2.1.21), α -glucosidase (EC 3.2.1.20), cellobiohydrolase
155 (EC 3.2.1.91), β -xylosidase (EC 3.2.1.37), N-acetylglucosaminidase (EC 3.2.1.30), phosphodiesterase
156 (EC 3.1.4.1), phosphomonoesterase (EC 3.1.3.2) and arylsulfatase (EC 3.1.6.1). Briefly, substrates
157 (40 μ l in dimethylsulphoxide) at final concentration of 500 mM were combined with three technical
158 replicates of 200 μ l of samples (diluted 1:10) in a 96 well plate. Background fluorescence was
159 determined by combining 200 μ l sample (diluted 1:10) with 40 μ l MUF standards. The 96 well plates
160 were incubated at 40 °C and fluorescence recorded at 5 and 125 min using a Tecan Infinite microplate
161 reader (Tecan, Switzerland) with an excitation wavelength of 355 nm and an emission wavelength of
162 460 nm. Quantitative enzymatic activities were calculated after blank subtraction based on a standard
163 curve of MUF. One unit of enzyme activity was defined as the amount of enzyme releasing 1 nmol of
164 MUF min⁻¹.

165 Laccase (phenoloxidase; EC 1.10.3.2) activity was determined by monitoring the oxidation of 2,2'-
166 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in citrate phosphate buffer
167 (100 mM citrate, 200 mM phosphate, pH 5.0), by monitoring the formation of green colouration
168 spectrophotometrically at 420 nm. Three technical replicates were performed for each sample.

169 Manganese peroxidase (MnP; EC 1.11.1.13) activity was determined by monitoring
170 spectrophotometrically at 595 nm the purple colouration from oxidative coupling of
171 3-methyl-2-benzothialone-hydrazone hydrochloride (MBTH) and 3-(dimethyl amino)-benzoic acid
172 (DMAB) in succinate-lactate buffer (100 mM, pH 4.5). Three technical replicates were performed for
173 each sample. The results were corrected by activities of samples without manganese, and with
174 ethylene diamine tetraacetate (EDTA) to chelate any Mn²⁺ present in the samples, allowing detection
175 of Mn²⁺-independent peroxidases (versatile peroxidase). The results were also corrected by activities
176 of samples in the absence of H₂O₂, allowing detection of oxidase (but not peroxidase) activity.

177 For each enzyme, n = 3 technical replicates were performed and enzyme activities were normalised
178 to the protein content of each sample, which was determined using Qubit™ fluorometric assays
179 (ThermoFisher Scientific Inc., UK).

180

181 *Metabolomics analysis*

182 As for enzyme assays, we selected blocks pre-colonised by *T. versicolor* (n = 5) upon which to perform
183 UHPLC-MS (Supplementary Table 2). 0.5 g of sawdust was added to 1666 µl of each of H₂O, methanol
184 and chloroform, vigorously vortexed and sonicated for 15 min (Elmasonic S150, Singen, Germany).
185 The extracts were allowed to sit until the polar (containing H₂O and methanol) and non-polar
186 (containing mostly chloroform) layers separated, then removed 1500 µl of the upper layer containing
187 the polar metabolite extracts. The extracts were centrifuged for 5 min at 17,000 x g (Biofuge, Thermo
188 Fisher Scientific, MA, USA), and 200 µl of supernatant removed and dried *in vacuo* (Thermo Savant,
189 NY, USA) for ca. 3 h. We then stored extracts at -80 °C until metabolomics analysis.

190 An intrastudy quality control (QC) sample was prepared by pooling small aliquots of all samples, and
191 the single extract was removed and dried down by centrifugation (20,000 x g for 10 min at 4 °C,
192 Biofuge). UHPLC-MS based metabolomics was performed (20 µl per sample) on a Thermo Dionex
193 Ultimate 3000 RS system with a Thermo Scientific Q Exactive Orbitrap mass spectrometer. Samples
194 (20 µl) were separated over a 100 x 2.1 mm, 1.9 µm particles, C18 column (Thermo Hypersil Gold) at
195 a flow rate of 400 µl min⁻¹ using a 14 min linear gradient programme from 0.1 % formic acid in water
196 to 0.1 % formic acid in methanol. MS acquisition started at 0.1 min, with the flow up to 0.45 min
197 directed towards waste. We acquired data in positive ion and profile mode from *m/z* 100-1000 at
198 70,000 resolution. Samples were analysed in a controlled randomised order, with the intrastudy QC
199 sample repeatedly analysed equidistantly between the biological samples.

200

201 *Metabolomics data processing*

202 To process the UHPLC-MS data, the Thermo.raw data files in profile mode were converted into mzML
203 format in centroid mode using MSConvert (Proteowizard 3.0.7665). Data were then aligned using an
204 R (3.2.0) based XCMS and CAMERA script (both R packages [26,27]) which resulted in a csv file intensity
205 matrix (containing 9309 features, i.e. peaks in the mass spectra). This matrix was imported into
206 MatLab and inserted into a direct infusion mass spectrometry (DIMS) SIMStitch workflow [28] where
207 a blank filter of > 2x sample over blank signal was applied, and a sample filter of peak-presence of at
208 least 50 % of all samples [29]. The matrix was further processed by probabilistic quotient
209 normalisation (PQN) and subsequently missing values were imputed using K-nearest neighbour (KNN)
210 with $k = 5$. The imputed data matrix was used as an input to univariate statistics, including calculation
211 of fold-changes. For multivariate statistics, a g-log transformation of the imputed data matrix was
212 additionally applied, using an assessment of the technical variance across the repeated measurements
213 of the intrastudy QC sample [29].

214 We putatively annotated UHPLC-MS features by inputting m/z values and their associated mean
215 intensities into MI-Pack software version 2 beta [30], where metabolites were compared against the
216 Kyoto Encyclopaedia of Genes and Genomes (KEGG) database. A set of highly significant metabolites
217 (ANOVA: $p < 0.001$) were further searched against the KEGG database to determine possible roles
218 within metabolic pathways.

219

220 *Statistical analysis*

221 To analyse the rate of decay and progression of interactions, we used R statistical software [31]. For
222 each interaction, we assigned every species an individual score of combative ability, expressed as the
223 percentage of the total system that it finally occupied. Briefly, each competitor scored between 0 and
224 2 for each block within a system (since two regions of every block were isolated; no outgrowth of a

225 competitor from either isolation point scored 0, outgrowth from one isolation point scored 1,
226 outgrowth from both isolation points scored 2). Scores for all blocks within a system were combined
227 for each competitor individually, normalised to the number of replicates and converted to a
228 percentage of the total system colonised. The data were analysed using a General Linear Model
229 combined with Tukey *post hoc* tests, with individual block position (i.e. number of faces of that block
230 involved in direct combat) and access to water (water was added to the perlite, as such, in 27-block
231 interactions the layer laid on the perlite has greatest access to water) factored into the model. The
232 rate of decay of wood in all interactions was compared using a one-way ANOVA followed by Tukey
233 *post hoc* tests.

234 For enzyme activities, we used a one-way ANOVA with Tukey *post hoc* tests to compare mean activity
235 (from five replicates), or Kruskal-Wallis tests followed by a Dunn's test *post hoc* procedure when data
236 were non-normally distributed, in R statistical software [31].

237 For GC-MS (VOCs) data, mass spectra of the entire data set were analysed by principal components
238 analysis (PCA) to check for clustering of the QCs and, therefore, robustness of the data set (see
239 Supplementary Fig. 2 for QC clustering), using MetaboAnalyst 3.0 [32]. We then removed QCs from
240 the matrix, and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) ($Q^2 = 0.93$,
241 $R^2 = 0.96$), chosen for its cross-validation method which reduces false-positive results [33], was applied
242 to the standardised binned data to determine the degree of separation between the four major
243 sample groups: pair-wise samples 1 d and 28 d after interaction set up, and 27-block samples 1 d and
244 28 d after interaction set up. Next, we separated the data and applied OPLS-DA to pair-wise ($Q^2 = 0.57$,
245 $R^2 = 0.61$) and 27-block ($Q^2 = 0.37$, $R^2 = 0.55$) sample groups separately. The modelled covariance and
246 correlation were used to identify the compounds contributing most to the discriminant model
247 separation, and one-way analysis of variance (ANOVA) with a 5 % Benjamini-Hochberg false discovery
248 rate (FDR) correction for multiple comparisons [34], and Tukey *post hoc* tests were applied to those
249 compounds.

250 Lastly, for UHPLC-MS data we applied PCA to the g-log transformed data to explore the separation
251 between control samples (*T. versicolor* growing alone), interaction samples and QCs. The median
252 Relative Standard Deviation (RSD) for the intrastudy QC samples was 11.15 %, indicating that the MS
253 data were of sufficiently high quality for further statistical analysis (see Supplementary Fig. 3 for QC
254 clustering). After removing the QCs, analysis of variance simultaneous components analysis (ASCA)
255 was applied, and the model was permutation tested (5000 permutations) to determine the
256 significance of factors (sample day, and block position within systems) [35,36]. An additional ASCA
257 model was tested to determine the significance of species distribution patterns within cubes, i.e.
258 species being dispersed, *V. comedens* occupying a larger adjacent volume, or *T. versicolor* comprising
259 the entire system, but did not include spatial location within assemblages as a factor. Pair-wise
260 comparisons using ASCA were carried out for *post hoc* testing of significant effects, and the *p*-values
261 were adjusted for multiple testing using a 5 % Bonferroni-Hochberg multiple testing correction [37].
262 Univariate analysis of variance (ANOVA) was applied to the whole normalised matrix with a 5 % FDR
263 correction [34] to test for significant metabolites. Finally, we determined fold changes (FC) between
264 significant groups.

265

266 *Network analytics*

267 We investigated the synergy of metabolites produced by *T. versicolor* during all interactions by
268 creating a co-occurrence Force Atlas2 [38] network analysis plot using Sci2 [39] and Gephi [40]. Data
269 were filtered such that only significantly abundant compounds (ANOVA/ASCA: adjusted $p < 0.05$)
270 relative to the baseline were included in the analysis, and abundancies of < 10 % the maximum were
271 removed from the matrix, resulting in 908 retained variables. The weighted degree of nodes was
272 calculated, and nodes were partitioned based on their weighting to facilitate removal of those which
273 did not cluster into a discrete module. A final network was constructed from the refined dataset, with
274 edges weighted by count of occurrence and clusters coloured by weighted degree. The average

275 abundance of all compounds within a cluster was calculated, and we used a one-way ANOVA with
276 Tukey *post hoc* tests with a 5 % FDR correction [34] to compare mean abundance between interacting
277 systems after 28d, in R statistical software [31].

278

279

280 **Results**

281 *Hierarchy and coexistence dynamics*

282 Typical transitive hierarchy of the focus decay species, *T. versicolor*, was established in paired block
283 interactions (Fig. 1), i.e. *H. fasciculare* > *T. versicolor*, *H. fasciculare* > *V. comedens* and *T. versicolor* >
284 *V. comedens* (Fig. 3). This hierarchy reflected the general niche occupied in wood decomposition: least
285 competitive *V. comedens* an early/primary decay species, *T. versicolor* a secondary colonising species,
286 and *H. fasciculare* a late/tertiary decay species, the most competitive. Transitivity was also exhibited
287 when the three fungi were dispersed throughout more spatially heterogeneous 3-dimensional
288 systems (Fig. 1e): 15 % of the original territory (defined as the relative proportion of a block occupied
289 by a single fungus) of *T. versicolor* was captured by *H. fasciculare*, and 69 % of the original territory of
290 *V. comedens* was captured by its competitors. However, when spatial dynamics within the
291 3-dimensional cubes were changed such that *V. comedens* occupied a larger adjacent, but same total,
292 volume as its competitors (Fig. 1f), *V. comedens* was displaced from just 6 % of its original territory,
293 and *T. versicolor* was displaced from 46 % of its original territory. The three species coexisted without
294 *V. comedens* being driven to near extinction (within a closed community network loop) as it was when
295 territory was patchy, which is a characteristic of an intransitive relationship (Fig. 3).

296

297 *Metabolite network function*

298 In addition to our 12 targeted enzyme assays, our untargeted analyses yielded 67 VOCs, and 2825
299 LC-MS signals of which 1597 were putatively annotated against existing compound libraries
300 (Supplementary Table 3). Univariate ANOVA analysis (with false discovery rate correction of p-values)
301 and ASCA multivariate analyses provided evidence of significant differences in the quantity of
302 individual functional metabolites from blocks originally colonised by *T. versicolor* between interactions
303 with divergent coexistence dynamics, which are summarised in Supplementary Information. Network
304 analysis of the detectable metabolome, VOCs and enzymes throughout all interactions (average
305 weighted degree = 3230), revealed 10 distinct clusters of synergistic metabolites (both antagonistic
306 and lignocellulose decaying), plus an additional 11 independently functioning compounds with a high
307 network degree. Namely, toluene (VOC: C66), an undefined oxidase enzyme, manganese peroxidase
308 (MnP), manganese independent peroxidase (peroxidase), arylsulfatase, phosphodiesterase, and five
309 unidentified small metabolites (Fig. 4; Cluster Identity Table 1).

310 By comparing the average abundance of individual clusters of metabolites produced in blocks
311 pre-colonised by *T. versicolor* in pair-wise competitive systems, we found that production of 7 clusters
312 of antifungals was induced in significantly greater quantities when *H. fasciculare* was *T. versicolor*'s
313 opponent, compared to 3 clusters when it was paired against the weaker *V. comedens*, relative to
314 single species controls (Fig. 5). This particular response was consistent, and in 3-dimensional
315 community interactions there was a stronger competition response shown in *T. versicolor*
316 pre-colonised blocks when *V. comedens* was more combative when occupying a larger adjacent patch
317 size (Clusters 6 and 9 were significantly more abundant (ANOVA: $p < 0.05$)), compared to when all
318 fungi were dispersed in 3-dimensions. Cluster 6 contained the putatively annotated metabolite
319 swainsonine, which functions in the biosynthesis pathway of piperidine- and pyridine-based
320 antimicrobial alkaloids, and isolongifolene, a sesquiterpene with known antifungal properties,
321 featured in Cluster 9 (Table 1). Although only putatively identified, the increased abundance of these
322 combative/defensive compounds in *T. versicolor*-colonised blocks correlates with the increased

323 combative ability of competitor *V. comedens* and longer coexistence of the three fungi within an
324 intransitive relationship loop (Fig. 3).

325 In addition to our findings in competitive systems, we found distinct metabolic differences between
326 2-dimensional and more spatially complex 3-dimensional controls where *T. versicolor* was the sole
327 occupant of these resource habitats. For example, the unclustered enzyme arylsulfatase and
328 Cluster 10, which comprised another six similarly functioning enzymes (Table 1), were significantly
329 more highly abundant (ANOVA: $p < 0.05$) when *T. versicolor* solely occupied a 3-dimensional cube
330 compared to when it was solely paired in 2-dimensions (Fig. 5).

331 The presence of other species affected metabolic function, as for example Clusters 7 and 8, amongst
332 others, comprising compounds such as antifungal sesquiterpenes (Table 1), were significantly more
333 abundant (ANOVA: $p < 0.05$) when *T. versicolor* was paired in an interaction compared to when it
334 decayed wood alone. Similarly, when we compared whole *T. versicolor* 3-d cubes with 3-d community
335 interactions, clusters 2, 3 and 4 which contain metabolites such as ankorine and fortimicin involved in
336 antibiotic biosynthesis pathways, were significantly more abundant (ANOVA: $p < 0.001$) in the more
337 species-rich systems. Within this experimental time frame the process of decomposition was not
338 affected by spatial dynamics or species diversity (ANOVA: $p > 0.05$; Supplementary Fig. 1).

339

340

341 **Discussion**

342 Our results indicated that coexistence dynamics and metabolic function are directly affected by spatial
343 occupation and patchiness of territory and can be translated into mechanistic functional processes.
344 Furthermore, we provide evidence of a metabolic response to shifts in community structure as a result
345 of altered connectivity. Landscape architecture changes combative mechanisms [2] and in return may
346 cause variation in expenditure of metabolic products between systems of varied levels of spatial

347 heterogeneity. Community composition may be altered by a change in combative mechanisms as a
348 result of landscape structural complexity [2], possibly due to stochastic effects within community
349 assemblages [3] or different gaseous regimes throughout 3-dimensional structures and altered edge
350 effects in 2-dimensions vs 3-dimensions. A study in which individuals of a community were paired
351 against each other in artificial media [21], found negligible effects of species diversity alone on
352 community function, but that a diverse community comprising weak competitors with high
353 intransitivity exhibited a positive diversity-function relationship, i.e. the structure of a competitive
354 network impacts community-level function. The more realistic complexity of the model system used
355 in the present study revealed very different relationships: *T. versicolor* changed its mechanism of
356 combat in systems where *V. comedens* occupied a larger adjacent volume, as clusters of metabolites
357 functioning in the biosynthesis pathways of antagonistic antimicrobial compounds were produced in
358 greater abundance. Presumably this emergent property was as a result of the greater combative
359 strength of *V. comedens* (i.e. more antifungals were needed to attack the connected *V. comedens*), or
360 as a result of a change of strategy by *H. fasciculare* which focused its attention on antagonising the
361 now weakest competitor with whom it shared the most antagonistic-fronts, *T. versicolor*, which
362 responded to this change with increased antimicrobial compound production, or, the emergent
363 property could have resulted from both simultaneously. Additionally, in this scenario *H. fasciculare*
364 was able to capture some of the territory of *T. versicolor* which would result in a change to
365 species-specific biochemical production. When *V. comedens* was dispersed it was less stable than
366 when connected, and connectivity of *V. comedens* altered the community dynamic such that the
367 usually stronger competitor, *T. versicolor*, came under survival pressure. The change in mechanisms
368 led to coexistence of the three species with more similar relative abundancies and a closed community
369 network loop (characteristic of intransitivity), compared to transitive community dynamics when the
370 individuals were dispersed, where *V. comedens* was outcompeted to near extinction. It is worth noting
371 that in natural deadwood species diversity would be far greater than that presented here, and the
372 presence of other fungal species as well as bacteria would influence interactions outcomes and,

373 therefore, metabolism [41]. Fungal-bacterial interactions have been shown to be mutualistic [42],
374 which could give individual fungal species a competitive advance against their competitors, and
375 further alter expected community hierarchies.

376 In the present study, production of seven cellulolytic enzymes functioning in resource exploitation was
377 boosted when spatial scale was increased from linear 2-dimensions to more heterogeneous
378 3-dimensions. The differences in functional metabolic processes across spatial scales, and between
379 the more diverse community exhibiting intransitive characteristics and the transitive community is,
380 therefore, reflective of a relationship between diversity and function that is regulated by space. The
381 impact of the nature (space occupancy) of communities in directing community structure should be
382 considered when looking at complex communities where outcomes are less predictable, and
383 metabolic quantification to potentially inform predicted outcomes is, therefore, very useful.

384 That species diverse communities promote coexistence and positively impact community function is
385 well known [21,43,44,45]. Our results confirm this relationship between function and diversity, i.e.
386 metabolic function changed significantly between systems with different numbers of species (single
387 species assemblages vs multi-species assemblages), but we also show that spatial scale and
388 distribution of species (patch dynamics) affect metabolic function as well. While these effects were
389 not directly translated into ecosystem services (i.e. the rate of wood-decay was not significantly
390 affected), decay in the natural environment occurs over much larger time-scales than used here [46].
391 So it might be predicted that given a greater length of time spatial heterogeneity and species diversity
392 would have resulted in changes to the rate of decomposition, since substrate utilisation was affected
393 over the short time-scale measured in this study. The decomposition activities of wood decay fungi
394 determine the rate of nutrient cycling in forest ecosystems which impacts forest function [47]. The
395 relationship between spatial dynamics, species diversity and function highlighted in our study is,
396 therefore, a key mechanism in the release of carbon from organic substrates into the carbon cycle,
397 which drives global change [48]. Microbial communities in every global ecosystem carry out an array

398 of functions as important as that of wood decayers [49,50,51], but the effects of spatial dynamics and
399 species diversity on these functions have not previously been measured and quantified by
400 experimental studies. The ecologically pertinent systems presented here are pioneering in their
401 quantitative capture of 3-dimensional spatial dynamics into the experimental study of microbial,
402 community and landscape structural ecology, which can be adapted, reconfigured and reimagined for
403 the study of communities with a range of interaction types (e.g. neutral, mutualistic, facilitative). Our
404 3-dimensional experimental design, and the finding that spatial dynamics directly impact coexistence,
405 diversity and function, are not only translatable to the understanding of diversity in existing
406 microbiomes but may also provide key insights into extinctions and predictions of future ecological
407 trends and community-level evolution.

408

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413

414 **Author contributions**

415 LB, DCE, CTM and JO'L designed the experiment. JO'L and KJ set up the experiment, KJ, JO'L and CTM
416 analysed VOCs, JO'L analysed enzymes. JO'L, US and MRV analysed small metabolites (LC-MS). J'OL, JE
417 and KH contributed to analysis of the untargeted LCMS data, and J'OL analysed the block interactions
418 data, enzymes data and VOC's data. J'OL, LB, DCE and CTM drafted the paper, and all authors had
419 editorial input.

420

421 The authors declare no competing interests.

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Figure Legends

Fig. 1: Spatial distribution of species in pair-wise (a-d) and 3-dimensional 27-block (e-g) interactions. a-d: pair-wise interactions in all conceivable combinations, plus *T. versicolor* self-pairing; e: dispersed cube (fungi were dispersed throughout the system and arranged so that no two blocks containing the same species had adjacent faces); f: “wall” distribution cubes (all fungi occupied the same total volume of wood but the adjacent territory occupied by *V. comedens* was larger; the other two competitors were arranged so that no two blocks containing the same species were adjacent); g: single species, *T. versicolor* 27-block cube. Cut vessels in pair-wise interactions and rows within 27-block layers were touching so that the wood grain ran in the same parallel direction as denoted by arrows.

Fig. 2: Sampling and deconstruction of wood blocks. Individual blocks were split along the grain into quarters, and three of the quarters from each block were foil wrapped, flash frozen in liquid nitrogen and stored at -80 °C. For the remaining quarter, two chips (~2 mm³) were taken from an inside face and inoculated onto 2 % malt agar, then emerging mycelia identified morphologically.

Fig. 3: Interaction progression after 28 d in pair-wise interactions (*T. versicolor* (Tv) against *V. comedens* (Vc); *T. versicolor* against *H. fasciculare* (Hf); *H. fasciculare* against *V. comedens*), and 3-dimensional cubes in which all three species were evenly dispersed (Fig. 1e), and where *V. comedens* occupied a larger adjacent volume (Wall; Fig. 1f). Bars show the proportion of territory occupied by each species at the end of the experiment (mean of n = 5, SEM = ± 12.2).

Fig. 4: Metabolic network of the full complement of significant compounds produced by *T. versicolor* throughout all interactions. Synergistic clusters of putatively annotated metabolites are clearly visualised and grouped based on their weighted degree (WD) (number of weighted edges, i.e. connections to other nodes, for a node. Average WD = 3230) and grey nodes labelled with compound

names denote metabolites that did not form clusters (note that unlabelled grey nodes lack putative identifications). Clusters and their WD are detailed in Table 1. Edges are weighted by the count of occurrence of synergistic metabolites within samples, and node sizes and cluster colours represent weighted degree.

Fig. 5: Significant differences in the average abundance of metabolic clusters (details of composition of clusters in Table 1) between interactions. Orange denotes cluster abundance is significantly higher (ANOVA: $p < 0.05$) in the interaction on the left of the colon; Blue denotes significantly lower (ANOVA: $p < 0.05$) cluster abundance in the interaction on the left; and no colour denotes no significant difference (ANOVA: $p > 0.05$) between a pair of interactions. Tv, *T. versicolor*; Vc, *V. comedens*; Hf, *H. fasciculare*; Wall, 3-dimensional cube where *V. comedens* occupied a larger adjacent volume; Dispersed, 3-dimensional cube where all three species were dispersed; TvCube, 3-dimensional cube colonised entirely by *T. versicolor*.