



## How many myxomycete cells are there in wood?

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### Abstract

Following proof that myxomycetes are to be found within wood we have counted the minimum numbers of cells in four surveys including wood that has not been in contact with the ground (three sites and two species of tree; one site samples from the ground and aerial). We found that 1). Numbers of cells increased with decay as measured by the C:N ration; 2). The largest population density was  $>64,000 \text{ cm}^{-1}$ ; 3). The preponderance of protists in wood that had not touched the ground were myxomycetes and the aerial dispersal of dry spores gives a colonization advantage to myxomycetes and 4). It is possible to find widely differing densities of myxomycetes in the same piece of wood.

**Key Words** – aerial dispersal – protozoa – slime moulds – wood decay

### Introduction

Taylor *et al.* (2015) reported that myxomycete cells can be found within the wood and thereby confirm the work of Ostrofsky and Shigo (1981). They made no mention of the population density of the wood dwelling cells only presence or absence in the wood of eight different species of tree. The ecology and the ecosystem functional relevance of myxomycetes would depend on the evidence of the density of myxomycete cells, since presence alone would not indicate any significant ecological role in the decomposition of woody tissues. Until the work of Taylor *et al.* (2015) the assumption of reported work was that myxomycetes were present as plasmodia, but the authors state that, despite numerous isolations of myxomycetes, plasmodia were never found before myxomonads (myxomycete amoeboid-flagellates) had been recorded and plasmodia had never been seen to emerge from wood slivers.

Since Taylor *et al.* (2015) have reported that myxomycetes can be recovered from the internal tissues of wood it becomes possible to devise a methodology to count the number of cells in these tissues. Initially we used methods to replicate the method of Singh (1946) whereby direct counts were made of emerging myxomonads from slivers of wood from the centre of a piece of wood. This proved to be difficult to maintain over a period of time and the method militated against the possible extensive use in a survey of wood.

Feest and Madelin (1985) reported that the population of myxomycetes in soil could be determined by the use of a most probable number technique and we therefore used variant of their

methodology in the current work.

The role of myxomycetes in wood decay would seem to relate to their predation of bacteria growing in wood either as a result of the mobilisation of nutrients by fungi or their own decomposition activities. If this is so, then the more decomposed a wood sample the greater the possible numbers of myxomonads. A useful indication of the state of wood decay is given by the carbon: nitrogen (C:N) ratio (Swift, Heal & Anderson, 1979, Anderson, & Ineson, 1984;) and this could be assessed by relating the numbers of myxomonad cells to the C:N ratio.

The first aim of this paper is therefore: a) to apply a methodology based on Feest & Madelin (1985) to samples of wood to enumerate the population of myxomonads in wood samples and b) to test for association with state of decay, source and species of tree and thus give some indication of the role of Myxomycetes in wood decay.

We adopted the methods described in Taylor *et al.* (2015) to determine the density of a piece of wood and this was supplemented by an assay of the nitrogen and carbon content to give a C:N ratio as an assessment of the state of decay of the wood. The retention of nitrogen in decaying tissues will imply that lower C:N ratios would be found in more decayed wood (Anderson, Rayner and Walton, 1984). There are limitations to this measure if nitrogen fixing bacteria are present and if nitrogen is translocated from soil by fungal hyphae this may confuse the issue although the opposite may also happen.

Whilst myxomycetes have been recovered from wood (Taylor *et al.* 2015) they have been shown by Feest to be only part of the whole microbiocoenosis found in soil and the situation might be the same in wood so material was tested for countable numbers of both amoeba-flagellates (as a general category minus the myxomonads) and ciliates. The capacity to form dry air dispersible spores should provide an advantage to myxomycetes compared to the other two taxa which could be tested at the same time as for myxomycetes by analysis of branches that have not been on the ground

Variables that might influence recovery of myxomycete cells from wood might include: size of sample, culture medium and sample shaking time and these culture conditions were tested to provide an optimum methodology as detailed below.

## **Materials and Methods:**

Samples of branches were collected from three sites: Leigh woods, Wetmoor wood and Weston Big wood all situated near Bristol in the SW of the UK. Oak (*Quercus* sp.) samples from Leigh woods were picked from the ground. From Wetmoor both from the ground and still attached (aerial) branches were collected. Samples from Weston Big wood were of Lime (*Tilia europea*) picked from the ground.

A sample 3-5 cm long was cut from one end of the branch to determine the density of the wood. The branches had diameters of between 3-9 cm and 20-40 cm length and slivers of wood 5 x 4 x 2 mm size were aseptically removed from 30 sites across the surface of a split branch. Care was taken to avoid wood surrounding insect bore holes and other damaged areas and a total sample represented 0.5 to 1.5 g of wood. These slivers were placed in a conical flask containing 100 cm<sup>3</sup> of Evian water and shaken on a wrist action shaker for 30 minutes and samples put through a dilution series to determine the Most-Probable Number (MPN) of myxomycetes (Anon 1969). The wood chips were retrieved by filtering the suspension and drying at 60°C to constant weight and weighed. The dry weight of the wood sample was used to calculate the numbers of protozoa (myxomycetes, flagellates and ciliates) per g of dry wood. Combining the weight with the density of the wood allowed calculation of the numbers of organisms per cm<sup>3</sup> of wood as a constant measure as opposed to the variable dry weight of the wood dependant on state of decay.

The suspensions of organisms were serially diluted to give  $\times 10^{-1}$ ,  $\times 10^{-2}$  and  $10^{-3}$  dilutions of the original solution and 1cm<sup>3</sup> was added to each of five quarter strength Corn Meal Agar plates overlain with a dilute suspension of *Aerobacter* sp.. The plates (15 per wood sample) were then incubated in unsealed plastic bags at 20°C for 2 weeks. The plates were observed from above in phase contrast optics (x200) after one and two weeks for the presence of myxomonads, amoeba-flagellates and ciliates. The resulting "score" of positive plates was applied to Most Probable

Number tables (Anon, 1969) and the number of organisms calculated per gram and per cm<sup>3</sup> of wood.

Nitrogen content of the wood was calculated by a micro-Kejldahl assay of powdered wood. Carbon was assayed by placing in a Perkin Elmer elemental analyser and completely burned and the amount of carbon in the gases was deduced by the change in volume of the combustion gases when the CO<sub>2</sub> was removed.

## Results:

Tables 1-4 give the results of the assay of 30-31 branches for each site placed in order of density.

**Table 1** Physical characteristics of wood and numbers of protozoa recovered from samples of Oak wood collected from the floor of Wetmoor wood near Bristol UK. Ordered by density.

Sample number	Density g cm-3	C:N ratio	Myxos. cm-3	Amoebo-flagellates cm-3	Ciliates cm-3	Myxos. g-1	Amoebo-flagellates g-1	Ciliates g-1
10	0.12	93.54	5384	0	0	44871	0	0
1	0.12	154.1	78	5490	31	653	45751	261
11	0.12	582	3707	1752	94	30898	14606	786
4	0.13	85.11	1545	135	38	11387	1040	297
6	0.15	140.4	39	1578	0	263	10526	0
9	0.16	95.96	127	433	127	796	2707	796
7	0.17	160.8	0	704	0	0	4142	0
20	0.18	222.2	861	2708	196	4787	15047	1094
26	0.18	532.8	178	1228	111	992	6823	620
19	0.2	241.2	0	1162	0	0	5813	0
25	0.21	388.9	0	0	0	0	0	0
5	0.23	159.0	0	0	0	0	0	0
24	0.23	427.4	3356	0	98	14592	0	429
23	0.25	526.1	1984	4365	0	7936	17460	0
14	0.26	335	0	0	0	0	0	0
16	0.32	155.3	0	0	0	0	0	0
12	0.32	333.5	1875	0	0	6861	0	0
15	0.32	333.9	0	0	0	0	0	0
17	0.34	433.3	0	0	0	0	0	0
30	0.34	526.7	0	0	0	0	0	0
2	0.36	110.4	0	0	0	0	0	0
8	0.37	117.1	359	0	0		972	0
18	0.37	294.3	167	0	0	453	0	0
3	0.42	186	0	0	0	0	0	0
28	0.44	293.3	191	76	0	434	173	0
13	0.44	428.3	448	179	0	1018	407	0
27	0.47	294.6	0	0	0	0	0	0
22	0.48	1169	0	0	0	0	0	0
29	0.58	362.6	0	0	0	0	0	0
21	0.6	2350.	0	0	0	0	0	0
Mean	0.29	384.4	676.63	660.33	23.16	4342.79	4182.23	142.76

**Table 2** Physical characteristics of wood and numbers of protozoa recovered from samples of Oak wood collected from the floor of Leigh woods near Bristol UK. Ordered by density.

Sample number	Density g cm-3	C:N ratio	Myxos. cm-3	Amoebo-flagellates cm-3	Ciliates cm-3	Myxos. g-1	Amoebo-flagellates g-1	Ciliates g-1
10	0.1	98.63	11029	61	208	110294	612	2083
31	0.1	61.65	4180	33	0	41806	334	0
3	0.11	33.66	2577	360	20	23430	3280	187
11	0.11	78.39	4393	639	0	39941	5809	0
18	0.11	72.73	4850	6790	155	44091	61728	1410
4	0.13	79.55	683	15627	0	5259	120210	0
15	0.13	82	18	18	0	140	140	0
22	0.13	89.1	0	0	0	0	0	0
29	0.15	103.77	377	629	25	2518	4198	167
30	0.15	101.73	169	0	0	1128	0	0
12	0.16	110.67	2530	119	372	15813	744	2325
19	0.16	129.53	23263	25	452	145395	161	2827
28	0.17	109.93	13593	0	0	82079	0	0
5	0.19	124.81	20779	4545	0	109364	23923	0
1	0.21	111.54	0	187	0	0	893	0
24	0.21	133	340	0	0	1623	0	0
2	0.22	123.92	38076	0	0	173076	0	0
21	0.24	127.74	0	0	0	0	0	0
20	0.26	210.22	25215	0	0	96982	0	0
6	0.27	86.3	6528	130	913	24177	483	3384
14	0.32	178.62	64142	0	0	200445	0	0
23	0.34	196.88	7314	37615	104	21511	110633	307
7	0.36	121.39	0	0	0	0	0	0
9	0.37	109.52	64	0	0	173	0	0
13	0.37	219.95	76	0	0	206	0	0
25	0.4	164.54	114	0	0	285	0	0
17	0.41	166.64	19224	139	0	46888	341	0
26	0.42	186.04	0	0	0	0	0	0
27	0.57	159.48	520	0	0	912	0	0
8	0.58	164.39	167	0	0	289	0	0
16	0.7	270.29	0	0	0	0	0	0
Mean	0.26	129.24	8071.64	2158.61	72.54	38316.93	10757.71	409.35

Of the samples taken; 68 of 121 produced myxomycetes, 44 amoeba-flagellates and 20 ciliates. Tables 1-4 are ordered in order of density and show that the denser samples produced the greatest recovery of flagellates and ciliates but Myxomycetes were recovered from all densities. Of particular interest is the recovery from branches still attached to the tree where 14/30 had myxomycetes, 9/30 had flagellates and just 2/30 ciliates. Even more telling is that, excluding one sample yielding  $>32,000 \text{ cm}^{-3}$  amoeba-flagellates and probably affected by invertebrates, gives the following average populations: 593.5 myxomycetes; 74.1 amoeba-flagellates and 5.3 ciliates  $\text{cm}^{-3}$ . Clearly dry spore dispersal is effective in giving the myxomycetes an advantage in colonising wood. The largest population density of myxomycetes was  $64,142 \text{ cm}^{-3}$  representing over 200,000 per gram of wood. Since the wood samples tested were very small this will mean that the lowest value detectable is larger than for larger size samples and in this case for samples between 0.5 and 1.5 g

the minimum detectable values would be between 400 – 133 cells per g (based on the presence of one positive recovery plate out of the fifteen used).

**Table 3** Physical characteristics of wood and numbers of protozoa recovered from samples of Oak wood collected from the canopy of Wetmoor woods near Bristol UK. Ordered by density. Sample 10 probably taken from an invertebrate affected section of wood.

Sample number	Density g cm-3	C:N ratio	Myxos. cm-3	Amoeba-flagellates cm-3	Ciliates cm-3	Myxos. g-1	Amoeba-flagellates g-1	Ciliates g-1
8	0.13	97.39	784	0	0	6032	0	0
20	0.13	137.06	153	249	0	1179	1917	0
23	0.13	144	692	538	0	5325	4142	0
21	0.14	106.26	51	128	0	368	920	0
10	0.17	97.48	344	32419	0	2026	190703	0
3	0.17	192.08	205	0	0	1208	0	0
12	0.18	80.54	0	0	0	0	0	0
5	0.19	126.68	139	55	55	735	294	294
4	0.22	212.45	9527	0	0	43.307	0	0
16	0.24	191.92	0	0	0	0	0	0
30	0.25	183.04	0	135	0	0	541	0
24	0.26	189.04	394	0	0	1516	0	0
1	0.27	145.03	0	0	0	0	0	
15	0.3	104.61	0	97	0	0	326	0
9	0.3	195.5	0	0	0	0	0	0
29	0.31	0	0	0	0	0	0	0
28	0.31	139.38	225	0	0	726	0	0
6	0.31	143.38	0	0	0	0	0	0
26	0.31	302.6	0	0	0	0	0	0
7	0.34	169.18	0	0	0	0	0	0
11	0.36	117.71	297	0	0	827	0	0
18	0.36	141.32	0	578	105	0	1605	291
2	0.36	174.04	0	0	0	0	0	0
13	0.37	225.57	0	0	0	0	0	0
17	0.38	185.32	443	0	0	1167	0	0
27	0.38	203.78	2166	379	0	5702	997	0
14	0.39	189.76	2386	0	0	6119	0	0
19	0.4	187.8	0	0	0	0	0	0
25	0.54	177	0	0	0	0	0	0
22	0.72	421.27	0	0	0	0	0	0
Mean	0.297	166.04	593.53	1152.6	5.333	1099.11	6714.83	20.17

Excluding the aerial sample the mean density of the myxomycete population of the three ground sample sites followed the progress of decay as determined by the mean C:N ratio thus:

Site and source	Mean C:N	Mean Myxos cm <sup>-3</sup>
Leigh Woods Oak	129.2	8072
Weston Big Wood Lime	215.6	1240
Wetmoor Woods Oak	384.5	677

The more decayed a branch the higher the number of myxomycete cells. This was true for amoeba-flagellates and ciliates although their population densities were lower and the frequency of

recovery was also lower. Carbon: nitrogen ratio and density as measures of decomposition were tested for association by a linear regression of for example the data in Table 1 and produced a very highly significant ratio ( $p < 0.001$ ) Where samples had been taken from the same branch, albeit different ends, it was possible to compare results for both the density and myxomycete populations. Table 5 shows that densities did not differ greatly between ends but that myxomycete populations could (sometimes significantly) and therefore one would assume that the populations of myxomycetes were patchily distributed throughout a single branch.

**Table 4** Physical characteristics of wood and numbers of protozoa recovered from samples of Lime wood collected from the canopy of Weston Big woods near Bristol UK. Ordered by density. Samples 11 & 4 probably from an insect affected piece of wood.

Sample number	Density g cm-3	C:N ratio	Myxos. cm-3	Amoebo-flagellates cm-3	Ciliates cm-3	Myxos. g-1	Amoebo-flagellates g-1	Ciliates g-1
13	0.14	101.23	984	0	0	7032	0	0
27	0.14	60.6	38	0	0	273	0	0
7	0.15	125.82	0	0	0	0	0	0
29	0.15	96.47	663	0	0	4424	0	0
30	0.17	79.67	9444	5085	145	55555	29914	854
12	0.2	159.93	0	0	0	0	0	0
9	0.21	247.52	743	0	0	3539	0	0
6	0.22	110.93	251	2517	251	1144	11441	1144
23	0.22	214.32	0	430	0	0	1955	0
24	0.22	111.83	0	0	0	0	0	0
3	0.23	124.83	4079	0	0	17738	0	0
28	0.23	244.47	0	87	0	0	380	0
2	0.27	209.5	1476	84	0	5468	312	0
5	0.31	509	13225	105	0	42662	341	0
16	0.33	220.67	0	0	0	0	0	0
21	0.34	184.2	0	0	0	0	0	0
11	0.35	165.57	2075	10379	0	5931	29655	0
4	0.36	139.24	1691	12080	96	4697	33557	268
22	0.36	119.25	230	92	0	886	354	0
19	0.38	169.74	0	0	0	0	0	0
8	0.41	247	0	0	0	0	0	0
14	0.44	144.66	2140	0	0	4864	0	0
15	0.48	244.05	0	0	0	0	0	0
10	0.5	384.25	163	0	0	327	0	0
18	0.52	379.58	0	0	0	0	0	0
20	0.52	327.79	0	0	0	0	0	0
25	0.59	270.18	0	0	0	0	0	0
17	0.64	327.79	0	0	0	0	0	0
26	0.69	238.79	0	0	0	0	0	0
1	0.75	510	0	0	0	0	0	0
Mean	0.351	215.62	1240.06	1028.63	16.4	5151.33	3596.96	75.53

## Discussion

We have shown that it is possible to estimate the myxomonad population in wood samples and that the population is within the wood and not superficial as indicated by Taylor *et al.* (2015) thus satisfying our first research aim. This has also been seen to relate to state of decay with more myxomonads (and other protozoa) in the lower C:N ration samples of wood thus the population

increases with decay and there is a presumed role for myxomycetes in the decay process satisfying our second research aim. At no time was a plasmodium recovered from the wood samples before myxomonads had developed and thus one can assume that the population is largely composed of myxomonads (as in soil see: Stephenson & Feest 2012).

**Table 5** Pairs of samples from the same branch where in three cases there is a highly significant difference of myxomonad population estimate between ends.

Sample Number	Density g-3	Difference between densities	Myxos cm-3	Difference between pairs	Significance
2	0.36	0.06	0	0	0
3	0.42				
16	0.32	0.02	0	0	0
17	0.34				
23	0.25	0.02	1984	1372	NS
24	0.23		3356		
25	0.25	0.02	0	178	0
26	0.23				
27	0.21	0.03	0	191	0
28	0.18		191		
34	0.13	0.06	683	20096	p<0.01
35	0.19		20779		
43	0.37	0.05	76	64066	p<0.01
44	0.32		64142		
64	0.17	0.05	205	9322	p<0.01
65	0.22		9527		

It is possible that some of these population estimates are greatly underestimating the actual population if myxomonad cysts in wood respond in the same way as they do in soil when frozen. Freezing induces excystment of dormant cysts in soil that do not become active even when in a wet environment (Feest & Stephenson, 2013). This underestimation can be up to 90% thus the highest population we recovered of c.64,000 cm<sup>-3</sup> might be even greater. At these densities it is clear that myxomonads are of relevance to the decay of wood and the remobilisation of nutrients from wood.

The advantage that myxomycetes have in the possession of dry air-dispersible spores is clearly shown in the still attached samples of wood where colonisation by myxomycetes was greater than for other protozoa thus the possession of complex sporangia, with on occasions active spore release mechanisms, can be explained as having considerable “fitness” advantages.

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