VISUALISING MACROFUNGAL SPECIES ASSEMBLAGE COMPOSITIONS USING CANONICAL DISCRIMINANT ANALYSIS

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Abstract

Recent generalisations of canonical variate analysis allow the technique to be applied to typical ecological data sets such as the presence or absence, or the abundance, of each species in a long list of species. This provides a useful tool for testing and visualising differences in assemblage compositions obtained from macrofungal surveys. In this paper, one such advance, the canonical analysis of principal coordinates (CAP), is illustrated using data sets obtained from macrofungal surveys conducted in the silvicultural treatment areas of the Warra long-term ecological research site in southern Tasmania, Australia. Differences in species assemblage compositions are tested using non-parametric permutation tests that provide exact probability values, and the similarity or dissimilarity of the various sampling units are graphically displayed in a manner easily understood by ecologists. The CAP ordination diagrams in the two illustrative examples clearly display the differences in the mycota between the earliest stage of regeneration and the mature forest, and their associated statistical tests quantify these differences.

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Introduction

Ordination techniques aim to display graphically the differences between experimental units in data sets involving two or more variables. The variables may be of a variety of types: measures or counts, continuous or discrete, ordered or disordered, etc. The units may have been sampled randomly or opportunistically, or may have been subjected to different treatment regimes. Conveniently, ordination techniques may be classified as unconstrained or constrained. Unconstrained ordinations include multidimensional scaling, whether non-metric (nMDS) or metric, the latter including principal component analysis (PCA), which is based upon a Euclidean distance measure, principal coordinate analysis (PCoA), a generalisation of PCA that allows other definitions of distance (or dissimilarity) such as ones in use in ecology, and correspondence analysis, whether detrended (DCA) or not (CA). These unconstrained ordinations share the common feature that the experimental units are unstructured, i.e. they are not divided into groups and, furthermore, they do not incorporate any null hypotheses that can be tested statistically. Despite these restrictions, they still may display the variation that exists within a cloud of data points, and perhaps suggest hypotheses that may be subjected to statistical tests by other procedures.

Constrained ordinations, by comparison, operate on structured experimental units, which may typically be predefined groups. One standard ordination technique used by biometricians for testing and displaying differences between predefined groups of experimental units is multivariate canonical variate analysis (CVA). When coupled with the associated feature of discriminant analysis, CVA can assign new individuals (i.e., experimental units) to the group to which they are closest, using discriminant functions based upon a linear combination of a set of explanatory variables. Traditionally, these statistical techniques have necessarily been limited to variables that follow multivariate normal distributions, making them unsuitable for ecological data arising from inventory sampling, where the presence or absence, or abundance, of each species in a list of species often forms the raw data set. A further constraint imposed by the traditional CVA is that the number of variables must be less than the number of observations. This may not apply to ecological data sets where the variables are species, the lists often being many times longer than the number of observations (e.g., the total number of visits made to the sampling units).

Anderson and Willis (2003) recognised the need for a flexible method of constrained ordination that could be applied to any distance or dissimilarity measure, so that the limitations imposed by the traditional CVA could be overcome. This was achieved by coupling metric principal coordinate analysis (PCoA), which allows any definition of distance or dissimilarity, with CVA to produce canonical analysis of principal coordinates (CAP). That coupling leads to two pathways, one of which is a canonical discriminant analysis, which tests hypotheses concerning groups, and the other is a canonical correlation analysis, which tests hypotheses regarding relationships with additional sets of quantitative predictor variables such as environmental or genetic factors or measures. It is the first use of CAP with which the present communication is concerned. The mathematical details behind CAP are given in Anderson and Robinson (2003), whereas the ecological perspective is provided in Anderson and Willis (2003). Both papers offer practical examples drawn from sampling fish assemblages in a marine reserve. To date, applications in which CAP has been used for data on fungi are rare, although two recent papers (Bastias *et al*. 2006a, 2006b) applied CAP to data obtained from fungal DNA from forest plots that had different prescribed burning treatments to test whether the burning regimes altered soil and ectomycorrhizal fungal community structure. In the present paper, examples are drawn from macrofungal surveys in forests subjected to different silvicultural treatments and the examples contrast unconstrained and constrained ordinations of the data. In particular, CAP tests the null hypothesis that there are no differences between fungi in forests subjected to different silvicultural treatments or visited at different times of the year.

Materials and Methods

The present paper illustrates the application of CAP to macrofungal species assemblages. It contrasts the ordination diagrams from CAP with those from an unconstrained ordination, PCoA. Although other unconstrained ordinations could be used, for example nMDS, the latter is not an objective procedure, relying on random starting points and a selection of one solution amongst many based upon the minimisation of the “stress” criterion. This lack of objectivity means that different implementations of nMDS may result in different ordination diagrams. In contrast, PCoA as originally devised by Gower (1966) is an objective procedure that should produce the same ordination diagrams irrespective of which computer package computes them. Moreover, PCoA is the starting point for a CAP analysis, so that a single computer run will produce both a PCoA and CAP analysis if the appropriate options are chosen.

The examples are drawn from two surveys conducted at the Warra LTER (long-term ecological research) site in southern Tasmania. The first survey involved a comparison between macrofungal species in a regenerating coupe after a clearfelled, burnt and sown (CBS) silvicultural treatment had been applied, and in a mature forest in close proximity to the CBS coupe (Gates *et al*. 2005). 307 species were found in the two coupes. The second survey also involved a comparison of silvicultural treatments, in this case between 387 macrofungal species found collectively in an “aggregated retention” coupe and in a mature forest (Gates and Ratkowsky 2006). In this aggregated retention coupe, the trees in ca. 70% of the area were harvested, followed by a low intensity burn to stimulate regeneration. No artificial sowing of seeds took place. The remaining 30% of the area was retained in eight islands, of which three were

Table 1. Number of visits to the experimental units of each coupe in each of the two surveys (for more details, see Gates *et al*. 2005, Gates and Ratkowsky 2006, respectively).

Survey	Autumn (Mar-May)	Winter (Jun-Aug)	Spring (Sep-Nov)	Summer (Dec-Feb)
First	7	7	7	6
Second	13	8	7	7

surveyed for fungi. Separate lists were made of the fungi in the harvested and unharvested portions of the aggregated retention coupe, so that together with the mature forest, three distinct groups of experimental units could be compared. For comparability, the total length of the transect within the three groups was approximately equal. It was hypothesised that the fungi in the harvested portions of the aggregated retention coupe would be very distinct from the fungi in the unharvested islands and in the mature forest, with the latter two units showing some degree of similarity as the mature forest and the aggregated retention coupe were adjacent to one another and were of the same forest type.

In both surveys, multiple visits were made in all four seasons, providing some degree of replication, although the replication was pseudoreplication (see Hurlbert 1984) and an unequal number of visits was made. Nevertheless, this replication made it possible to test for coupe differences or seasonal differences using CAP by means of a permutation test and also to test for misclassifications using its “leave-one-out” allocation procedure. Table 1 summarises the number of visits made during each season. In both surveys, visits to each sampling unit in each coupe were made on the same day, thereby eliminating biases that would occur had this not been possible.

In both surveys, fungal species records from each visit were converted to presence/absence and Bray-Curtis dissimilarities were used, without standardisation or transformation.

Results

First Warra survey:

Figs 1 and 2 display the first two principal coordinate axes of a PCoA analysis and the first two canonical axes of a CAP analysis for the first Warra survey, respectively. The first axis in both diagrams clearly separates the fungal assemblages in the two coupes, with the points from the harvested coupe (H) all having positive scores, and the points from the mature forest (M) all having negative scores. The second axis in both procedures has only partial success in separating the sampling units with respect to seasonal effects, but almost succeeds for the mature forest using the CAP analysis. Plotting the third canonical axis from CAP (not shown) confirms the separation of the assemblage compositions in the four seasons within the mature forest, as the third axis clearly separates the spring from the summer results. Within the harvested coupe, the separation remains unclear. Note that in some instances there are fewer points in Figs 1-2 for a combination of coupe and season than the number of visits given in Table 1 indicate, e.g. four points for the regenerating coupe in summer compared to six visits actually made. This was caused by the fact that there were no fungi observed during some visits to that coupe, these “zero lists of species” occasionally occurring during the drier months.

The PCoA axes presented in Fig. 1 display information about the similarities between individual visits, but do not quantify differences between groupings. As PCoA is unconstrained, there are no tests of significance embodied in the procedure. CAP, on the other hand, provides an exact test of the null hypothesis that there are no differences between the eight groups formed by combining coupe and seasonal effects, using random permutations. The P-value obtained from the permutation test performed by CAP was $P=0.00020$ using 4999 permutations. Since $1/(4999 + 1)=0.00020$, this means that no randomly permuted data set had a more contrastingly different macrofungal species assemblage than that of

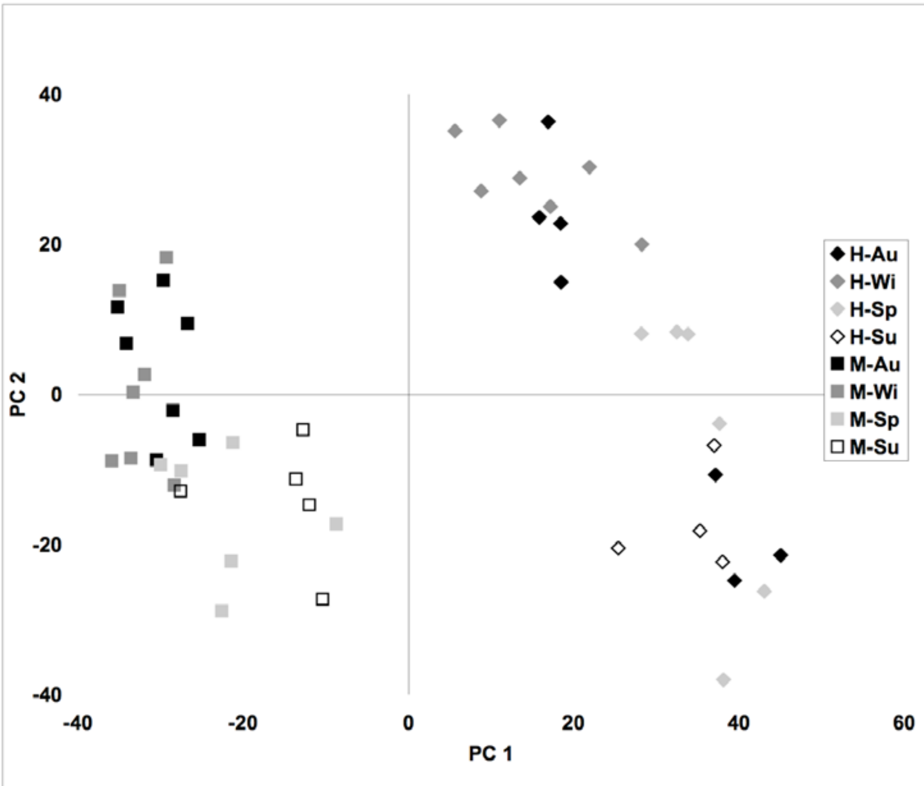

Figure 1. The first two principal coordinates of the PCoA analysis, first Warra survey.

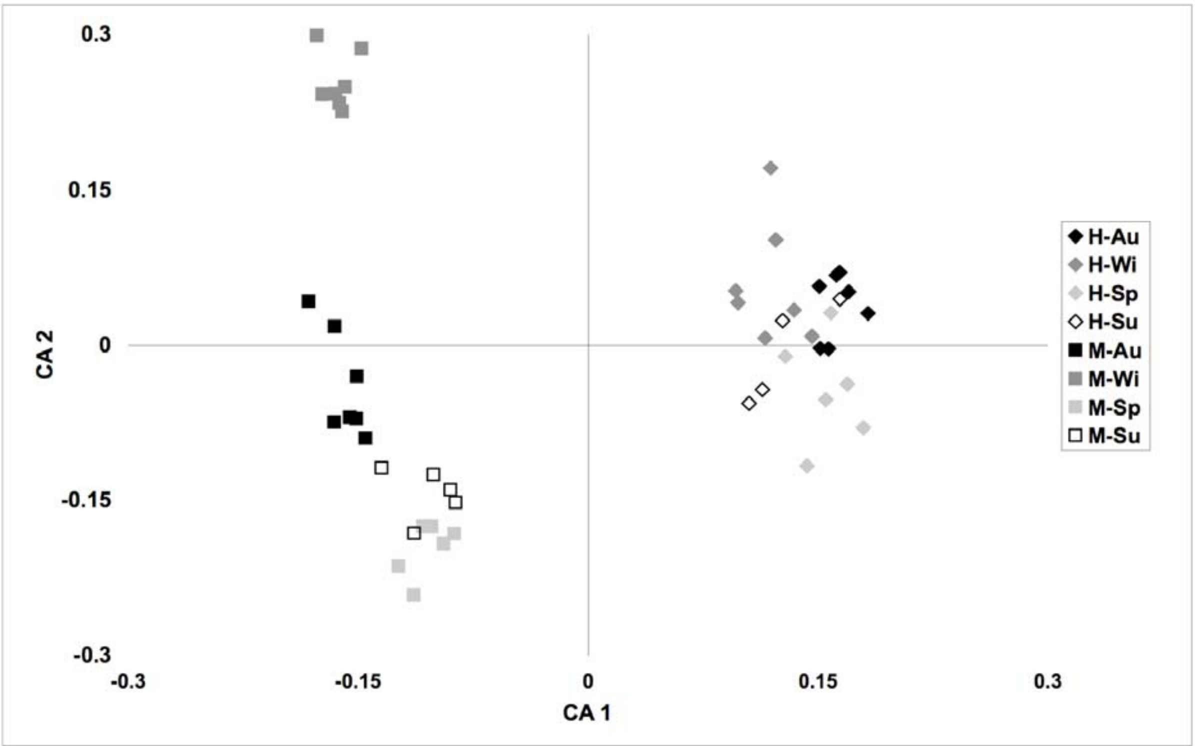

Figure 2. The first two canonical axes of the CAP analysis, first Warra survey.

[Note: In Figs 1 & 2, the first letter represents the plot (H=CBS coupe; M=mature forest) and the next two letters represent season (Au=autumn; Wi=winter; Sp=spring; Su=summer)].

Table 2. Classification table, first Warra survey, showing the reclassification of the original visits into groups, and the success and misclassification rates of the CAP analysis.

Original group	Classified into groups									
	HAu	HWi	HSp	HSu	MAu	MWi	MSp	MSu	Total	%correct
HAu	4	1	1	1	0	0	0	0	7	57.1%
HWi	0	7	0	0	0	0	0	0	7	100%
HSp	1	0	4	1	0	0	0	0	6	66.7%
HSu	0	0	0	4	0	0	0	0	4	100%
MAu	0	0	0	0	7	0	0	0	7	100%
MWi	0	0	0	0	0	6	1	0	7	85.7%
MSp	0	1	0	0	0	1	4	0	6	66.7%
MSu	0	0	0	0	1	0	0	4	5	80.0%

Total correct = 40/ 49 = 81.6%
Misclassification error = 18.4%

[Note: The first letter represents the plot (H=CBS coupe; M=mature forest) and the next two letters represent season (Au=autumn; Wi=winter; Sp=spring; Su=summer)].

Table 3. Selected list of species occurring at least seven times and only in one or other of the two coupes, first Warra survey.

Species exclusive to the Clearfelled, Burnt, Sown regenerating coupe	Species exclusive to the unharvested mature forest
Bovista brunnea Berk.	*Ascocoryne sarcoides* (Jacq.) J.W.Groves & D.E.Wilson
Galerina nana (Petri) Kühner	*Auriscalpium* 'warrensis'
Gerronema 'pink-buff'	*Chondrostereum purpureum* (Pers.) Pouzar
Gymnopus 'dark brown, hygrophanous'	*Dermocybe kula* Grgur.
Psilocybe 'dark brown, pellucid, in moss'	*Galerina* 'small, umbonate with sphaeropedunculate cheilocystidia'
Pycnoporus coccineus (Fr.) Bondartsev & Singer	*Hydnum repandum* L.: Fr.
Schizophyllum commune Fr.:Fr.	*Lactarius clarkeae* Cleland
Scutellinia aff. *margaritacea* (Berk. ex Cooke) O.Kuntze	*Lactarius stenophyllus* Berk.
Stereum ochraceoflavum (Schwein.) Sacc.	*Marasmiellus affixus* (Berk.) Singer
Trametes versicolor (L.:Fr.) Lloyd	*Mycena albidocapillaris* Grgur. & T.W.May
	Phellodon niger (Fr.: Fr.) P.Karst.
	Ryvardenia campyla (Berk.) Rajchenb.

the original data set. Therefore, one would be justified in concluding that there are at least some significant differences among the eight groups. Using a leave-one-out approach (see Appendix A of Anderson and Willis 2003 for the details), one can take each of the original visits in turn and determine to which of the eight groups its fungal assemblage is closest. The result of this reclassification is presented in Table 2. Only one misclassification occurred between coupes, with one of the spring visits to the mature forest being classified as a winter visit to the regenerating coupe. All the other misclassifications occurred within the same coupe, with some assignments of the seasons being erroneous. Overall, the classification success rate was 81.6% (Table 2). For completely random data, the classification success would be expected to be only 100/8 = 12.5%, suggesting that the groups are different.

Some of the more notable differences between the species of fungi in the two coupes are summarised in Table 3, which includes a selection of the most frequently occurring species that were found only in one or the other of the two coupes. The entries in each column comprise species that occurred exclusively within the coupe indicated, and were obtained directly from lists of the 307 fungal species in the database. Alternatively, one may employ the correlations between each of the species and the canonical axes that are part of the output of CAP to assist in interpreting the axes. However, such correlations are often not readily interpretable, as the axes maximise the separation between all eight groups, not just the major groupings, and recourse to the original data is often a better option. A full list of the 307 fungal species may be found in Gates *et al.* (2005).

Second Warra survey:

For the data set from the second Warra survey, Figs 3 and 4 display the first two principal coordinate axes of a PCoA analysis and the first two canonical axes of a CAP analysis, respectively. In both ordinations, the first axis clearly separates the harvested portions (H) of the aggregated retention coupe from the unharvested islands (U) and from the mature forest (M), but the two uncut areas are not separated on that axis. The second principal coordinate axis of PCoA (Fig. 3) does not succeed in separating those sampling units, but the second canonical axis of CAP (Fig. 4) appears to largely separate them, a fact that can be confirmed from an examination of the classification matrix (Table 4), which uses a leave-one-out allocation procedure to determine to which of the 12 experimental units (i.e. combinations of the parts of the coupe and season) each species list is the most similar.

From Table 4, it is seen that misclassifications among the major sampling units are rare, with only two visits being reclassified wrongly to a major sampling unit. No misclassifications of visits to the harvested coupe (H), or to the mature forest (M), occurred, the two misclassifications being for visits to the unharvested portion (U) of the aggregated retention coupe, both of which were mistakenly reclassified by the discriminant functions as visits to the mature forest. Aside from these two, all other misclassifications were between seasons within the same coupe. As the total misclassification error was 34%, erroneous assignments to season were more frequent than in the first Warra survey.

The P-value obtained from the permutation test performed by CAP was P=0.00020 when the number of permutations used was 4999, so that, in common with the first Warra survey, no randomly permuted data set had a more extreme macrofungal species assemblage than that of the original data set.

Table 5 summarises some of the most notable differences between the species of fungi in the two coupes, the list including a selection of the most frequently occurring species. The entries in the first column comprise species that occurred only in either the mature forest or the uncut portion of the aggregated retention coupe, or both, whereas the second column lists species that occurred only in the harvested areas of the aggregated retention coupe. The full list of 387 macrofungal species, and the coupes, habitats and substrates on which they occurred, is given in Gates and Ratkowsky (2006).

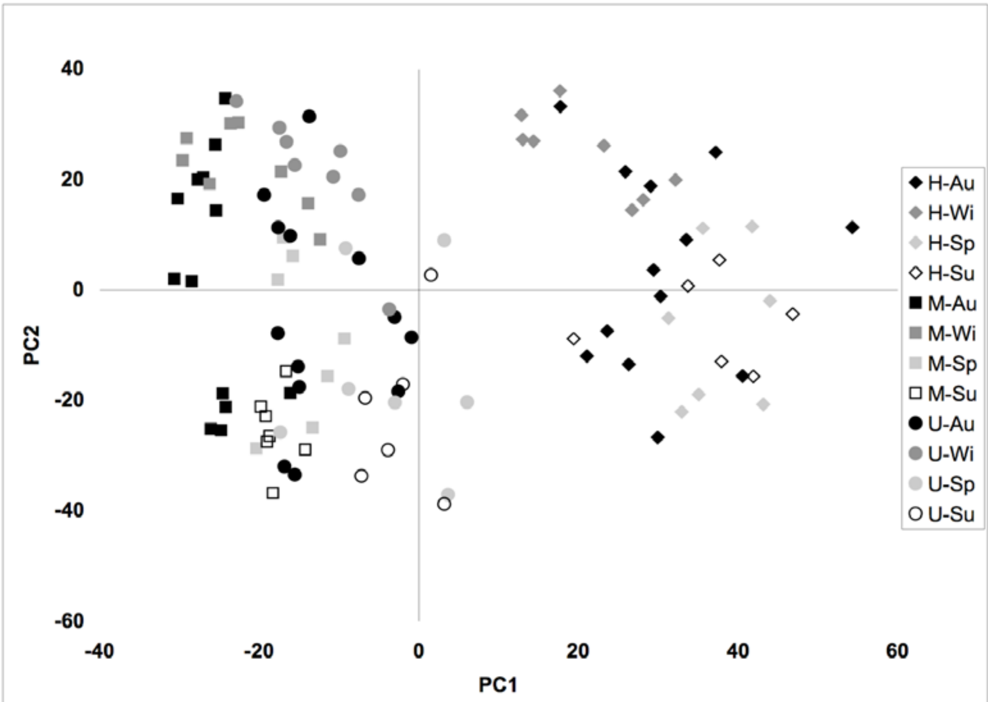

Figure 3. The first two principal coordinates of the PCoA analysis, second Warra survey.

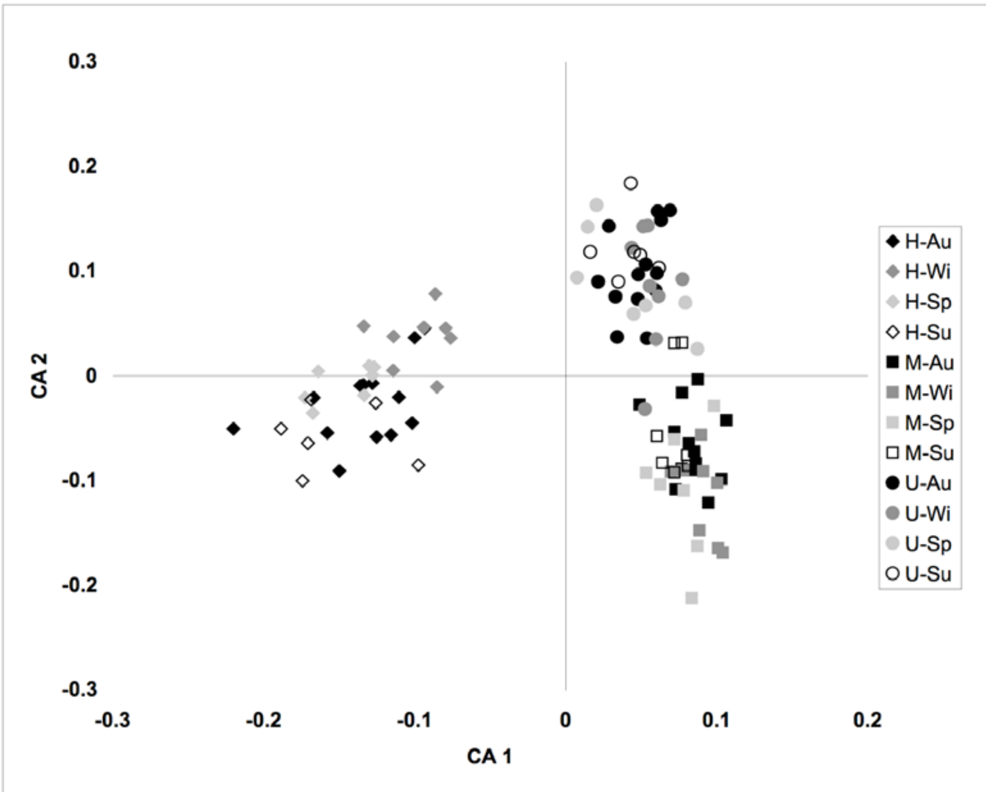

Figure 4. The first two canonical axes of the CAP analysis, second Warra survey.

[Note: In Figs 3 & 4, the first letter represents the plot (H=harvested portion of aggregated retention coupe; M=mature forest; U=unharvested portion of aggregated retention coupe) and the next two letters represent season (Au=autumn; Wi=winter; Sp=spring; Su=summer)].

Table 4. Classification table, second Warra survey, showing the reclassification of the original visits into groups, and the success and misclassification rates of the CAP analysis.

Original group	Classified into groups													
	HAu	HWi	HSp	HSu	MAu	MWi	MSp	MSu	UAu	UWi	USp	USu	Total	%correct
HAu	6	2	1	4	0	0	0	0	0	0	0	0	13	46.2%
HWi	0	8	0	0	0	0	0	0	0	0	0	0	8	100.0%
HSp	1	1	3	2	0	0	0	0	0	0	0	0	7	42.9%
HSu	0	0	2	4	0	0	0	0	0	0	0	0	6	66.7%
MAu	0	0	0	0	11	1	0	1	0	0	0	0	13	84.6%
MWi	0	0	0	0	1	7	0	0	0	0	0	0	8	87.5%
MSp	0	0	0	0	0	1	3	3	0	0	0	0	7	42.9%
MSu	0	0	0	0	0	0	1	6	0	0	0	0	7	85.7%
UAu	0	0	0	0	1	0	0	0	9	1	1	1	13	69.2%
UWi	0	0	0	0	0	0	0	0	1	5	2	0	8	62.5%
USp	0	0	0	0	0	0	1	0	0	1	3	2	7	42.9%
USu	0	0	0	0	0	0	0	0	3	0	0	3	6	50.0%
					Total correct = 68/103 = 66.0%									
					Misclassification error = 34.0%									

[Note: The first letter represents the plot (H=harvested portion of aggregated retention coupe; M=mature forest; U=unharvested portion of aggregated retention coupe) and the next two letters represent season (Au=autumn; Wi=winter; Sp=Spring; Su=summer)].

Table 5. Species occurring at least 10 times and only in the mature forest and/or in the uncut parts of the aggregated retention coupe versus those in the harvested parts of the aggregated retention coupe, second Warra survey.

Species exclusive to the mature and/or uncut forest	Species exclusive to the harvested areas
Boletellus obscurecoccineus (Höhn.) Singer	*Aleuria aurantia* (Pers.) Fuckel
Cantharellus concinnus Berk.	*Byssomerulius corium* (Pers.: Fr.) Parmasto
Cortinarius 'C48, lilac and brown, *Phlegmacium*'	*Coprinellus angulatus* (Peck) Redhead, Vilgalys & Moncalvo
Cortinarius 'C62, varnished, golden brown with sharp reddish umbo'	*Galerina nana* (Petri) Kühner
Entoloma austroprunicolor G. Gates & Noordel.	*Loreleia marchantiae* (Singer & Clémençon) Redhead, Moncalvo, Vilgalys & Lutzoni
Galerina 'small, umbonate with sphaeropedunculate cheilocystidia'	*Lyophyllum* 'small, brown'
Lactarius clarkeae Cleland	*Mycena* 'brown striate, becoming sulcate'
Lactarius eucalypti O.K. Mill. & R.N. Hilton	*Pholiota highlandensis* (Peck) A.H. Sm. & Hesler
Marasmiellus affixus (Berk.) Singer	*Pycnoporus coccineus* (Fr.) Bondartsev & Singer
Mycena interrupta (Berk.) Sacc.	*Schizophyllum commune* Fr.: Fr.
Mycena toyerlaricola Grgur.	
Phellodon niger (Fr.: Fr.) P. Karst.	
Pholiota squarrosipes Cleland	
Podoserpula pusio (Berk.) D.A. Reid	
Pulveroboletus ravenelii (Berk. & M.A. Curt.) Murrill	
Ryvardenia campyla (Berk.) Rajchenb.	
Stereum ostrea (Blume & Nees: Fr.) Fr.	

Discussion

In the two examples from macrofungal surveys at the silvicultural treatment trials in the Warra LTER in southern Tasmania, there was a large measure of agreement between the PCoA and CAP ordination diagrams. Both techniques succeeded in visualising the separation between the major experimental units but were more limited in separating seasons. The latter result is not surprising, given the arbitrary nature of the boundaries between seasons, the seasons being based upon a contiguous three months' period (Autumn: March-May; Winter: June-August; Spring: September-November; Summer: December-February). However, CAP does more than just provide an ordination diagram, expressed as canonical axes. By incorporating a permutation test, the user can test the null hypothesis of no difference between the macrofungi lists in the sampling units and obtain an exact, distribution-free P-value. In each of the illustrative examples, 4999 random permutations were used, and a P-value of 0.00020 resulted. This is interpreted as meaning that the differences among the macrofungal assemblages in the eight sampling units of the first trial and the 12 sampling units of the second trial are real and not due to chance.

The canonical discriminant analysis procedure applied by CAP maximises the differences among the predetermined structural units and provides a test of significance, unlike nMDS, PCoA, DCA and other unconstrained ordination procedures, where the data are unstructured. Although there may seem to be little justification for use of an unconstrained ordination when the experimental design has a clearly defined structure and one has an explicit multivariate hypothesis to test *a priori*, Anderson and Willis (2003) argue that there may be additional patterns in the multivariate data cloud that are not detected by CAP. Therefore, they suggest that a robust unconstrained ordination be used in conjunction with the constrained CAP analysis. This recommendation has been followed here, and the similarity between the CAP and PCoA diagrams suggests that CAP is capturing the most important factor influencing the assemblage structure.

In this paper, PCoA was chosen in preference to nMDS to illustrate the unconstrained ordination procedure, as it is an objective method of MDS and is available as an option when using CAP. To compare the results to those from a nonmetric procedure, the first two axes of a three-axes nMDS ordination of the data from the second Warra survey, carried out using Primer 6 (2006), are presented in Fig. 5. The original data matrix is the same as that for the CAP analysis presented in Fig. 3, i.e. presence/absence data were used, not transformed and not standardised, with Bray-Curtis dissimilarities.

In Fig. 5, the separation between the cloud of points for the harvested portion (H) of the aggregated retention coupe and that for the mature forest (M) is very clear, but the overall interpretation of the ordination is muddied by the very wide scatter of the data points for the unharvested portion (U) of the aggregated retention coupe. Aside from being located in more or less the right position, i.e. somewhere between the clouds of points for the other two treatments, there is little else to be learned from this diagram. Seasonal effects are also not revealed, with points for each season being spread over a wide range. Displaying the third axis (not shown here) of the three-axes solution does not add anything further to the interpretation.

Some further remarks

In the first CAP example presented here (see Fig. 1), the spread of the points within the mature forest (M) and within the clearfelled, burnt and sown group (H) appear to suggest that the distances between the observations, and hence the variability, is greater for the control group. However, such a conclusion would be unjustified without carrying out a formal statistical hypothesis test, as the distances displayed on a canonical variates diagram are not Euclidean distances, but Mahalanobis distances (see Legendre and Legendre 1998). That is, the distances between points take account of the variances and covariances of the species. The consequence of this is that differences in dispersion cannot be deduced from how the points are arranged on the axes of a CAP diagram, but must be tested using a formal statistical test. A procedure (PERMDISP2) for

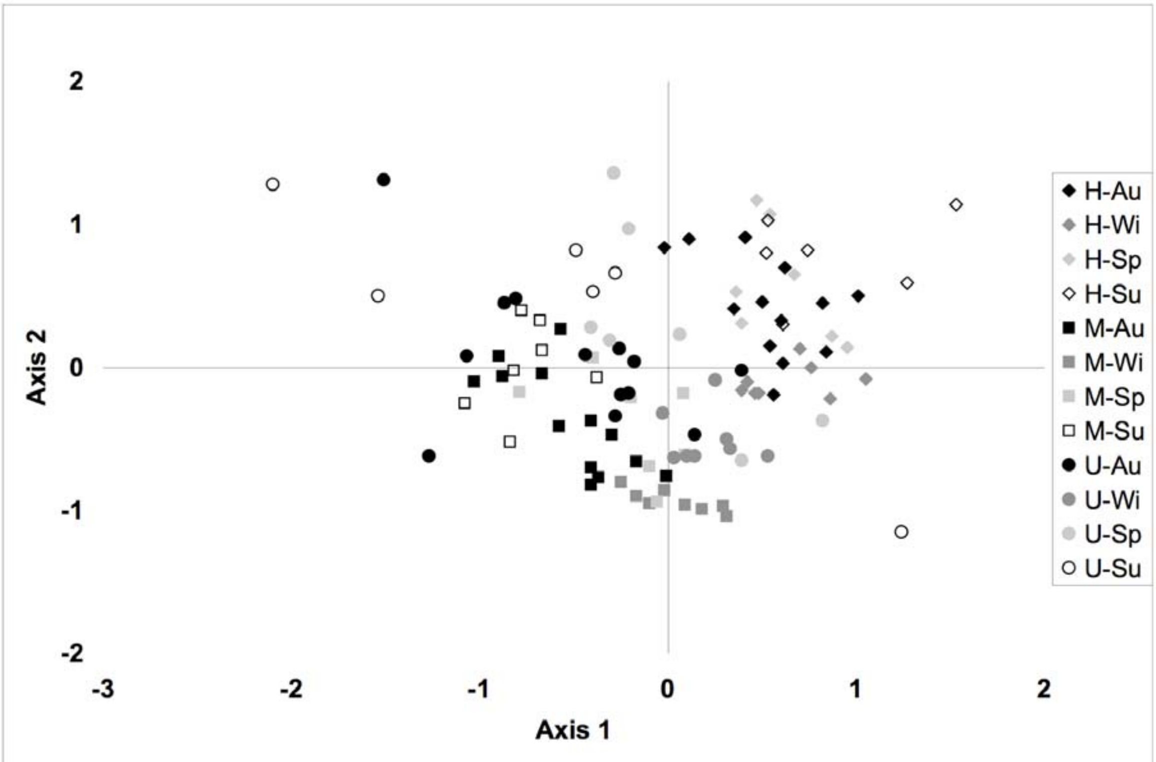

Figure 5. Axis 2 vs. Axis 1 of a nMDS analysis of macrofungal data, second Warra survey.

[Note: The first letter represents the plot (H=harvested portion of aggregated retention coupe; M=mature forest; U=unharvested portion of aggregated retention coupe) and the next two letters represent season (Au=autumn; Wi=winter; Sp=spring; Su=summer)].

doing this has been developed by M.J. Anderson, which performs a distance-based permutation test for the homogeneity of multivariate dispersions in groups of unequal sample sizes and is based upon any definition of similarity (such as Bray-Curtis).

CAP is available for downloading as freeware from M.J. Anderson's home page at the University of Auckland. The address is

http://www.stat.auckland.ac.nz/~mja/

User notes explaining step-by-step how the programs are used may also be downloaded from that website.

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