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Bias in the calculation of myxomycete species mean substrate properties

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Abstract: If during a myxomycete survey the substrate properties of samples are measured, then the mean substrate properties associated with the common species of myxomycetes can be calculated. These mean properties for each species are biased on datum and scale because the histograms of the substrate properties are too limited in range and yield a peak that is too high. However, the effects of the datum and scale bias can be determined. The data from a new Australian survey and data on pH in previous surveys around the world are consistent with a myxomycete species corrected substrate mean properties being the same around the world. These corrected mean properties, and their standard deviations, give the relative probability of occurrence of myxomycete species on substrates of known properties.

Keywords: acidity, density, water retention, environmental modelling

Introduction

Most myxomycetes survey papers report which species are present in a particular area, on what substrate types and plant species are present, and discuss questions of relative species richness. Some ecologically oriented studies look at specific problems, such as the variation of myxomycete species along distances up a tree (Everhart et al. 2009), the return of dominant myxomycetes after a fire (Adamonyte et al 2016), and the effect of acid rain (Wrigley de Basanta 2004). Some large studies (e.g., Stephenson 1989; Schnittler 2001) collect myxomycetes from all obvious types of substrates, estimate the environmental attributes of these substrates, and by using multivariate analysis, determine the relative importance of the environmental attributes in terms of myxomycete species occurrence.

Previous studies have shown that the type of dead substrate (bark, wood, or twigs) is an important control of myxomycete occurrence. This study derives a statistical model that describes the probability of occurrence of a myxomycete species based on the substrate's chemical and physical properties. Using this statistical model and data from suitable myxomycete surveys, it should be possible to integrate the data of myxomycete species occurrences and their substrate properties from surveys around the world and obtain an accurate model of the effect of substrates on the occurrence of the common myxomycete species. This

is not a study of the relative importance of substrate properties to the occurrence of myxomycete species. It is a study of how to obtain unbiased estimates of the properties of these substrates and integrate the unbiased estimates from multiple surveys.

Materials and methods

In this study the myxomycete substrates investigated were pieces of dead bark from a living tree. The three bark properties studied were acidity, density, and water retention. Acidity was a known constraint of myxomycete occurrence. The dead bark is partly weathered, so the bark cell walls are partly broken. The physical properties of density and water retention both represent rough measures of the proportion of fibres to air in the dried substrate, which is a measure of the myxomycete's access to the body of the substrate for feeding and are also a measure of the duration of myxomycete growing time if rainfall events are well separated in time.

Bark was the preferred substrate to use in this study because its myxomycete productivity is relatively high, its physical properties are similar within a tree species, and where sampled it is isolated from other substrates and thus unlikely to contain myxomycete resting phases derived from them. Bark can be collected in the same state of decay at any time, as there is a steady-state between little-decayed bark next to the wood and increasingly decayed bark towards the bark surface. The bark properties studied (acidity, density, and water retention) can be measured rapidly and with low expense.

Acidity has been known for a long time as an important control on myxomycete species occurrence (Härkönen 1977). In this paper acidity was measured as pH by a relatively inexpensive pocket pH meter that was calibrated with standard pH solution of 5.0 and 7.0 before and after the measurements. The pH measurements were made about 24 hours after the start of bark substrate cultivation. At that time the free water was poured off the five Petri dishes of each sample into a single container, and the pH of the water in the container was measured. Measurements were made with the water at about 22^{o}C, and the pH meter had been in a solution for long enough that its readings had stabilized. The accuracy of measurements is likely to be about 0.1 pH units.

For each substrate sample density and water retention were measured on one small piece of bark. The bark pieces were 1 to 10 g in mass and were irregular in shape because most pieces were too fragile to shape to a regular body. The measurement of density was by the water displacement method. Measurement of water retention was derived by measuring the mass of the sample when it was dry, and after having a film of water over it for 24 hours. Water retention is reported as a percentage by volume of the bark piece. Twenty-three of the samples were less fragile, so for each sample a representative piece could be cut with rectangular sides. For these pieces density was measured by both the displacement method, and by using the mass and the length of the rectangle sides. The differences between the two types of density estimate have a mean of zero and a standard deviation of 30 kg/m^3, so the two methods of calculating density gave similar results. The accuracy of measurements is likely to be about 30 kg/m^3 for density and 6% by volume for water retention.

The pattern of water uptake with time was studied on five bark pieces with a wide range of water retention. For each piece there was initially a fast uptake of surface water with bubbles of air surfacing. Then at about 30 sec, or a little later, there was a much slower uptake of internal water, the intake being approximately linear with the log of the time since the initial wetting. This linear uptake continued to the

end of the measurements at 80 hours. The amount of fast uptake water had a mean value of 18%. The amount and ratio of the fast and slow uptake water varied greatly between samples, but on mean they are about equal in importance. Importantly, the physical measurement of water retention used in this paper is the result of two distinct processes. Most of the water retention measurements were in a relatively small range of generally 14 to 40%, so, when the measurement errors are considered, there was little useful dispersion in the values. Because of these two factors, water retention is not an ideal substrate quantity.

When the sample property pH is plotted against density the properties were found to be largely independent. When density is plotted against water retention, the retention has a small range of generally 18-40% except at near mean density values. There are few samples with high density and high water-retention, because fibre density is about 1200 kg/m^3 (Bootle 1983), so when bulk bark density is high there is little space for voids and interconnections. There were also a few samples with low density and high retention, and few samples with retention below 18%.

In order to obtain pH, density and water retention measurements on Australian tree bark, and to determine the myxomycete species associated with them, 57 bark samples of Australian trees were collected within 400 km of Canberra in southeast Australia (2020 NSW survey). The samples were collected between 0.5 and 1.5 above ground level from old trees. The samples were cultivated by the moist chamber method (Wrigley de Basanta 2017). In this survey the procedure was as follows. For each sample five Petri dishes (90 mm diameter x 15 mm high) were used. The dishes were lined with a sheet of filter paper, pieces of bark were placed to cover the filter paper with the bark surface uppermost. The pieces of bark placed in each Petri dish were from as wide a range of types of the sampled pieces as possible, with the intention of obtaining a high competition culture. The average sample cultivated consisted of 50 pieces of bark with a total mass of 40 g. The dishes were half filled with de-ionized water, covered, and left for 24 hours. Then the water was poured off from the five Petri dishes into another container, and pH was measured. The Petri dishes were then kept at about 22^{o}C in a room with diffuse daylight. They were checked about 6 times at intervals of seven days. The checking consisted of finding all mature fruiting bodies of myxomycetes, removing them for study and adding extra water to keep the bark material in a damp state. The collected myxomycete fruiting bodies were identified to morphological species.

The other major myxomycete data set used is that of all the Australian Survey bark samples collected previously on traverses: about 247 samples giving 1646 myxomycete records from mainland Australia, representing five states/territories (Wellman 2019). Along these traverses the collection stops were made about every 50-100 km, and at each stop one to three trees of different species were sampled. Traverses were positioned to go cross the gradient from high to low annual rainfall. In Western Australia there is a traverse with a gap in the middle with 1000 km of actual sampling (WA data), in Northern Territory and Queensland two traverse lines 1500 and 940 km long (NT + Qld data), in South Australia a traverse line 1100 km long, mainly in SA but extending north to Alice Springs in NT (SA data), and in New South Wales there are numerous traverse lines, in total more than 1500 km long (NSW data).

Results

The mean substrate properties of pH, density and water retention have been calculated for the common myxomycete species as follows. The myxomycete species mean pH values were calculated using the pH values of only the WA, NT + Qld areas of the Australian survey. Data from other areas were not

used, because there are no pH values for the SA survey and because the NSW pH data gave a histogram of the substrate pH with two distinct peaks. Consequently, the myxomycete species mean pH values were considered unreliable. The myxomycete species density and water retention mean values were calculated using the property values of 57 samples of the 2020 NSW survey. The myxomycete species mean values for substrate pH, density and water retention are listed in Table 1.

Table 1. Myxomycete species mean values of pH, density, and water retention (WR). The mean substrate property values are listed in two scales: samples, and a corrected scale. N is the number of samples.

Species of myxomycetes	Number of pH measurements	Number of density and WR measurements	Uncorrected data			Corrected data		
			pH	Density (kg/m^3)	WR (%)	pH	Density (kg/m^3)	WR (%)
Arcyria cinerea	22	11	5.2	410	56	5.4	317	62
Arcyria sp.	7	6	4.4	330	38	4.2	181	33
Arcyria pomiformis	7		4.3			4.0		
Badhamia versicolor	5		6.5			7.5		
Badhamiopsis ainoae	14	3	5.7	640	40	6.2	708	36
Calomyxa metallica	12	16	5.4	640	45	5.8	708	44
Comatricha elegans	37	15	4.4	510	37	4.2	487	31
Comatricha ellae	37	15	4.7	540	50	4.6	538	53
Comatricha laxa	7	6	4.9	520	39	5.0	504	35
Cribraria confusa	9	5	4.0	290	56	3.5	113	62
Cribraria minutissima	14	8	4.1	320	43	3.7	164	41
Cribraria violacea	5	5	6.3	540	59	7.2	538	67
Dianema corticatum	7	7	6.8	660	46	8.0	742	46
Didymium dubium	11	7	5.4	650	46	5.8	725	46
Echinostelium minutum	15	6	4.5	490	46	4.3	453	46
Enerthenema papillatum	15	7	4.5	380	46	4.3	266	46
Licea biforis	15	3	5.6	790	36	6.1	963	30
Licea kleistobolus	34	23	5.1	590	46	5.3	623	46
Licea operculata	28	4	5.1	760	38	5.3	912	33
Licea pygmaea		8		560	56		572	62
Licea scyphoides		4		570	49		589	51
Macbrideola oblonga	10	11	5.7	720	41	6.2	844	38
Paradiacheopsis fimbriata	10	7	4.4	490	27	4.2	453	15
Perichaena vermicularis	6	11	6.1	550	48	6.9	555	49
Physarum decipiens	22	12	5.7	610	49	6.2	657	51
Physarum leucophaeum	13	12	5.7	660	45	6.2	742	44
Stemonitis fusca	7	2	5.5	455	51	5.9	394	54
Trichia contorta	5		6.1			6.9		
Trichia varia	5		5.2			5.4		

Discussion

To calculate unbiased mean substrate properties for a myxomycete species, the property values used must have properties that form a random variable. This requires that, relative to the range in property values acceptable to a myxomycete species, the substrate samples collected must have a larger range in

each property value and that the histogram of each of the property values must be flat, not peaked. In this survey a histogram of a substrate property has standard deviations of 0.77 to 1.3 pH units for acidity for four areas of Australia, and 200 kg/m^3 for density and 14% for water retention for the 2020 NSW survey. The scatter of the myxomycete species' means have standard deviations of 0.8 pH units for acidity, 140 kg/m^3 for density and 8% for water retention. The scatter of values about an individual myxomycete species mean substrate property is given by standard deviations of 0.65 pH units for acidity, 130 kg/m^3 for density and 11% for retention. For each substrate property, when the scatter of the myxomycete mean species values and scatter about these mean species values are combined, then that standard deviation is similar to that of the substrate samples, so the collected samples' range in properties is insufficient to calculate unbiased mean values of myxomycete species means. Also, for each sample property a histogram of the sample values is close to a normal curve with short tails and is not a flat high. Hence, in this survey the range of properties in the substrate samples is not wide enough, and histograms of substrate sample properties are not flat enough, for the calculation of myxomycete species mean substrate properties to be unbiased. Surveys elsewhere have similar sampling statistics, so they will also give myxomycete species mean substrate properties that are biased.

A description of the bias mechanism is as follows, using pH as the substrate property. The myxomycete species mean values of substrate pH are calculated. If a myxomycete species mean pH value is near the peak of the histogram of pH of all the samples, then the pH values averaged will be in equal numbers higher or lower than the survey mean, and the calculated species mean value will be unbiased. If the myxomycete species mean pH is on either flank of the histogram, then samples are much more likely to be in the direction of the peak of the histogram than of the nearby tail, so the calculated value of the mean pH will be biased towards the mean pH of the survey, so will be wrong in scale. Relative to the species' unbiased values, the biased species' values are (allowing for random errors) in the correct order, with the correct relative spacing, but their mean values (datum of the values) are not correct, and the distance the values are apart (scale of the values) is not correct, being shorter.

The bias in datum is calculated as follows. The effect of a variation between surveys in the mean survey pH can be calculated from the data of 11 myxomycete surveys located around the world (Table 2 of Wellman 2021). For each survey the original papers give a list of myxomycete species with their mean substrate pH values. Within the surveys 26 species occur two or more times. These common species can be used to align the myxomycete species mean pH values of the various surveys, by adding a constant to all the myxomycete species mean pH values of a survey. A calculation alternately adjusted the mean pH associated with each myxomycete species, and the constant that adjusted the pH values in each survey, to minimise the residuals from the model. The adopted results have residuals with a standard deviation of 0.2 pH unit. The various surveys had a mean survey substrate pH ranging from 5.2 to 7.7 pH units, and corresponding survey pH corrections ranging from +0.58 to -1.45. When the mean survey pH was correlated with the survey pH corrections for the 11 surveys the slope was -1.0 within experimental error ($r = 0.98$). Hence, if the mean pH of the substrates of two surveys differs by one pH unit, then this results in the myxomycete calculated species mean pH values of the two surveys differing by one pH unit. This is the bias correction for the pH datum. This correction is what is expected. The recorded myxomycete species mean pH values is calculated from the survey's substrate properties. If the mean pH of the survey substrates of two surveys is one pH unit different, then the calculated myxomycete species mean pH values

will differ by one pH unit. This datum bias correction method has not been independently checked to be the accurate correction for density and retention, but there is no reason for it not to be true.

The biases in scale are calculated as follows. For each sample in the 2020 NSW survey there is a measured value for each of the sample substrate properties, and for each myxomycete species recorded on the substrate there are potentially an estimate of its substrate property mean values. For those samples with a reasonable number (≥3) of myxomycete records with mean values, then, on average, one would expect that, if the myxomycete properties are correctly scaled, then the mean of these species' records mean substrate properties (species value) would approximate the measured substrate properties of the sample. The scaling for the properties was calculated as follows. For each property a list was prepared of the accepted samples, giving for each sample the measured substrate value of the bark (subscript c) and the mean of the myxomycete species' average value for the property (subscript s). These two estimates are thought to have errors of similar magnitude, so equal weight was given to each axis. The following linear equations were obtained:

$$pH_c = pH_s \times 1.91 - 4.63,\ r = 0.83,\ n = 54$$
$$Den_c = Den_s \times 2.15 - 0.63,\ r = 0.63,\ n = 29$$
$$WR_c = WR_s \times 4.05 - 140,\ r = 0.76,\ n = 35$$

All these relationships are statistically significant. However, there seems to be a problem with the WR_c equation. Use of this equation results in the mean water retention values of three myxomycete species that are close to, or outside of the possible range of retention. It is thought that this equation is incorrect because of the biases introduced into the calculation due to bark having only a small range in retention. The slope of the retention relationship is likely to be similar to that of pH and density, and be about 2.0, as this gives the correct scatter in relation to bark retention compared with the equations for pH and density. The preferred equation is:

$$WR_c = WR_s \times 2.0 - 47.$$

The myxomycete species corrected mean values for each of the properties are listed in Table 1. The corrected mean substrate values are associated with standard deviations of 1.3 pH units for acidity, 260 kg/m^3 for density and 22% for water retention. The myxomycete species raw calculated mean substrate property values are biased in scale, so if one were to be strict the units (pH unit, kg/m^3, %) given in Table 1 are incorrect in scale.

When the data for a substrate property are integrated using data from many surveys, then a reference datum should be adopted to list the unbiased substrate values of the myxomycete species. The correct reference datum for a property is thought to be a datum such that the mean of the myxomycete species mean values is similar in value to the mean of the measured properties for the substrates that the species are occurring on worldwide (Wellman 2021).

An independent measure of the influence of substrate properties on the occurrence of myxomycete species can be achieved by selecting samples with reasonably constant substrate properties and looking at the variation in myxomycete species recorded on these substrates. Wellman (2023) studied the relative abundance of myxomycetes species on tree bark substrates, the substrates being *Acacia*,

Eucalyptus of the 'box' or 'coolabah' type all over Australia, and *Pinus* from six sites around the world. For each of these three bark substrates, the most common myxomycete species provided over 60% of the records. This dominance of a common myxomycete species is consistent with, over continental sized areas, the four bark substrates each being reasonably consistent in substrate properties, and with the myxomycete species mean substrate properties being consistent over the sample area.

This paper used a survey where the myxomycete fruiting bodies have been identified to morphospecies. It is possible that, if identified using molecular methods, some of these morphospecies would be species complexes (Leontyev et al. 2022). If morphospecies were present, then there would be little effect on the pH relationship because there is generally a narrow range in average substrate pH for myxomycete species within a genus (Wellman 2021).

If substrate properties are measured in future myxomycete surveys, then it should be possible using the methods of this paper to integrate myxomycete species unbiased mean substrate properties from numerous surveys around the world to a single model. This single model would give the relative probability of occurrence of myxomycete species on substances of known properties.

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