Plant diversity assessment and monitoring

Handout

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Day 1: Theoretical background on biodiversity

Biodiversity is a multifaceted concept and difficult to describe in simple terms. Thus, in order to get a common idea of what biodiversity is, we started the workshop with the official definition by the Convention on Biological Diversity (CBD) signed in Rio de Janeiro in 1992. The convention defined biodiversity as:

> *'Biological diversity' means the variability among living organisms from all sources including*, inter alia (=among others), *terrestrial*, *marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.'*

Hence biodiversity deals with the variability of life at the level of genes, species and ecosystems.

Secondly, we explored the question "why is biodiversity important" and why should we care about it? We found that biodiversity as several types of uses, namely "direct use values", "indirect use values" and "non-use values" (Gaston & Spicer 2003). We discussed the following points with some examples.

Direct use values are :

- Food
- Medicine
- Construction / Materials
- Fire wood – livelihood
- Ecotourism

Indirect use values are:

- Water filtration & regulation
- Nutrient cycling
- Pollination
- Photosynthesis
- Soil maintenance

We discussed biodiversity within the concept of ecosystem functions and ecosystem services in the light of indirect use values. We found out that biodiversity has an insurance function. The more species the more likely it is that the ecosystem that harbours those species, will be a functioning ecosystem. We also connected the use-value concept to ethnobotanical research examples from western Africa. Finally, non-use values are

Non-use values:

- Bequest value, i.e. the value of passing intact nature to our future generations. We are obliged to use nature in a sustainable manner. We found the example that capitalism is not sustainable and is linked to unlimited growth, which is impossible on a planet with endless resources.
- Intrinsic value, i.e. the value of the pure existence of a large biota that strives and lives and gives joy to many people in the world. We should not decide whether a certain bug is useful,

because we do not know its role in the ecosystem. Hence, we should appreciate all species relatively equal, of course this is difficult.

- Option value, i.e. the values of the hidden potential of many species for potential medicine or as inspirational source for bionics or biomimetic.

We then discussed the fact that biodiversity is globally declining. We found that climate change is an important issue. Already this year, all four months were significantly above average temperature of the last 80 years. However, climate change is working slowly and is not (yet) the main cause for species decline. This is in fact the land use problem, i.e. the transformation of natural forests into agricultural land, plantations, urban areas, etc. Deforestation and land degradation are often the consequence of bad land management and thus uncontrolled land use seems to be the main problem for species decline. One thing that was not discussed in the course is the problem that taxonomical knowledge is lost, too. I urged the students (several times during the course) that it is very important to have good species knowledge and to be able to identify at least one taxonomical group (e.g. plants, spiders, birds, reptiles, etc.). This is particularly true for all the soon-to-be forestry graduates who should be able to identify the trees they work with.

After discussing what the main sources of species decline are, we turned to the question on how to measure biodiversity. I started with a classical example that most 20 out of 22 students answered as expected. There are two different forests, A and B (Fig.1). A has two species and B has three species. Most students were convinced that B is more diverse because of higher species richness. I observe the same pattern of student answer also in Germany and elsewhere, because species richness is logical and intuitive concept. However, I then reminded the students to look at each species frequency. In A, species are equally frequent, while in B one species (X) is dominating, while the two other species only occur with one single individual. Thus, it is unlikely to find many species in forest B, but the species X. I then introduced the concept of evenness which describes the distribution of abundances within one community. A has an evenness of 1 (maximum) while B has an evenness of about 0.2.

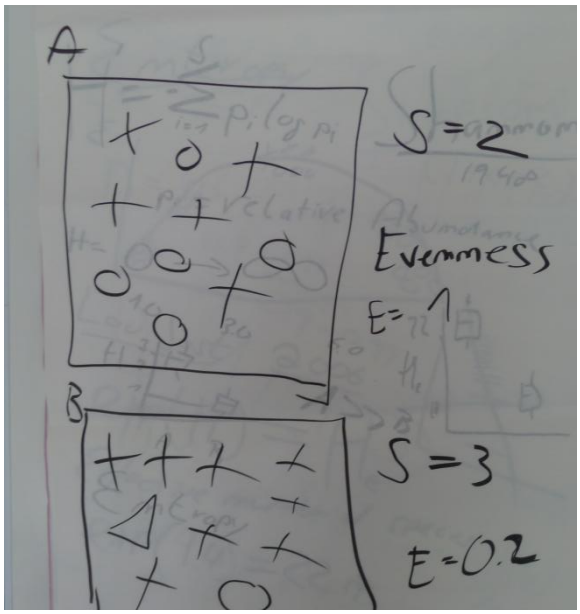

Fig. 1 Example of Richness and Evenness

So until here, I have introduced species richness and evenness as central concepts of biodiversity measurement. Then I introduced the Shannon Diversity index, invented by Shannon in 1948. I explained the formula (Fig 2.) which includes pi which is the relative abundance of species i.

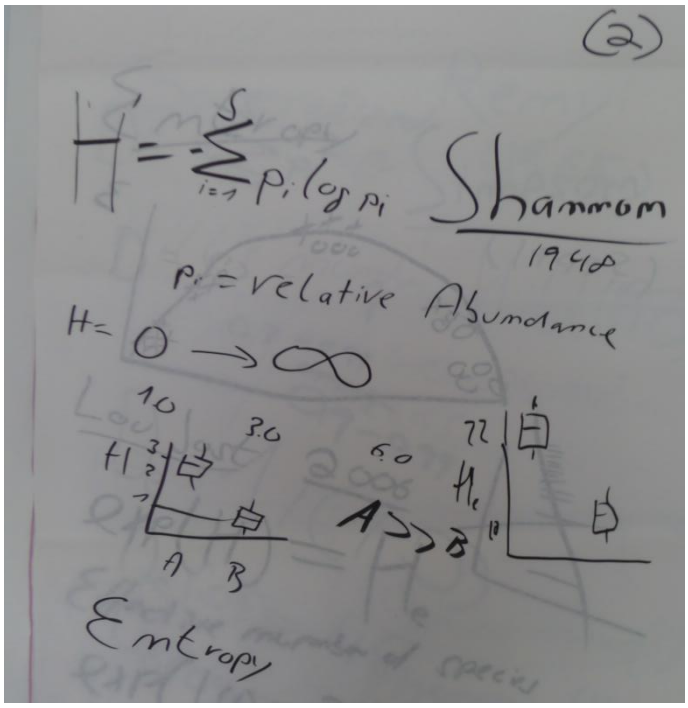

Fig 2. Formula and boxplot examples of Shannon index.

The Shannon diversity index, expressed in real numbers, has usually values from 0 to theoretically unlimited, i.e. there is no upper bound. I used a boxplot (left) to show how Shannon values can be visualized to show differences between forest A and B. In Figure 2, A is clearly more diverse than B. However, the interpretation of H in biological terms is very difficult. Many authors actually advocate against the usage of Shannon, because it "confounds" richness and evenness. Yet, as will be shown, this is only due to a (long lasting) misunderstanding of the Shannon index. It is important to note that Shannon (H) is not a diversity index, but an entropy index. I thus made a quick excursion to explain the concept of entropy with an easy to understand example (figure 3).

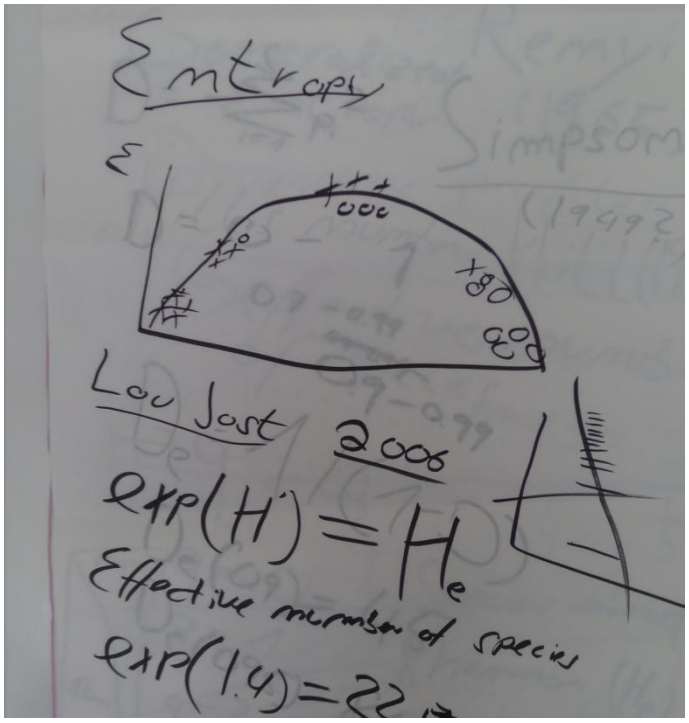

Fig. 3 Entropy and the concept of effective number of species (He) for H

Entropy is maximum when an equal amount of information (abundance) is available for any state (species). In the example in figure 3, we have two species, circles and crosses. At both ends the x-axis we have either X or O. The more me mix the two species, the more entropy there is. The Shannon index (H) is actually a measure of entropy. As can be seen on the figure 3 on the right hand side, Shannon entropy is increasing linearly, thus making interpretation very difficult. What is the difference between H=1.7 and H=1.5. Is this a lot or neglible? I then introduced the work of an Ecuadorian mathematician and biologist, called Lou Jost. Jost (2006) came up with a long-lost knowledge about the effective number of species, a concept introduced by MacArthur 1967 and developed further by Hill (1973). Jost said that a simple transformation of the Shannon index by its exponential i.e., exp(H), leads to a linearization of the entropy and transforms the original entropy values into diversity values, in units of species richness. However, the transformed values are called the effective number of species, which means that for example exp(1.4) = 22.17, a Shannon value of 1.4 equals 22.17 effective species with an equal abundance. Hence, we need 22.17 equally abundant species to generate an entropy of 1.4. The effective number of species has two important properties. 1) It is linear and thus can be compared easily with other transformed Shannon values, and 2) it is in units of species richness and thus can be compared to other measures of effective number of species. It thus becomes easier to interpret than standard Shannon values.

Besides the Shannon index, there are many other indices, invented in the 70s and 80s. However, many of them are not in use today because of strange behaviour (see Peet, R. 1974 and 1975 for good reviews). Another commonly used diversity measure is the Simpson index (Simpson, 1949), also called the Gini-Simpson index (Figure 4) .

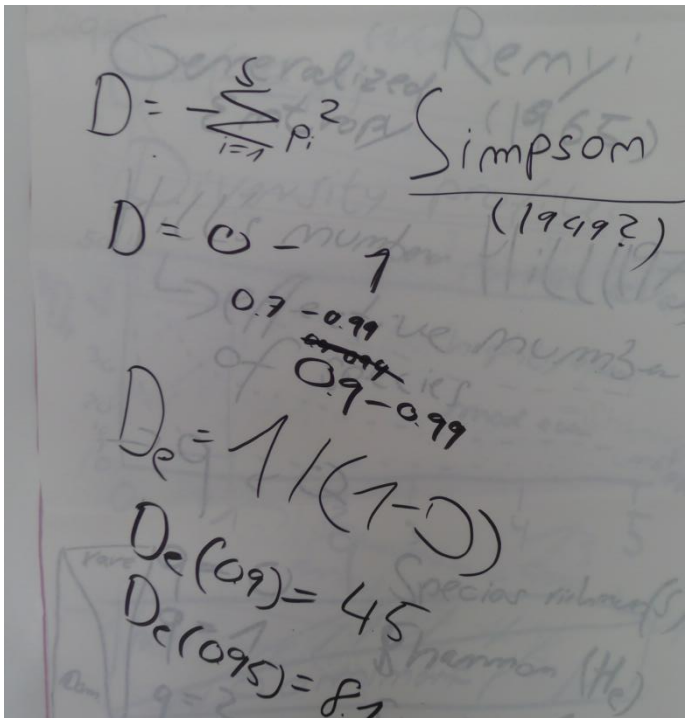

Fig. 4 Simpson index and its transformation (De) into effective number of species

The Simpson index (D) is similarly hard to interpret as the original Shannon values. As it is a so-called dominance index, the values are often between 0.7 and 1 while they can in theory range from 0 – 1. Also here, a transformation into effective number of species (De), helps to better interpret the Simpson index values on a species number basis. Small changes in D can lead to large changes in De.

Hence it became clear that the concept of effective number of species Is useful to better interpret the diversity or better say entropy values. I then noted that all three indices so far, e.g. S, H and D, are linked mathematically by a so called generalized entropy formula (Fig 5) which was identified in different versions by Renyi in 1961, Patill and Tailie 1982 and Hill 1973. These generalized entropies allow calculation of all possible diversity indices of an order of q. The order of q can be read as follows:

- q = 0 → Species richness
- q = 1 → Shannon index
- q = 2 → Simpson index

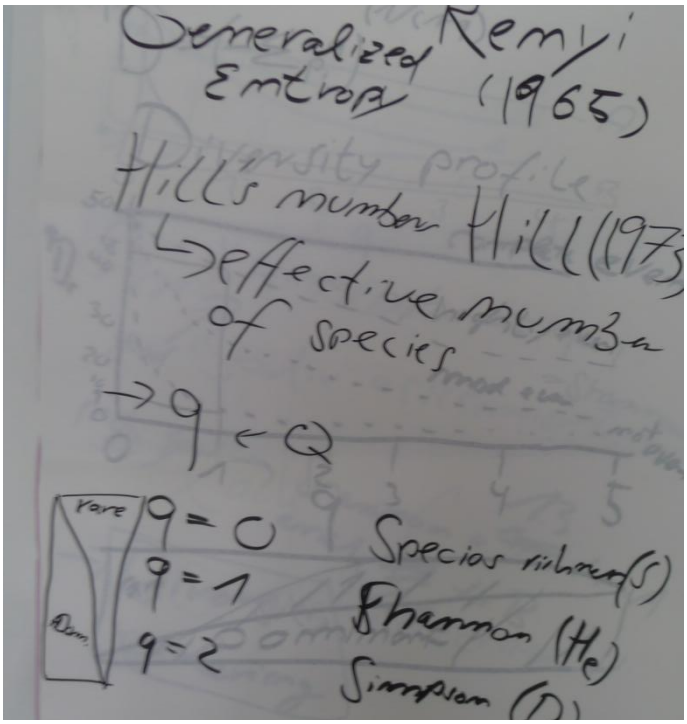

Fig. 5 Generalized entropies and the order of q

The order of q is sensitive to abundance. An order of q = 0 weights all species equally, thus rare species are of high importance and have more weight than in other orders of q. The higher the order of q gets, the more important becomes the dominance effect of single species. I then introduced the generalized entropy formula developed by Hill (Fig.6), which transforms an diversity index (D) of the order of q (^{q}D) into an effective number of species.

I then introduced an important tool, that in combination with the effective number of species concept is a valuable tool form comparing and ranking biodiversity across different communities / assemblages. In figure 6, I developed the idea of the diversity profile piece by piece by showing first a community that has 50 species and all of them are equally abundant and thus leads to a straight line which I called “completely even”. Next, I draw the dashed lines for a highly even community, a moderately even and a not-at-all-even community, all with 50 species. The importance of abundance becomes eminent in this figure and the patterns of evenness obvious. Also the lines are comparable across the whole profile in terms of effective number of species. For example, at q=1 we see that ^{q}De is only 10 effective species, while the completely even community has also 50 effective species. It is also possible to compare orders of q like 0.5 0.7 and so on. Usually values for q are used from 0 to 5 in 0.1 steps.

It is important to note that the order of q can be read as an importance of rarity / dominance, as portrayed in the lower part of figure 6.

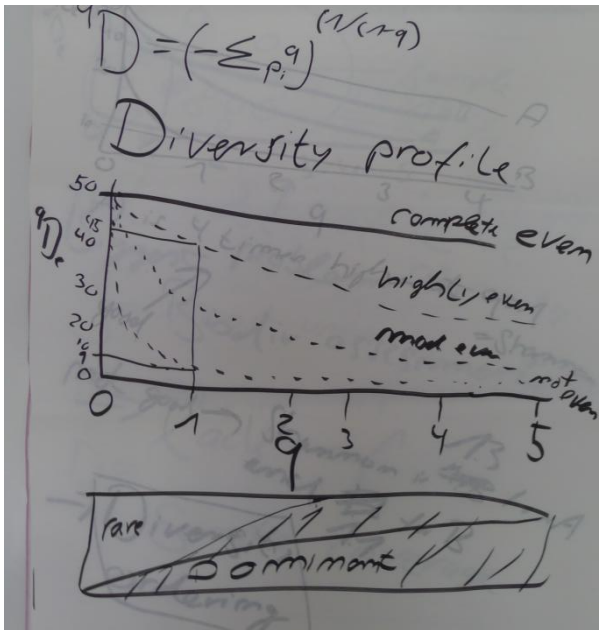

Fig. 6 Hills generalized entropy and diversity profiles for comparing diversity

We then looked at an example of two non-intersecting diversity profiles, of forest A and B (Fig.7). We see that forest A is always more diverse across all orders of q then B. We can also quantify in terms of effective number of species, how many times more diverse A is over B for a given order of q (A is 4 times more diverse than B at an order of q=1). I introduced the method of diversity ordering, which simply consists of comparing or ranking diversity profiles.

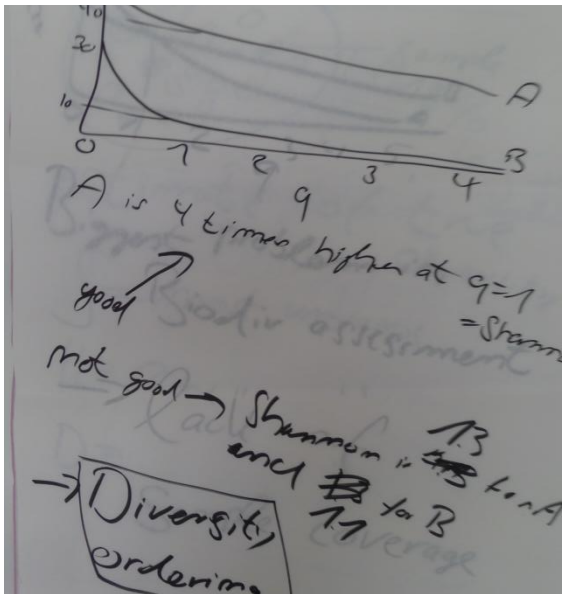

Fig. 7 Example of a comparison of two diversity profiles

However, in the next slide (Fig.8), I showed that diversity ordering is not always trivial and that diversity profiles might intersect. Then it is not possible to state that A is truly more diverse than B across all orders of q, but that in some orders of q A or B is more diverse.

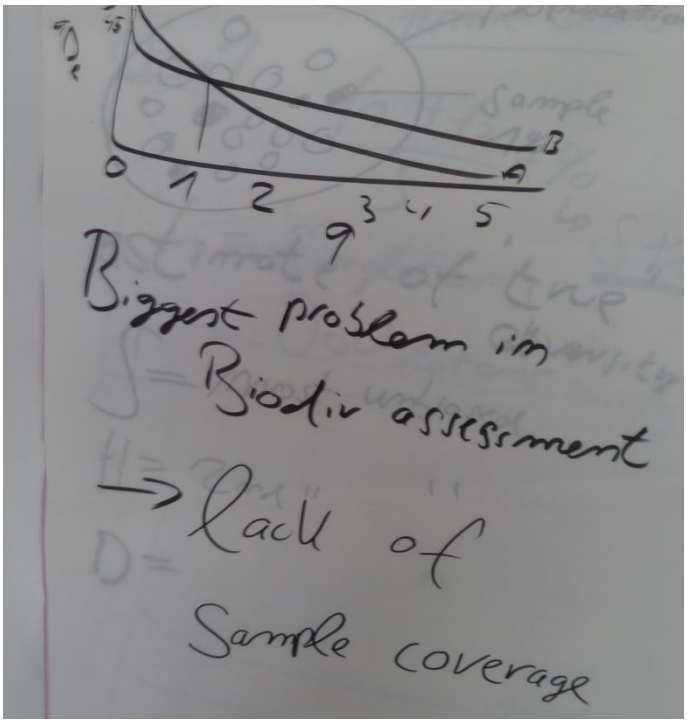

Fig. 8: Another example of diversity profiles and the biggest problem in biodiversity research

I then asked, what is the biggest problem in biodiversity assessment? Nobody had an answer, but the most important problem is that of lack of sample coverage. We have to understand that the sampling we are doing has the ultimate aim to estimate the true diversity of an assemblage. I used the example of a statistical population from which we sample (Fig.9) in order to estimate the true diversity from. Too few samples will lead to a strong bias in the diversity estimates along q because in particular rare species are often overlooked, but are essential part of the biodiversity.

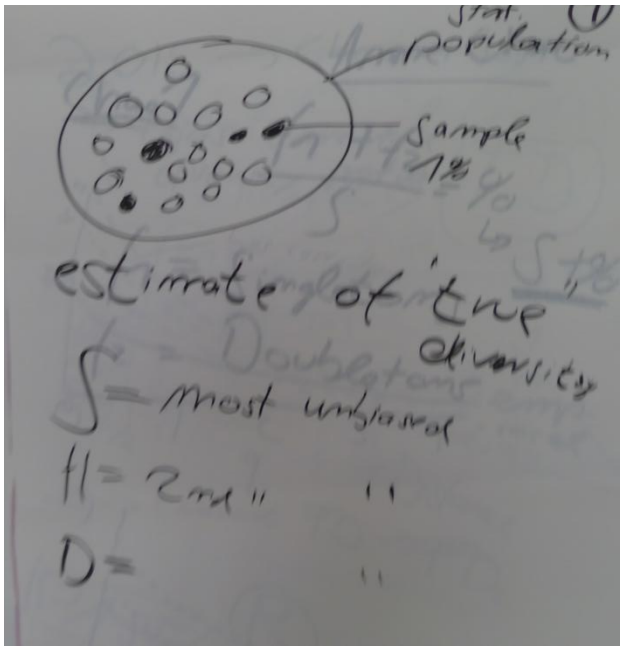

Fig. 9: Biased estimates caused by undersampling
(**it should read "S = most biased"! Not unbiased! Also see text**)

Hence, species richness is the most biased diversity index as it strongly depends on the rare species. Shannon index is the second most biased diversity index, while the Simpson index is relatively

unbiased. There exist a working bias-correction for the Simpson index that can provide unbiased estimates. After understanding that sampling impacts the quality of our diversity estimates, the question arises what we can do about that. I then introduced the work of Anne Chao, a professor for mathematics in biology from the University of Taiwan, who devoted most of her research to study biodiversity estimation. She developed a series of so called richness estimators, which try to estimate the true number of species, i.e. including the hidden or not found rare species. For example one of her earliest richness estimators called Chao1 used the number of singletons and doubletons (f1 and f2) to estimate the percentage of hidden species. This percentage is then added to the original value of richness in order to get a better estimate of species richness (Fig.10).

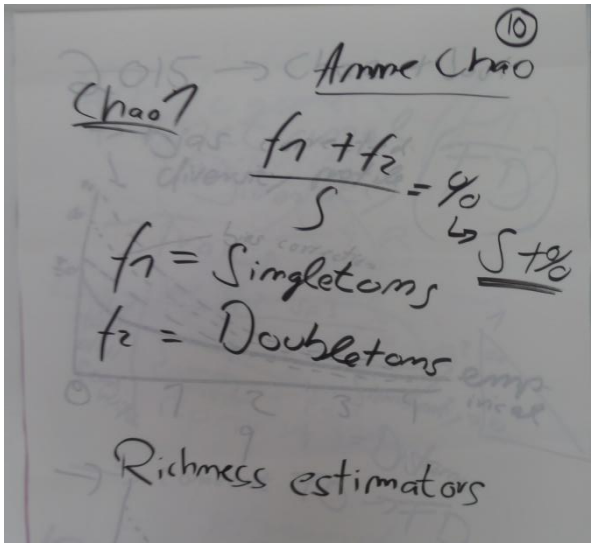

Fig. 10: Chao1 richness estimator to correct the biased richness estimates

In 2012 until today, Anne Chao and Lou Jost teamed up with some other prominent ecologists to develop bias correction for diversity profiles based on the effective number of species concept. They came up with several statistical procedures to improve the estimation of diversity profiles. One example is shown in figure 11.

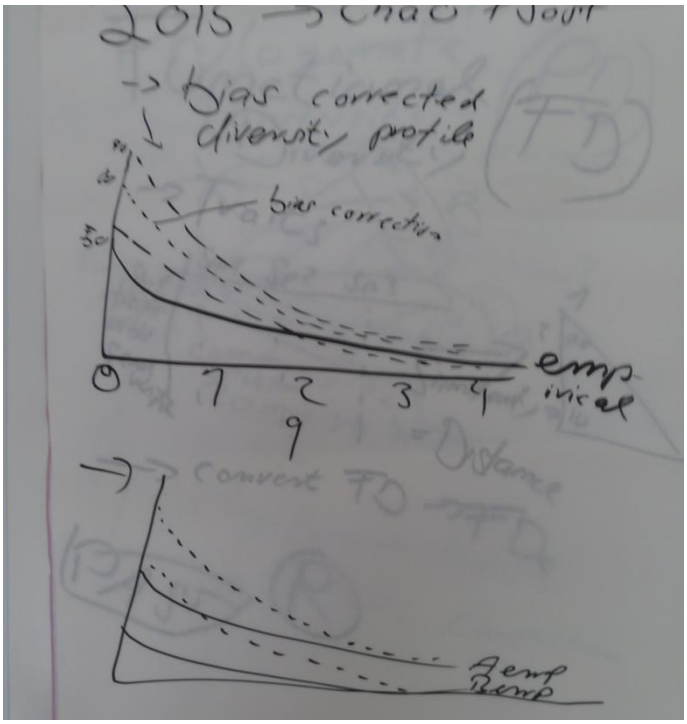

Fig.11: Bias corrected diversity profiles

The upper plot in figure 11 shows the empirical diversity profile with a solid line. The empirical profile is the one that we measure in the field. The bias corrected diversity profile has a dotted line, while the confidence interval for each order of q is shown as a dashed line. We can see that there is a big difference between the empirical and the bias corrected line at low orders of q. However, this effect diminishes around q=2 which supports the statement from earlier that Simpson index is the least biased diversity index of the q =0,1,2. In the lower plot, two empirical profiles and two bias corrected profiles are shown for A and B. This is what we have as an output after this week of exercise. An empirical and a bias corrected diversity profile for the Arboretum versus Natural Forest.

After having talked now a lot about taxonomical diversity, i.e. at a species level, we will now introduce two other broad concepts which are gaining importance in science; functional diversity and phylogenetic diversity. Functional diversity deals with the diversity in species characteristics, the so called traits. Traits can be anything that is measurable or classifiable in terms of species characters. Prominent examples for plants are life form, growth form, leaf type, specific leaf area (SLA), seed weight, average height, life history (annual, perennial), flower type etc. All these traits character ise the ecology of a species and the functional niche it occupies in nature. The concept of functional diversity was also identified to be in particularly important in the studies of ecosystem functions and services. Thus I felt it important enough to be mentioned here in the course (Fig 12).

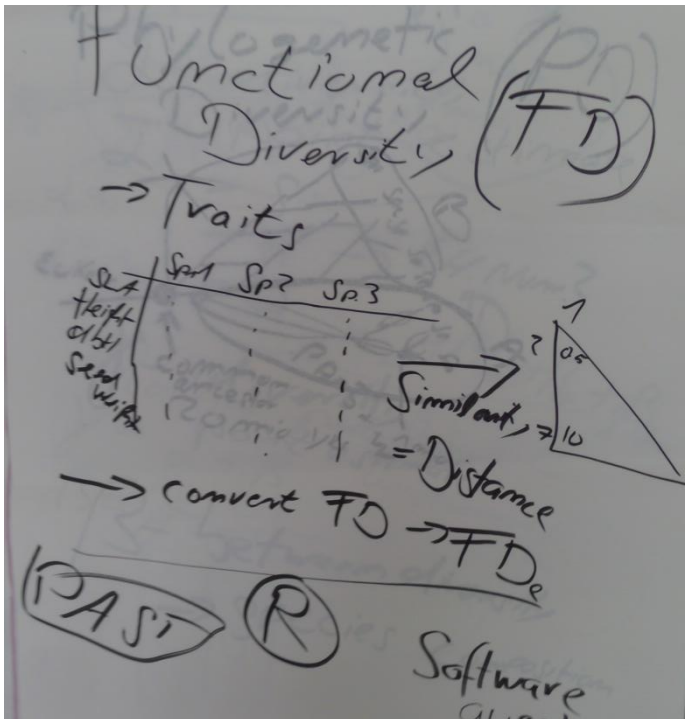

Fig 12: Functional diversity

The basis for functional diversity analysis is a species by trait matrix. From this matrix we use a similarity index or the Euclidean distance (usually Gower distance is used) to calculate a similarity triangular matrix which describes in the sum the functional diversity of a community, e.g. a forest type. Calculating multiple FD values for multiple forests allows to compare the functional diversity then. However, this is only a tiny glimpse on a huge topic with more than one solution with regard to how one can calculate FD. It is important to note that Chao and Jost also developed a effective number of species equivalent for Functional Diversity! Thus it becomes possible to compare multiple diversity types (Taxonomical, Functional and Phylogenetic) within a unified framework. A question came up about which software to use to calculate these things. I said that we will use PAST and R on Wednesday.

Potentially equally important for analysing biodiversity is the new strain of phylogenetic diversity (Fig 13). Phylogenetic diversity (PD) includes the evolutionary history of species as described by a phylogenetic tree (i.e. the APG III or IV tree). The idea of phylogenetic diversity is that a species within a tree has a certain distance in branch-lengths within a cladogram from the common ancestor (root node). This distance is counted as the number of branches within the dendrogram, or as the distance in million years from the root node. The latter is a better measure of evolutionary history but harder to get. So, phylogenetic diversity requires a phylogenetic tree, which includes all species which are considered in our sample. This is for sure difficult in Ethiopia, as potentially not all species are somehow listed in GeneBank, the online-repository for genetic information. Still I felt it important to mention this type of diversity as well, so that the people at least have heard of it. Furthermore, also PD can be converted to effective number of species, so it fits into our concept of the course as well.

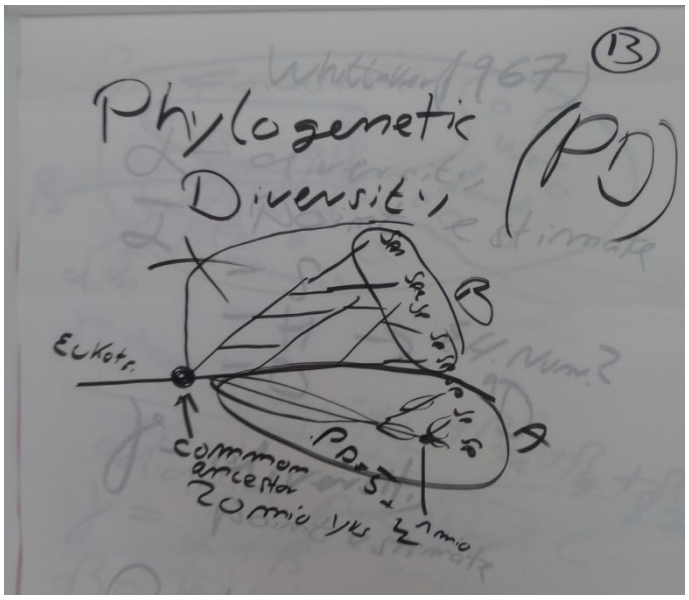

Fig 13. Phylogenetic diversity is based on a cladogram or a phylogenetic trees. Community A and B differ in their species composition and likely in their phylogenetic diversity.

I came up with the example of *Welwitschia mirabilis*, which is an extraordinary plant living in south west Africa (Angola, Namibia). This species is phylogenetically very distinct, thus if we loose this species in a sample, it would have a much stronger impact on phylogenetic diversity then if we loose a very young species, e.g. one out of the many Asteraceae. Of course this is all depending on context and ecosystem.

Finally, I wanted to make the students aware of the biodiversity levels introduced by Whittaker (1965), i.e. the alpha, gamma and beta diversity. Few of the students have heard of this concept, at least few of them raised their hands when asked. However, as this is such an important concept, as it is used everywhere in the world, I made an attempt to clarify what alpha, gamma and beta mean (Fig. 13).

- Alpha: point estimate of diversity within a habitat
- Gamma: point estimate of diversity for all considered habitats
- Beta diversity: between diversity between all habitats.

In fact, we talked about Alpha and Gamma already the whole day, but we did not lose a word about beta diversity, which actually makes use of the information on the species composition within the different habitats. This is an important difference. Species composition can tell us about the type of habitat much better than any species richness number or diversity profile.

In figure 14, I gave an example for a forest area how to locate alpha, beta and gamma on a map. Furthermore, I shortly introduced the idea of additive diversity partitioning which means that gamma consists of alpha + beta. However, likewise, we can derive beta from gamma-alpha. However, additive or multiplicative diversity partitioning is a hotly debated field of research and should not be taught to practitioners necessarily now.

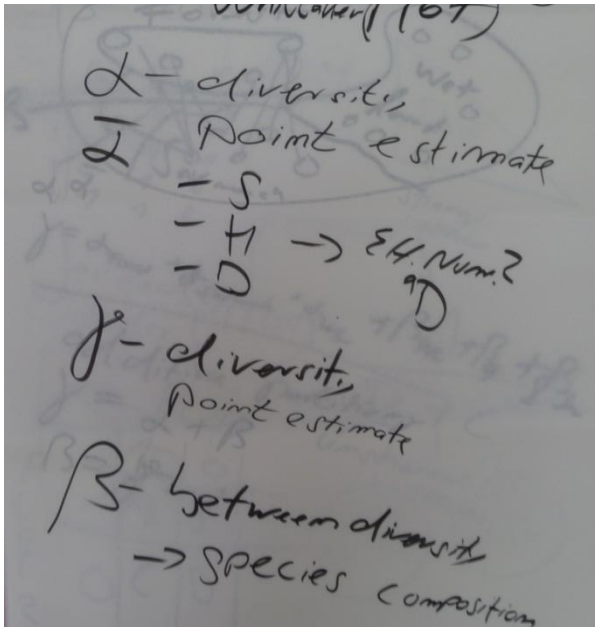

Fig. 14: Whittakers famous concept of the different levels of biodiversity

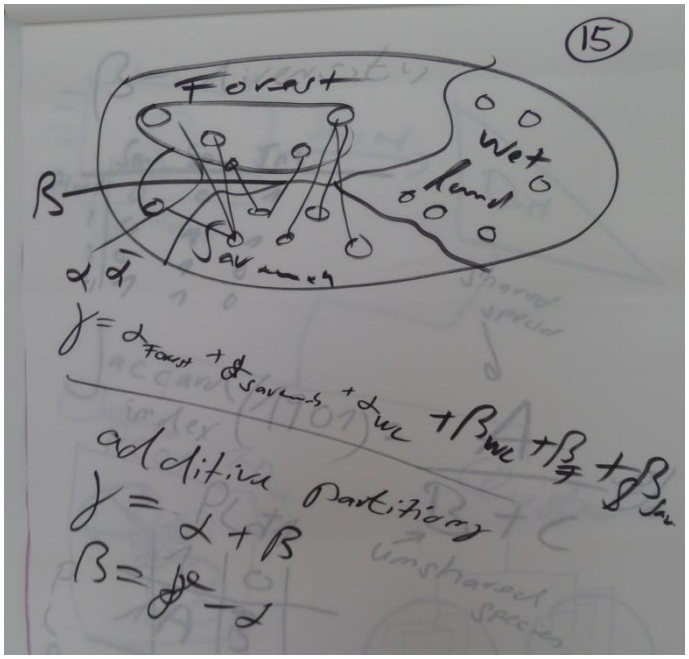

Fig. 15: Example of alpha, beta and gamma on a map, below; additive partitioning of diversity

In order to introduce the idea of beta-diversity, I first draw a species by plot matrix with species in columns and plots (samples) in rows. The cells of the matrix contain either 1 or 0 in order to inform about presence (1) or absence (0). I introduced one of the oldest similarity indices to describe such a species by plot matrix; the Jaccard Index (Jaccard 1901). The formula is shown in figure 16 and requires A, B and C. **However, the formula is WRONG! I must be A / (A+B+C)**! The meaning of the capital letters is shown in the small table in the lower right. A means the number of shared species between plot 1 and plot 2. B and C are the number of unshared species in the plots. Thus the Jaccard index shows the percentage of shared species in relation to all species in two samples. The Jaccard Index is then computed either for all sample pairs or only for the species list of Forest A and B.

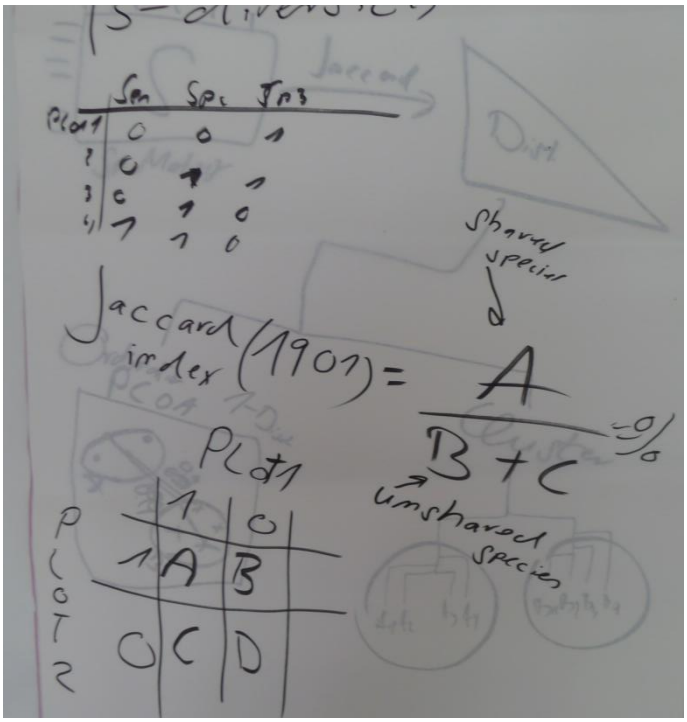

Fig. 16: Beta diversity as calculated by the Jaccard index.
NOTE: Jaccard formula is wrong! Has to be A / (A+B+C)

In the first case, one gets a similarity matrix out of the species by plot matrix by applying the Jaccard index (Fig 17.) The similarity matrix (S) can be converted into a distance matrix (D) by simply subtracting 1-S = D. This inverts the similarity matrix. The distance matrix is then usually put into an ordination analysis (e.g. a PCoA) to identify patterns in ordination space. For example, one can see whether samples of two different habitats overlap, how long the distance is from each point to the next point or from the farthest points. Furthermore, one can subject the distance matrix to a cluster analysis which produces a dendrogram. The dendrogram allows us to identify clusters of species in the dataset. If there are plots with similar species, they will cluster together in the dendrogram. In the example in figure 17, there were two distinct groups of samples coming from forest A and forest B. In the ordination the showed a clear separation in ordination space. In the cluster analysis they also differed clearly in the dendrogram, with the forest A on the left, forest B on the right hand side of the dendrogram. However, the dendrogram suggests that there might be a third cluster within forest B. Thus cluster analysis can potentially help us to find subgroups within known groups. This approach of beta diversity analysis is called distance based beta diversity.

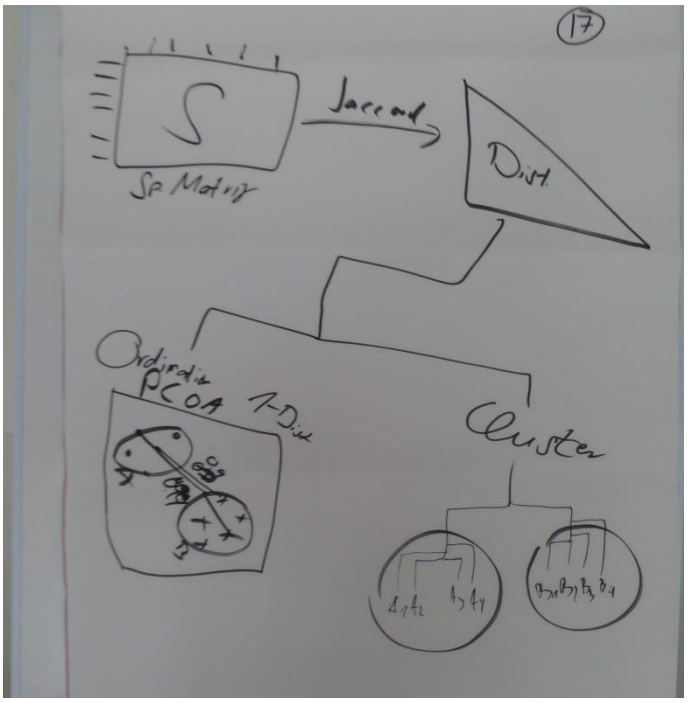

Fig. 17: Examples for the use of a distance matrix, ordination and cluster analysis.

I ended the first part of the course on the theory of biodiversity and its assessment with a wrap up from what we learned today. I hope this hand out will help the students to repeat some of the ideas provided here.

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Day 1: Field Training

After the theoretical part, we went to the Wondo Genet Arboretum to practice data assessment for the upcoming day of field work. We identified a 20x20m area within the Arboretum close to the entrance and practiced data assessment. Therefore, I marked the corners of the 20x20m plot using water bottles. In the Arboretum, this is a sufficient idea, however, in the natural forests, a tape measure (100m) is a must. Without the tape measure one gets lost easily. In the Arboretum, the water bottles were well visible and the trees within the plot were all countable. I then showed how to take pictures from the corner (1 across the plot, OR from all four corners), and where and when to take a GPS waypoint. It is important to write down to coordinates (in WGS84, i.e. decimal degrees) into your fieldbook. If the GPS gets lost during field work, your coordinates will be lost, too. Finally, I showed how to write down the data into the field book. Other people prefer having printed document sheets, with one paper per plot. However, in my experience, one can easily have 5-10 plots on one page in a fieldbook. If you come up with 400 plots, It would be a big pile of paper to carry if you do not use a fieldbook! One note, write with pencil (sharp!) if possible. This allows to eradicate any errors later. Use field names for unknown species.

We focused here on tree species diversity, as identifying herbaceous species would have been too troublesome. But already with the trees, it became clear that one of the most important skills of a researcher working on biodiversity is to able to identify trees, if possible already in the field. Without any knowledge about flora keys, taxonomy etc. please DO NOT do this kind of research. You will be frustrated (and your supervisors, too).

After the Arboretum exercise, we went into the natural forests to understand the situation there. We decided that it would be better to have 10x10m plots, because of the high density of the natural forest stands. The day ended with the view from the red tower over the campus.

Day 2: Field work

On the second day, we split up into four groups. Three groups were selected to measure at least five, maximum ten plots of 10x10m in the natural forest. One group was sent to the Arboretum so we have two major types of systems to compare. In addition to the tools from yesterday, we had cameras and tablets for making photos, chalk to mark counted trees, a 100m tape measures to delineate the plots in the forest and free field books and sharpened pencils for the group leaders. Each team had one experienced tree expert, to be sure that in each group was one proficient taxonomically trained person.

Fig. 18: Natural forest vegetation close to the Wondo Genet College for Forestry & Natural Resources

Fig. 19: An even denser natural forest plot.

The field work was scheduled from 9:30 until 15:30. At 15:30 we met again in the class room and discussed the problems discovered so far. Interestingly, it was not very difficult for the natural forest groups, and we even could have chosen 20x20m, which I would recommend for future studies of tree communities. Most trees were identified, however several groups had several unidentified species. The natural forest group made 4-5 plots while the Arboretum group made ten plots, totalling to n = 24.

Day 3: Data entry

In the morning of the third day, we met for entering the data in the classroom. The aim was to produce one flat spreadsheet table with species as columns and plots as rows (Fig 20). Additional data such as GPS coordinates etc. could not be used, because the data was not provided in the correct format. Hence the only information we had besides the species counts was the grouping into natural forests (NF) and Arboretum (AR), which we aimed at comparing. Sadly, plot G4_9 was completely empty and had to be removed during the analysis.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	PNR	Cl.african	Afrofalc	C.macrsta	F.sur	Ficuvast	A.abyss	M.ciolata	C.africana	A.gumef	Senna sian	C. arabica	Juniperus
2	G1_1	3	1	0	0	1	0	1	2	0	0	0	0
3	G1_2	1	0	3	1	0	1	0	0	2	0	0	0
4	G1_3	3	1	2	0	1	1	0	1	2	0	0	0
5	G1_4	6	0	1	0	0	0	3	2	0	0	0	0
6	G1_5	2	0	2	0	0	0	0	2	2	0	0	0
7	G2_1	4	0	0	0	0	0	0	0	0	1	0	0
8	G2_2	4	0	2	0	0	0	0	2	0	4	0	0
9	G2_3	7	0	0	0	0	0	0	0	0	0	0	0
10	G2_4	5	0	0	0	0	0	0	3	0	4	2	0
11	G3_1	0	0	1	0	0	0	0	0	0	0	0	1
12	G3_2	6	2	0	0	0	0	0	0	0	0	0	0
13	G3_3	2	0	2	0	0	0	0	0	0	0	0	0
14	G3_4	3	5	0	0	0	0	0	0	0	0	0	0
15	G3_5	0	2	0	0	0	0	0	0	0	0	0	0
16	G4_1	0	3	0	0	0	0	0	0	0	0	0	2
17	G4_2	0	0	0	0	0	0	0	0	0	0	0	0
18	G4_3	0	8	2	0	1	0	0	0	0	0	0	0
19	G4_4	0	0	2	0	0	0	0	0	0	0	0	0
20	G4_5	0	13	0	0	0	0	0	0	0	0	0	0
21	G4_6	0	0	0	0	0	0	0	0	0	0	0	0
22	G4_7	0	0	0	0	0	0	0	0	0	0	0	0
23	G4_8	0	0	0	0	0	0	0	0	0	0	0	0
24	G4_9	0	0	0	0	0	0	0	0	0	0	0	0
25	G4_10	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 20: Part of the final data set. The groups sampled 24 plots and 28 species, from which 2 were unknown species and two were only known with a local name.

Day 3: Data analysis with PAST

With this dataset ready, we were able to make our first analyses. As most of the students never used R, I found it important to provide an alternative to perform some diversity analysis. The free software PAST is a great tool for that purpose. It is freely available at: http://folk.uio.no/ohammer/past/ and can be cited as: Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9pp.

The current version we used is PAST 3.0. The import is made easy by simply copy and paste the whole excel table into PAST. However, before pasting, one has to check both “show” boxes in the upper left and then the upper left cell of the table has to be selected. See figure 21 for an example.

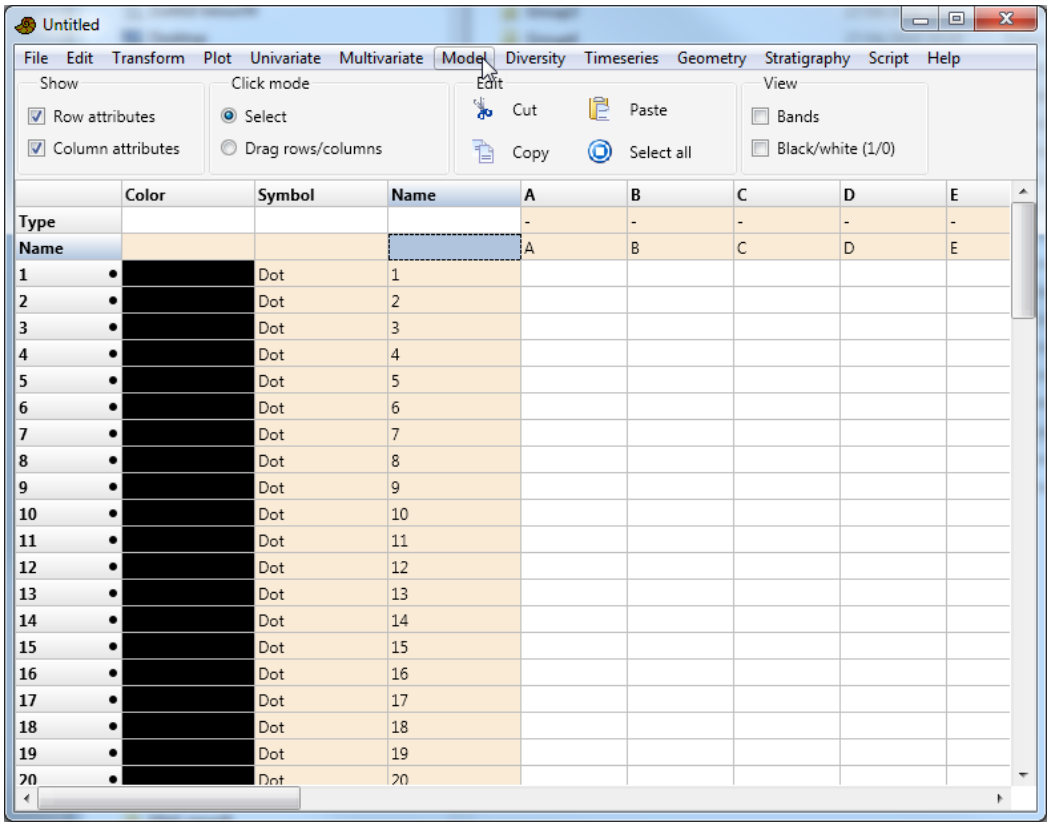


Fig. 21: PAST 3.0 while importing data. The “show” boxes are checked and the cell into which one has to paste the data is highlighted.

This is how data is usually entered. For the diversity menu, the format has to be reshaped however. We have to transpose the current table so that species are rows and columns are plots. Therefore, we choose EDIT->REARRANGE->TRANSPOSE.

In the diversity menu, we can now choose diversity indices, which calculates a set of 13 different alpha diversity indices. One can use “copy” to copy the result into excel, regroup the data for a certain parameter, e.g. Shannon and create a boxplot with PAST and even perform a t-test.

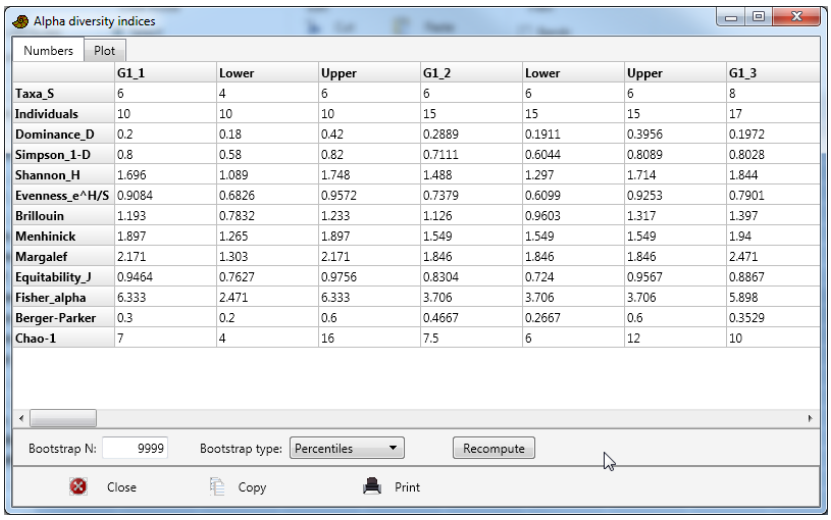


Fig. 23: Diversity indices calculated for all plots

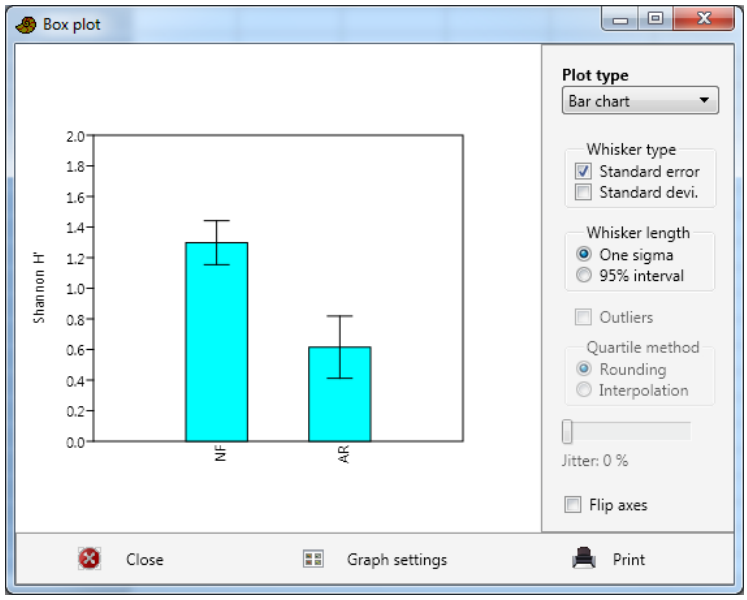


Fig. 24: Bar chart of Shannon diversity for natural forest and Arboretum. Made with PLOT->BARCHART/BOXPLOT

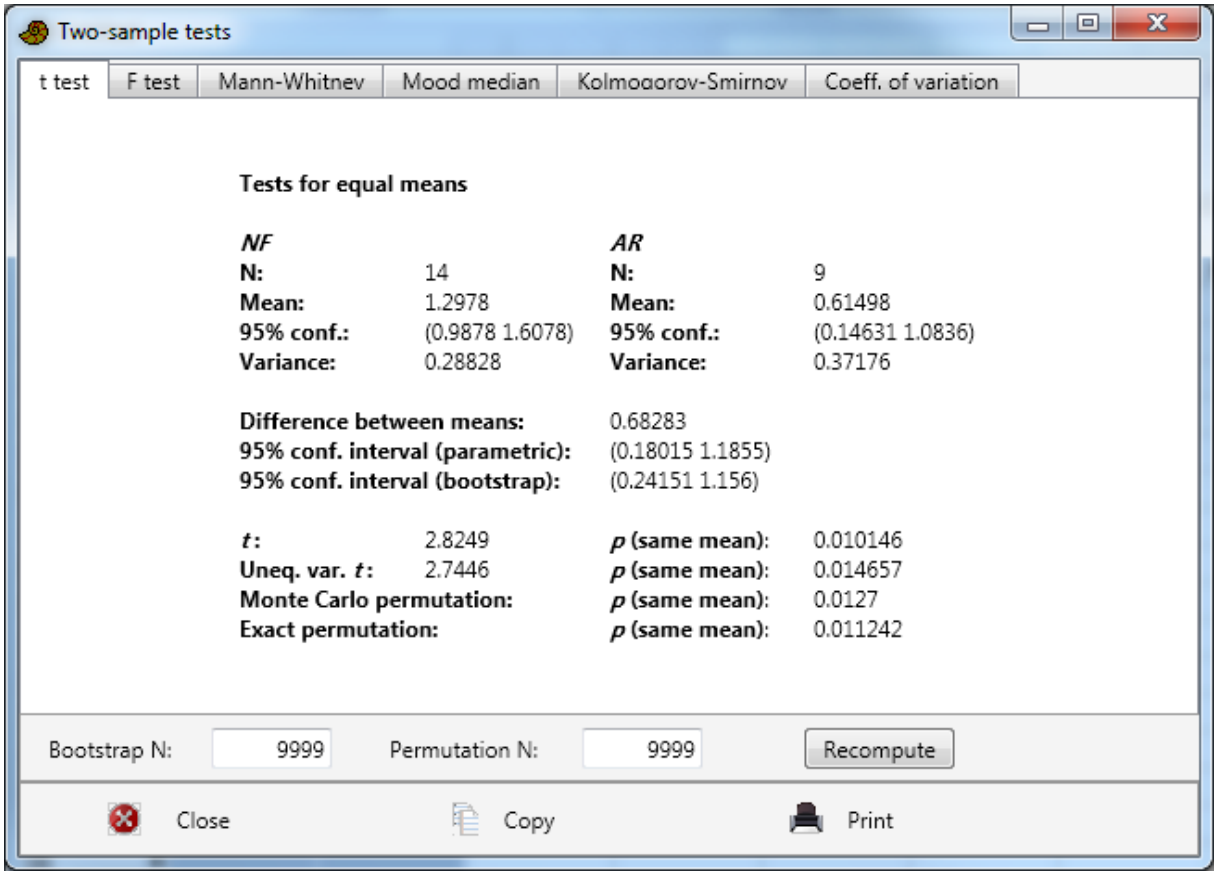


Fig.25: Results of the two sample t-test. Made with UNIVARIATE->TWO SAMPLE TEST

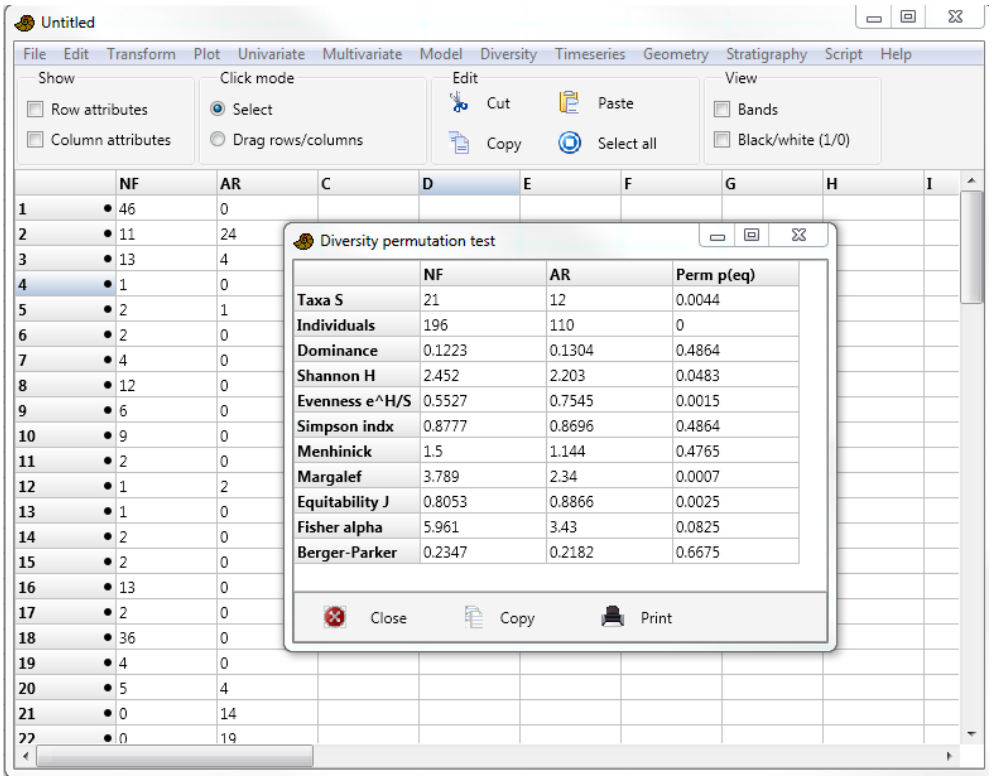
Furthermore, PAST allows to compute diversity profiles and perform also t-tests of two diversity vectors. However, therefore, we have to modify the data so that we sum up all the counts for each species per group, i.e. NF or AR. We do this in excel for convenience (Fig 26). The resulting columns (row 16 and 27) are then imported into a new empty PAST session as columns.

	A	B	C	D	E	F	G	H	I
1	PNR	Cl.african	Afrofalc	C.macrsta	F.sur	Ficuvast	A.abyss	M.ciolata	C.africana
2	G1_1	3	1	0	0	1	0	1	2
3	G1_2	1	0	3	1	0	1	0	0
4	G1_3	3	1	2	0	1	1	0	1
5	G1_4	6	0	1	0	0	0	3	2
6	G1_5	2	0	2	0	0	0	0	2
7	G2_1	4	0	0	0	0	0	0	0
8	G2_2	4	0	2	0	0	0	0	2
9	G2_3	7	0	0	0	0	0	0	0
10	G2_4	5	0	0	0	0	0	0	3
11	G3_1	0	0	1	0	0	0	0	0
12	G3_2	6	2	0	0	0	0	0	0
13	G3_3	2	0	2	0	0	0	0	0
14	G3_4	3	5	0	0	0	0	0	0
15	G3_5	0	2	0	0	0	0	0	0
16	**SUM NF**	**46**	**11**	**13**	**1**	**2**	**2**	**4**	**12**
17									
18	G4_1	0	3	0	0	0	0	0	0
19	G4_2	0	0	0	0	0	0	0	0
20	G4_3	0	8	2	0	1	0	0	0
21	G4_4	0	0	2	0	0	0	0	0
22	G4_5	0	13	0	0	0	0	0	0
23	G4_6	0	0	0	0	0	0	0	0
24	G4_7	0	0	0	0	0	0	0	0
25	G4_8	0	0	0	0	0	0	0	0
26	G4_10	0	0	0	0	0	0	0	0
27	**SUM AR**	**0**	**24**	**4**	**0**	**1**	**0**	**0**	**0**

Fig.26: Calculating sums of species counts per habitat group. The sum columns are then transposed and pasted as columns into PAST. This allows further analysis with the diversity menu.

Once the new two-column data set is imported into PAST as columns, one can work with some more of the tools in the diversity menu. Most notably, one can use the “Diversity permutation test” which performs a permutated two-sample t-test for all 13 diversity indices we used earlier. Figure 27 shows the layout of the data as well as the results of the t-test. Some of the indices show a significant difference, while others do not. Interestingly, Dominance, Simpson and Berger-Parker, i.e. those that rely on the dominance character of species do not show significant differences.

The final tool of the diversity menu we discussed was the Diversity Profile. PAST uses the exponential of the Renyi-Diversity Profile. The main reason for using diversity profiles is that evaluating differences in diversity by comparing one single diversity index could lead to different conclusions, depending on the index (Fig.27). Hence, a technique called diversity ordering, which uses diversity profiles for ranking groups of samples (each group as one column). The diversity profiles use the scale parameter alpha, which puts an emphasis on rare species (alpha close to zero) or dominant species (alpha far from zero). See also the discussion in day 1.



	NF	AR	C	D	E	F	G	H	I
1	46	0							
2	11	24							
3	13	4							
4	1	0							
5	2	1							
6	2	0							
7	4	0							
8	12	0							
9	6	0							
10	9	0							
11	2	0							
12	1	2							
13	1	0							
14	2	0							
15	2	0							
16	13	0							
17	2	0							
18	36	0							
19	4	0							
20	5	4							
21	0	14							
22	0	19							

	NF	AR	Perm p(eq)
Taxa S	21	12	0.0044
Individuals	196	110	0
Dominance	0.1223	0.1304	0.4864
Shannon H	2.452	2.203	0.0483
Evenness e^H/S	0.5527	0.7545	0.0015
Simpson indx	0.8777	0.8696	0.4864
Menhinick	1.5	1.144	0.4765
Margalef	3.789	2.34	0.0007
Equitability J	0.8053	0.8866	0.0025
Fisher alpha	5.961	3.43	0.0825
Berger-Parker	0.2347	0.2182	0.6675

Fig. 27: PAST 3.0 with the two columns dataset and the result of the diversity permutation test.

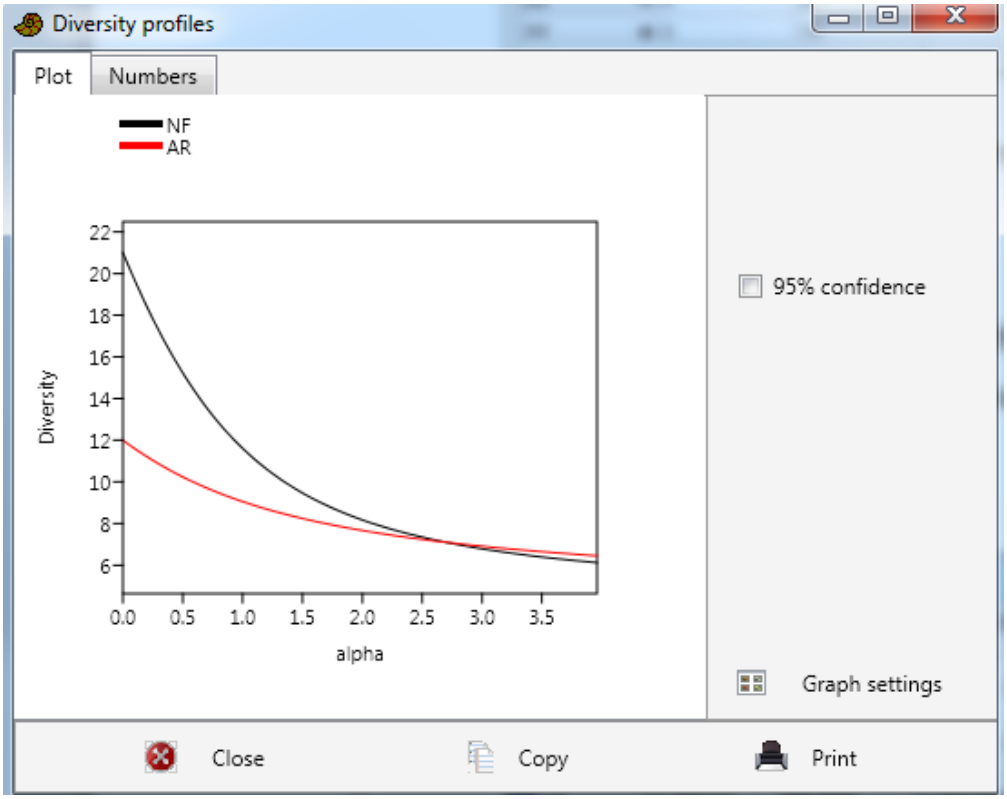
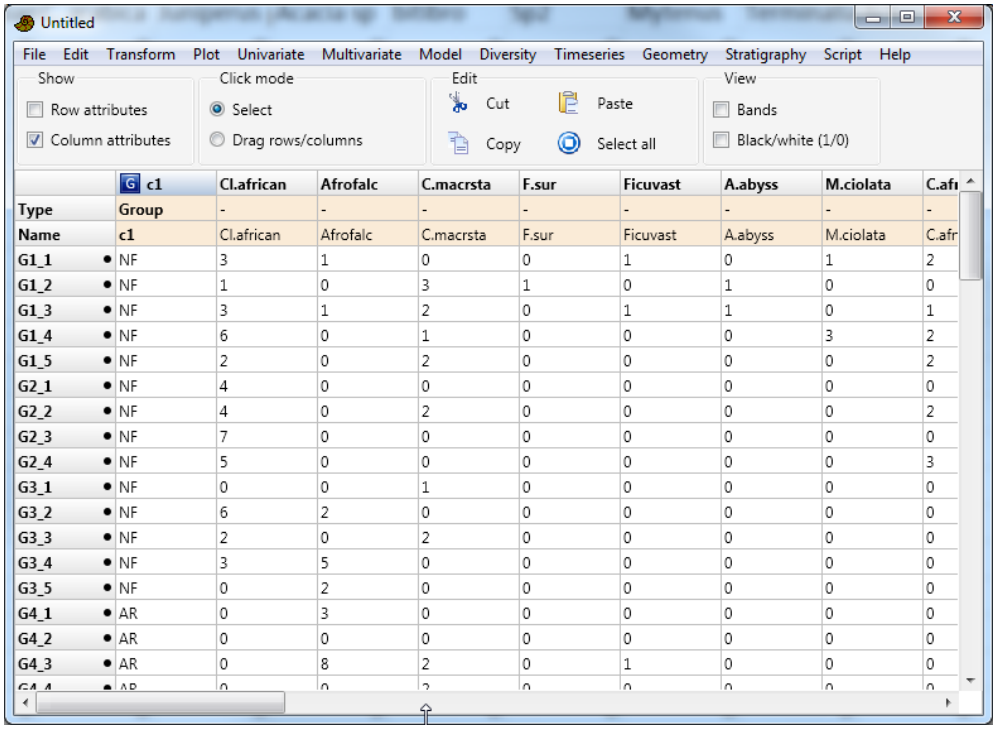


Fig. 28: Diversity profiles for the natural forest and the arboretum. The x-axis describes the scale parameter alpha, with low values giving more importance to rare species. At x = 0, the values become species numbers, AR has 12 species and NF has 21 species.

From the diversity profiles (Fig. 28), we can see that the main differences between NF and AR lies in the amount of rare species. While there are few rare species in AR, many rare species were found in the natural forest, which of course makes sense. Furthermore, the curves do not differ from alpha => 2 on. Alpha = 2 equals to the Simpson index, which was already not significantly different between the pairs in the permutation test (Fig. 27). However, we must be aware that we actually compare groups of different sizes. AR had only 9 plots (one was empty) and NF had 14 plots, meaning that we sampled more area in NF leading automatically to more species.

We also discussed ordination and cluster analysis for seeing how strong both groups differ in their species composition. Hence, we require our first species-by-plot matrix again. In order to provide the information on the groups, one has to add a new column, change its type (check box for column attributes) to group, and add for each plot the group name, NF or AR (Fig22).



	c1	Cl.african	Afrofalc	C.macrsta	F.sur	Ficuvast	A.abyss	M.ciolata	C.afr
Type	Group	-	-	-	-	-	-	-	-
Name	c1	Cl.african	Afrofalc	C.macrsta	F.sur	Ficuvast	A.abyss	M.ciolata	C.afr
G1_1	NF	3	1	0	0	1	0	1	2
G1_2	NF	1	0	3	1	0	1	0	0
G1_3	NF	3	1	2	0	1	1	0	1
G1_4	NF	6	0	1	0	0	0	3	2
G1_5	NF	2	0	2	0	0	0	0	2
G2_1	NF	4	0	0	0	0	0	0	0
G2_2	NF	4	0	2	0	0	0	0	2
G2_3	NF	7	0	0	0	0	0	0	0
G2_4	NF	5	0	0	0	0	0	0	3
G3_1	NF	0	0	1	0	0	0	0	0
G3_2	NF	6	2	0	0	0	0	0	0
G3_3	NF	2	0	2	0	0	0	0	0
G3_4	NF	3	5	0	0	0	0	0	0
G3_5	NF	0	2	0	0	0	0	0	0
G4_1	AR	0	3	0	0	0	0	0	0
G4_2	AR	0	0	0	0	0	0	0	0
G4_3	AR	0	8	2	0	1	0	0	0

Fig. 22: Setup of grouping column in PAST 3.0

After setting up correctly the grouping column, we can check the Multivariate menu. We can use a multivariate group test to see whether species composition differs and how strong the overlap between the groups is. We choose first MULTIVARIATE->TESTS->ONE-WAY-ANOSIM. We select the distance measure to be Bray-Curtis (see day 1!). This provides us with the ANOSIM statistic R based on two groups and the Bray-Curtis distance. The R value is 0.6049 and the p-value is 0.0001. The p-value is referring to the R-value not to a significant difference! The R-value shows that there is still some overlap between the groups (no overlap for R > 0.75). We can use the ONE-WAY-PERMANOVA to test for a significant difference in the values between the groups based on species composition. We receive an F-value (F=4.924) and a significant p-value (p=0.001), again with Bray-Curtis. One should always visualize the outcome using a PcoA or an NMDS. Both can be performed with MULTIVARIATE->ORDINATION->PCOA or NMDS. I prefer the PcoA over NMDS because NMDS is based on permutations and often delivers different outcomes. PcoA however has problems in showing semi-metric distances in 2D. This effect however also remains problematic in NMDS.

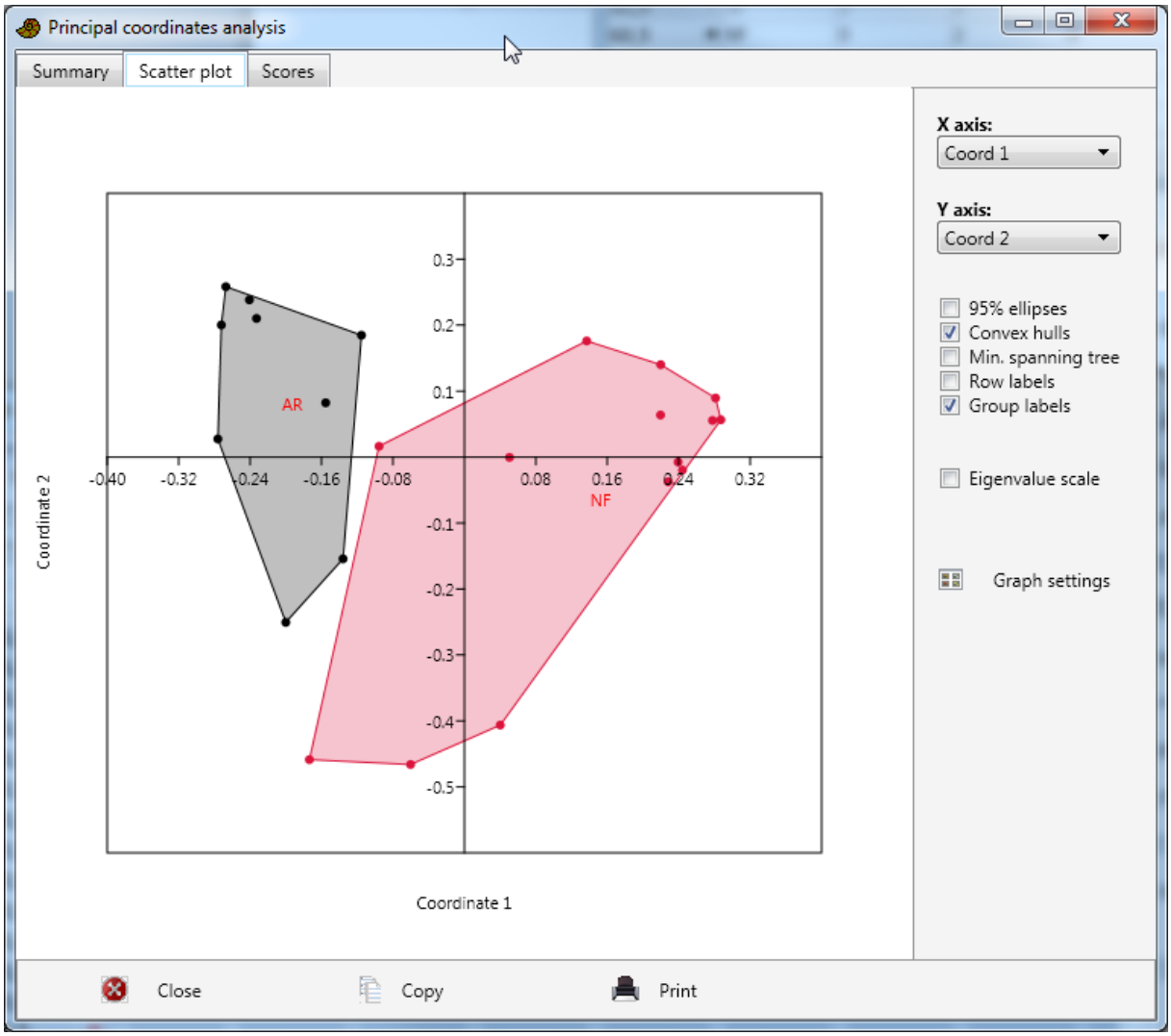


Fig.29: PCoA based on Bray-Curtis distances of the species composition with NF and AR. Convex hulls delimit groups which show no overlap in the first two dimensions (however in 2 and 3).

If we perform a hierarchical cluster analysis on the same dataset using MULTIVARIATE->CLUSTERING->CLASSICAL, we see the potential cause for the ANOSIM-R value of 0.6; two AR plots are nested within the NF group (Fig. 30). In the dendrogram the colours are the same as in the PCoA plot; red is NF and black is AR. Black has one cluster on the left and red three subclusters (our groups!?) on the right. This intermingling effect was not visible from the PCoA, however if one would have calculated a Minimum Spanning Tree in the PCoA plot, the lower two plots of AR would be located within NF, too!

In total, we see that PAST is a very flexible and mighty tool, easy to use and for free! It is of course not as full of functions as the software R, however it has most of the things one needs. Handling data is easy, and maybe is the limitation of functions also a good thing for starting diversity analysis.

If you are interested, subscribe to the mailing list at the PAST webpage. Then you receive news when there are any updates.

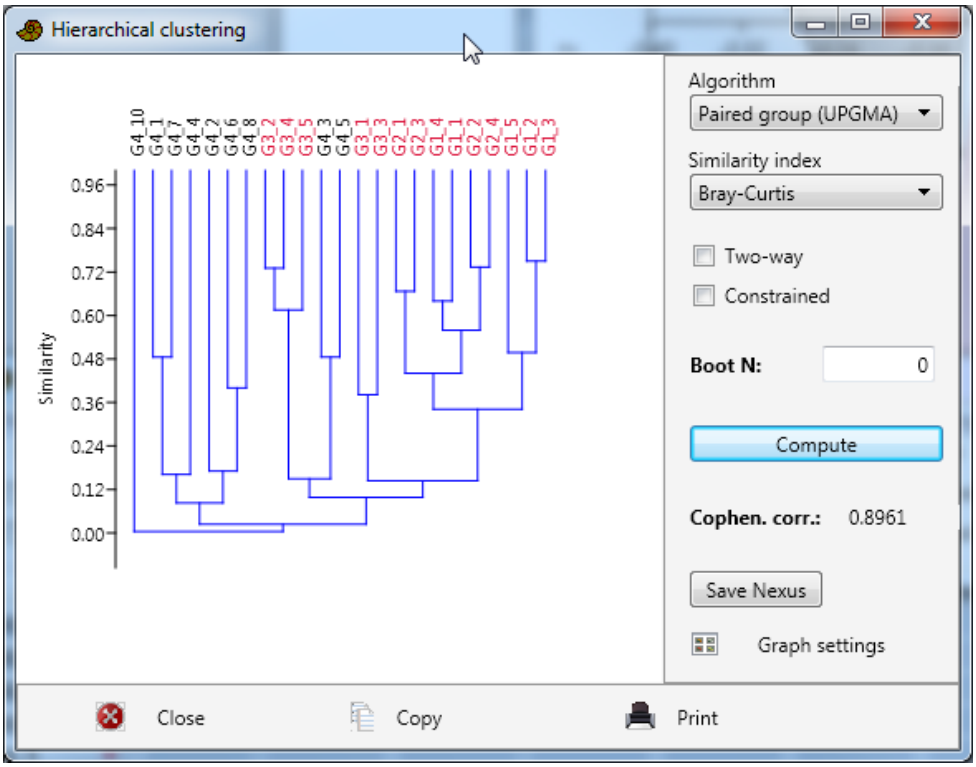


Fig. 30: Hierarchical clustering analysis for the species-plot matrix. Black are plots of AR and red are plots from NF. Two black plots are nested within the red cluster. A potential cause for the Anosim-R value of 0.6049.

Day 3: Data analysis with R

After having done the first steps with PAST, we have already achieved quite a bit. However, today, most scientists work with a software called “R”. R is a free statistical software that is actually a programming language. This sounds scary and truly it is not very easy to get warm with R if one is not comfy with coding and programming. Why do so many people still want to learn R? Because it is always up-to-date in terms of scientific methodology. For biodiversity science it means that the newest development in methods is most often available in form of a new function or a whole package. Books often use R to showcase methods and also provide datasets and code together.

Although I know that R is impossible to learn within 2 hours, we had the aim to produce a bias-corrected diversity profile following the most advanced technique developed by Anne Chao and colleagues (Chao and Jost 2015).

First of all, we have to download and install the software R. We find the installation files for windows here: https://cran.r-project.org/bin/windows/base/

The present version 3.3.0 but these change very often and one should always keep up with the new versions. I usually work in addition with the program RStudio, available for free at www.rstudio.com but for this course I felt it safer to work only within R. Please feel free to test RStudio. If you want to continue the path of R, then RStudio is a must have.

After the installation, this icon should appear on your desktop.

After starting the software, a screen like in Figure 31 will appear. This is the console window of R.

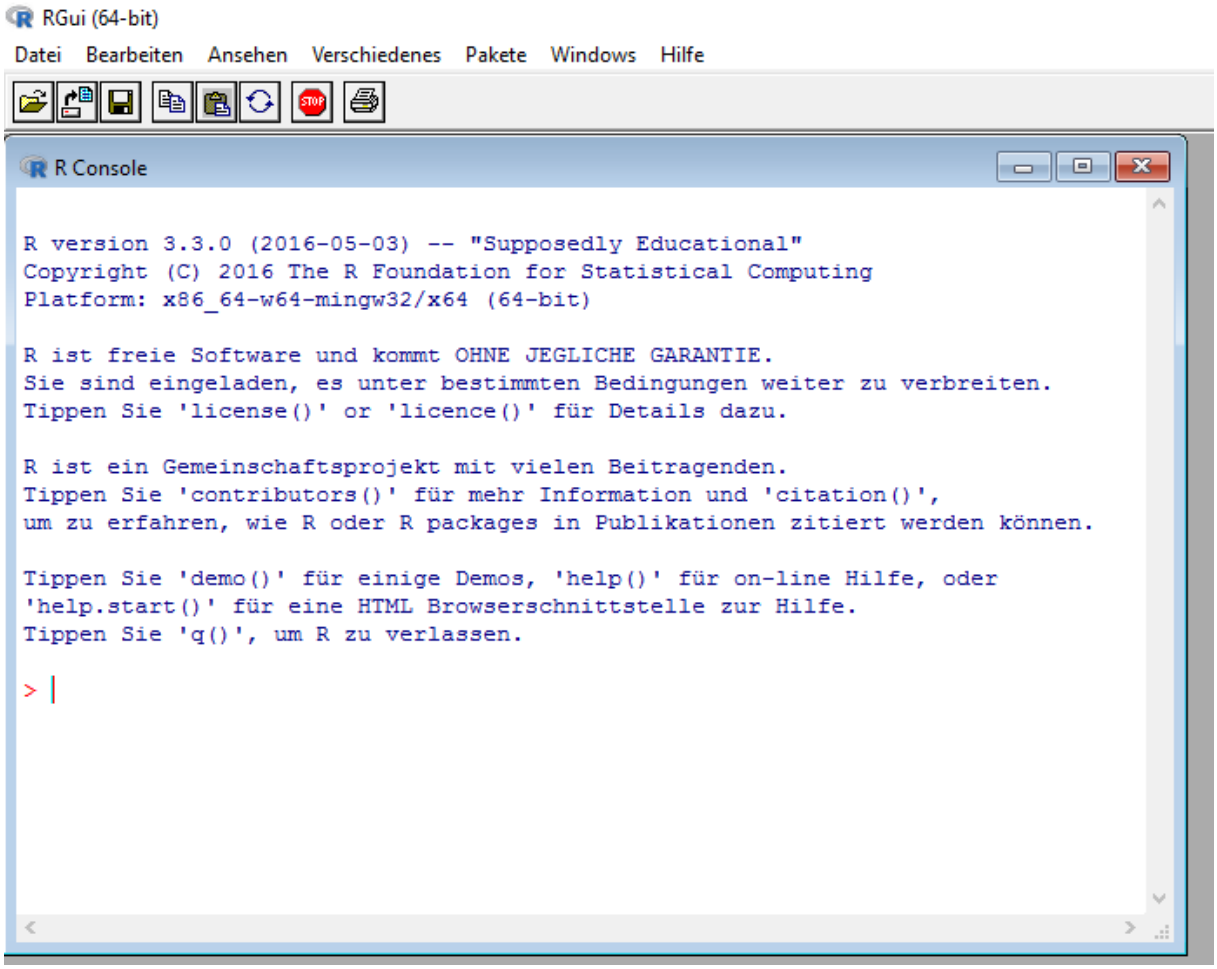


Figure 31: Console window of R 3.3.0. Sorry, my version is in German, but there is not too much to read anyway.

The most important menu point is packages (Ger. = Pakete). Packages are at the heart of R. These are collections of additional functions provided by researches all over the world. Everybody can write a function or a package, even you! ☺ We will also need several packages to work with. By clicking "packages", we can choose "Install Packages". Then, we are asked for a mirror site, which is another computer somewhere on the globe from where we can install packages. One should always choose the nearest one. Be aware, that for the current version of R, not all packages are already updated. So perhaps you cannot download a certain package at the moment, and you will have to either wait until it is available for the current R version or you install an older version of R. Today (21.05.16) the package SpadeR is for example not yet available for R version 3.3.0.

We have to install the packages "vegetarian", "vegan" and "SpadeR". Vegetatarian is a package that allows to calculate diversity indices according to Lou Jost (the Ecuadorian mathemagician). Vegan is a

defacto standard package for analyzing vegetation community data (see also the package dave!). SpadeR is the R version of the DOS-program Spade, provided by Anne Chao. SpadeR also includes the function for the bias corrected diversity profile.

After starting R, we have to first load the dataset. AS it is a CSV file, we use read.csv and put the data into an object called mydata. In R, everything is an object.

```
mydata <- read.csv("D:/WONDO/Group1234.csv", header=T, row.names="PNR")

mydata
```

We can see how large our object is by asking for the dimensions of the object.

```
dim(mydata)
```

We have 30 columns, although we only have 28 species. Somehow, Excel has smuggled in 2 extra columns. We can get rid of them by extracting the columns 1:28 from mydata using square brackets, and overwrite mydata.

```
mydata <- mydata[ , 1:28]
```

We also know that Plot G4_9 is empty. We check first the sum of the rows (shows 0), and then delete the 23rd row in mydata.

```
rowSums(mydata) # Plot G4_9 is empty!?

mydata <- mydata[-23,]
```

R is great for making figures easily. For example, a boxplot or a histogram a produced rapidly for the first column of the dataset by using:

```
boxplot(mydata[ ,1])

hist(mydata[ ,1])
```

Let´s start with our first biodiversity analysis using the package “vegetarian”. We load the package with the library() function.

```
##### Diversity Analysis with R

library(vegetarian)
```

The function H() calculates Hill Numbers, or species equivalents, at a certain level. For example this command:

H(mydata, lev="alpha", q=0)

Calculates species richness (q=0) at the alpha level, i.e. at the plot level for the whole dataset. The results is 3.913. This is the average Shannon over all plots. The next command:

H(mydata, lev="gamma", q=0)

Calculates species richness at the gamma level, so for all plots; the result is 28.

However, now we are mixing NF and AR plots. So we should separate them. Since we know that the first 14 rows belong to NF and the last rows to AR, we can separate the two areas by indexing the dataset.

Make subsets for Natural Forest (NF) and Arboretum (AR) plots

NF <- mydata[1:14 ,]

AR <- mydata[15:23 ,]

Now, we can calculate the average species richness for alpha and game for both habitats.

Average richness on plot level

H(NF, lev="alpha", q=0)

H(AR, lev="alpha", q=0)

NF: 4.78, AR: 2.55

Total richness per habitat

H(NF, lev="gamma", q=0)

H(AR, lev="gamma", q=0)

NF: 21, AR: 12

This can be repeated for Shannon (q=1) and Simpson (q=2),

Average shannon per habitat

H(NF, lev="alpha", q=1)

H(AR, lev="alpha", q=1)

NF: 1.29, AR: 0.61

Total shannon per habitat

H(NF, lev="gamma", q=1)

H(AR, lev="gamma", q=1)

NF: 2.44, AR: 2.072

We can do this also for the level of beta in form of species numbers.

Beta diversity in species numbers per habitat

H(NF, lev="beta", q=0)

H(AR, lev="beta", q=0)

NF=4.33, AR=4.69. Interestingly, the beta diversity seems to be higher in the Arboretum which may be logical as we often have very different groups of species in each plot, increasing the dissimilarity between the plots.

We can also calculate the effective number of species, i.e. the number of species with an equal abundance required to create the respective entropy value. For this, we need the function d().

Total Shannon per habitat in effective number of species

d(NF, lev="gamma", q=1)

d(AR, lev="gamma", q=1)

NF: 11.47, AR: 7.9.

Let´s try to redo some tricks we did with PAST. For example the PCoA. We load first the package "vegan". There we find the tools to calculate the distance matrix that shows the Jaccard-(dis)similarities between the plots.

VEGAN - beta diversity in form of ordination (similarity)

library(vegan)

calculate similarity measure

d <- vegdist(mydata, method="jaccard", binary=TRUE)

d

The object d is now our distance matrix object, we can use in another function called "cmdscale". This performs an ordinary PCoA. K=2 means that we only calculate 2 PCoA axes.

PCoA ordination

plot(cmdscale(d, k=2))

The output (Fig.32) seems a bit boring as there is no color and we do not know which point belongs to which habitat group. Therefore, we create first labels for the habitats, and store them in an object called hab.

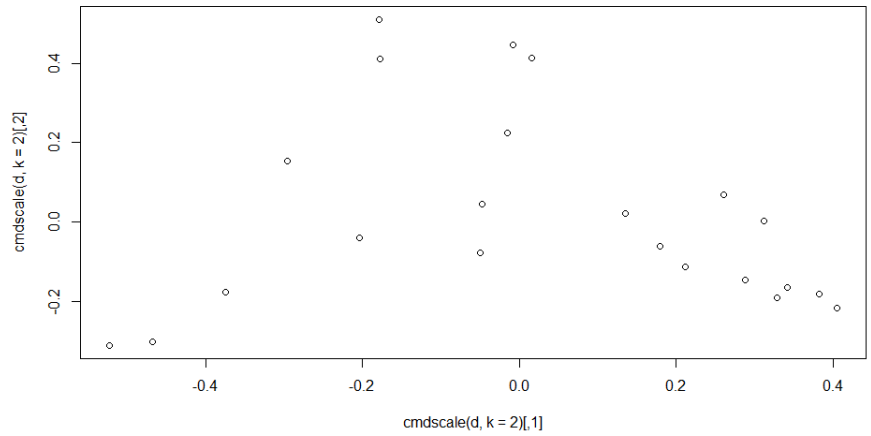


Fig. 32: PCoA of the vegetation data set based on Jaccard distances.

```
## create labels for each plot

NF <- rep("NF",14)

AR <- rep("AR",9)

hab <- c(NF,AR)

hab <- as.factor(hab)
```

Now let´s plot the points again. We know that AR is before NF (in the ABC), thus AR is black and NF is red.

```
## PCoA with Black = AR and red = NF

plot(cmdscale(d, k=2), col=as.integer(hab), pch=19)
```

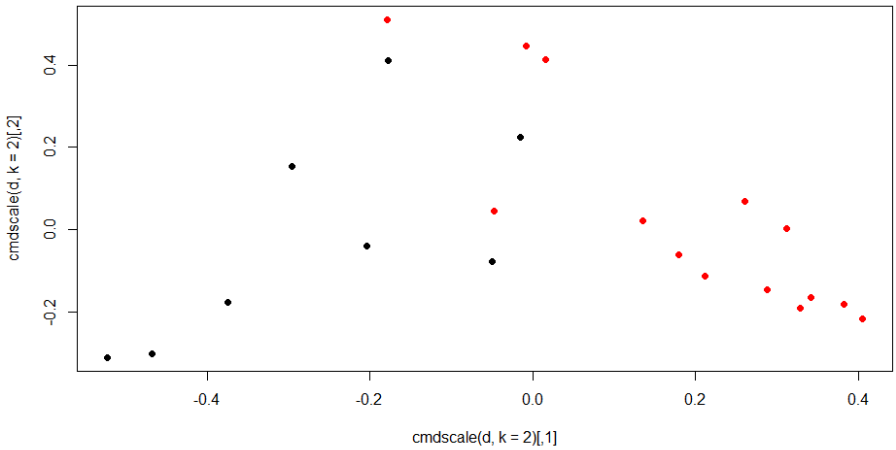


Fig. 33: Colored version of the PCoA of the vegetation data set based on Jaccard distances

It is now easy to use any other available distance measure such as “Bray Curtis”.

```
## Bray-Curtis measure for including abundances

d <- vegdist(mydata, method="bray", binary=FALSE)

plot(cmdscale(d, k=2), col=as.integer(hab), pch=19)
```

Try yourself at home. Finally we wanted to have the bias corrected diversity profiles for our two habitats. We will use the library SpadeR for this. However, this package requires a totally different data structure as we have at the moment.

```
### SpadeR - diversity profiles and richness estimators

library(SpadeR)

data(ChaoSpeciesDataAbu)

ChaoSpeciesDataAbu

# this is the required data format
```

```
752 276 194 126 121 97  95  83  72  44  39  16  15  13  9   9   9   8   7   4   2   2   1   1   1
```

This vector is from the example data set and shows the most abundant species first. It tells us that the most abundant species has 752 individuals. The second species has 276 and so on. Three species occur only once and two species only twice. These are the so-called singletons and doubletons. We also have to create this data. We can achieve this by summing the abundances for each species per habitat, sorting species from high to low and removing zero values. This is done in the next code block.

```
### create abundance vectors

NFabu <- colSums(NF) # sum of species abundances

NFabu <- sort(NFabu, decreasing=T) #sort

NFabu <- as.vector(NFabu) # turn into a vector

length(NFabu) # check where are zeros

NFabu <- NFabu[1:21] #only take nonzeros

NFabu  # ready
```

Now we can calculate a bias corrected diversity profile using the function Diversity.

```
#### Bias corrected diversity profile

Diversity(NFabu,datatype="abundance")
```

This leads to the figure 34, which shows us the bias corrected diversity profile. Interestingly, The empirical richness value is pretty close to the average of the estimated richness based on the profile. This holds throughout the whole range of q values. Let´s see how it works for the Arboretum

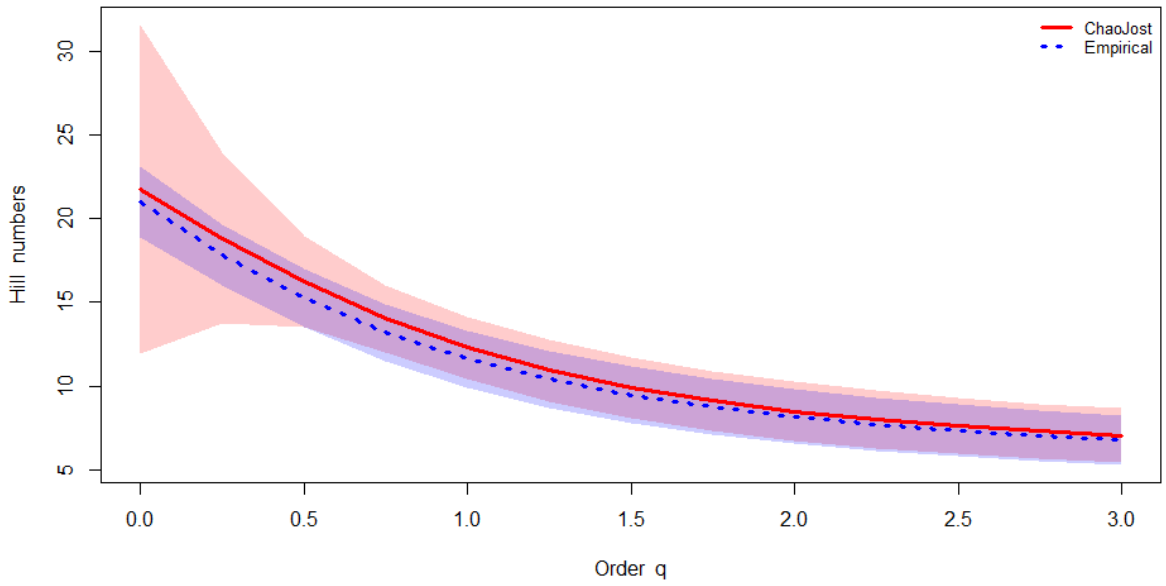


Fig. 34: Bias corrected diversity profile for the natural forest.

First, we create again another abundance vector for the Arboretum data. Figure 35 finally shows us how it looks like. Also here, the empirical diversity profile is very close to the estimated diversity profile.

```
## Create abundance vector for AR

ARabu <- colSums(AR)

ARabu <- sort(ARabu, decreasing=T)

ARabu <- as.vector(ARabu)

length(ARabu)

ARabu

ARabu <- ARabu[1:12]

ARabu
```

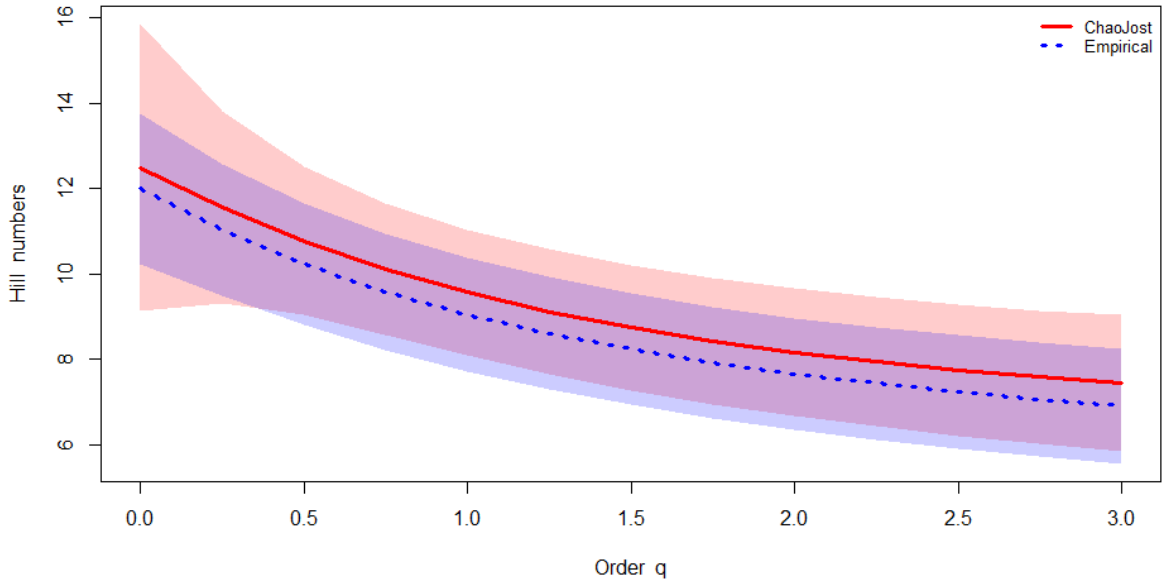


Fig. 35: Bias corrected diversity profile for the Arboretum data.

The diversity profiles tell us two interesting things. First of all, the bias in our data seems to be small. This is mainly due to the fact that we do not have that much rare species in the plots, in particular not in the Arboretum. The bias is usually highest for q=0, i.e. species richness. The effect can still be seen in Fig.34 for the natural forest where the confidence interval for the ChaoJost estimator is widening at q=0 and gets slimmer at q=0.5. Yet, the empirical values are still very close to the estimated ones. Second, we learned that we can compare now both diversity profiles and see whether one curve is always above the other along any order of q. Then, that curve would be truly more diverse. Let´s check this for our case, by plotting the results from both figures, 34 and 35 in one single plot (Fig.36).

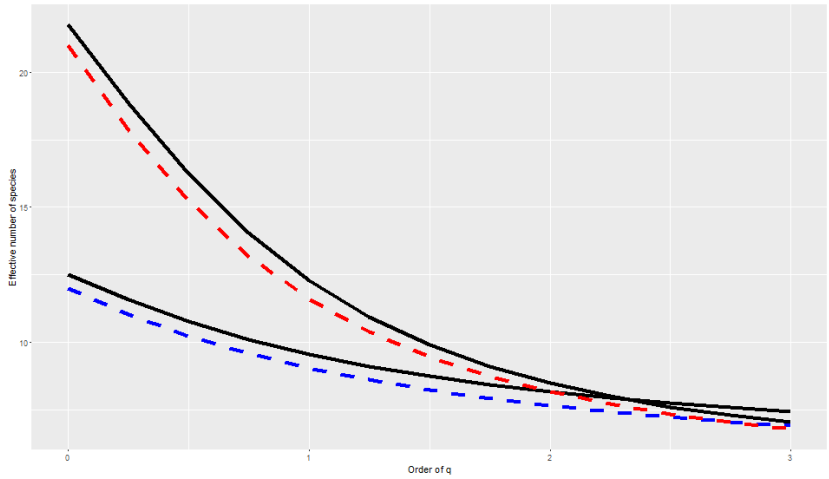


Fig. 36: Overlay of both diversity profiles. In red is the natural forest, in blue the Arboretum. NF is not more diverse after an order of q=2. Dashed lines are empirical lines and solid lines the ChaoJost estimators.

We see in Figure 36 that the Arboretum is equally diverse when we compare at an order of q = 2, which would be equal to the Simpson index. At higher values of q, e.g. larger than 3, the Arboretum could be even more diverse than the Natural Forest! Hence, we get different rankings dependent on which measure or diversity index we choose to compare two different data sets or habitats. This is very important for conservation issues. Only looking at species richness OR Shannon OR Simpson is not showing us the big picture. Diversity profiles are thus, in my opinion, the best way to understand a biodiversity data set.

What did we learn from this little R exercise? First of all, R is not trivial. Second, R is powerful. Third, it allows us to use very modern techniques, but we also have to understand them. Maybe for the beginning, you all are better off with PAST. After feeling comfortable with PAST and it´s capabilities, you can decide if you want to push yourself further using R. Good luck!

Literature

Chao, Anne, and Lou Jost. 2015. "Estimating Diversity and Entropy Profiles via Discovery Rates of New Species." Edited by Ryan Chisholm. *Methods in Ecology and Evolution* 6 (8): 873–82. doi:10.1111/2041-210X.12349.

Day 4: Biodiversity monitoring

The final session was devoted to Biodiversity Monitoring. I wanted you to a) understand the aims of monitoring, b) what are the costs and problems and c) who applied it and why (in examples).

The aims of biodiversity monitoring seem easy to grasp, i.e. look how biodiversity is developing over time at a certain location. However, this seemingly easy task can turn out to be a nightmare if not carefully planned. For example, you should have in mind, what kind of trends you expect and what could be possible driver of turnover in biodiversity. Trees for example, grow slowly and will directly answer in loss of individuals to an increase in temperature by 2°C. Maybe in hundred years? Climate change seems to be a good reason for doing biodiversity monitoring, however, then you need long term funding, and I mean long term. What could be a good reason for performing biodiversity monitoring? Typically, in practice, biodiversity monitoring is required for assessing the effects or impacts of construction (e.g. power plants, roads, train tracks) on vegetation. For this, before and after the construction data is gathered for a short time period (e.g. 2-5 years) and potential impacts are reported. Then, a clear potential impact can be defined, i.e. an estimated effect of species loss for example, by 20%. Nature conservation heavily relies on biodiversity monitoring. However, it is rarely applied, because it seems too costly, or it is unclear which indicator should be monitored.

In science, however, people often use biodiversity monitoring for testing theories. For example, the BIOTA Africa project (www.biota-africa.org) installed a number of so called Biodiversity Observatories (Jürgens et al. 2012) across Africa and monitor vegetation abundance annually since 2001. The GLORIA Project (Grabherr et al. 2000) monitors vegetation abundance at mountain summits all around the world. Both use standardized methodology and aim at understanding detecting climate change impacts on vegetation. The problem with scientific projects is that funding

is often only for a limited time frame and that the observers of plant diversity often change (e.g. PhD projects each 3-5 years) and thus continuity is not given.

A good example for biodiversity monitoring in the tropcis was provided by Dallmeier (1992). This book is available online at http://unesdoc.unesco.org/images/0009/000938/093876eo.pdf and should be read carefully if one is interested into monitoring tree biodiversity. Five sites where monitored from 1986 to 1992 in the Americas; four in south America and one in Northern America. Census is carried out every 5 years. One good example for the application of this biodiversity monitoring was the before-and-after comparison of the Hurricane Hugo. The impact became clear when comparing the number of living trees between the years (Fig.37).

Table 2. Basic statistics from Bisley Biodiversity Plot 0113 before and after Hurricane Hugo

	1988 CENSUS	1991 CENSUS
Area of plot (m^2)	10,000	10,000
Families represented	17	17
Species represented	33	31
Total number of trees	434	440
Live trees	434	332
standing	434	251
fallen	—	60
broken	—	21
Dead trees	—	108
standing	—	19
fallen	—	71
broken	—	18
Percentage survivorship	100	74.8
Sprouting trees	—	81
fallen sprouting	—	60
broken sprouting	—	21
Total basal area		
live standing trees (m^2 ha^{-1})	28.23	17.20
Trees with undetermined status	0	4

Fig. 37: Table 2 from Dallmeier (1992). Number of living trees was halved by Hurricane Hugo.

The sampling design of at the Bisley Plot was aiming at having a complete census of all tree species in a 500x500m (=25ha) area (Fig.38). The strongly nested design splits the area into hectares, which are then divided into 25 quadrats á 20x20m, which are again subdivided into 16 even smaller areas of 5x5m. Within these plots, all tree species were counted. Trees were tagged, measured for dbh etc. and identified to species level. Species identification can be difficult in the field, hence one should be prepared or should be able to collect and transport specimens for later identification.

The final message of this session is that, monitoring matters! But, it is a tricky job that needs careful thinking and planning. Do not approach lightly this job, but if you can install a biodiversity monitoring project, you will have also a long-term job perspective.

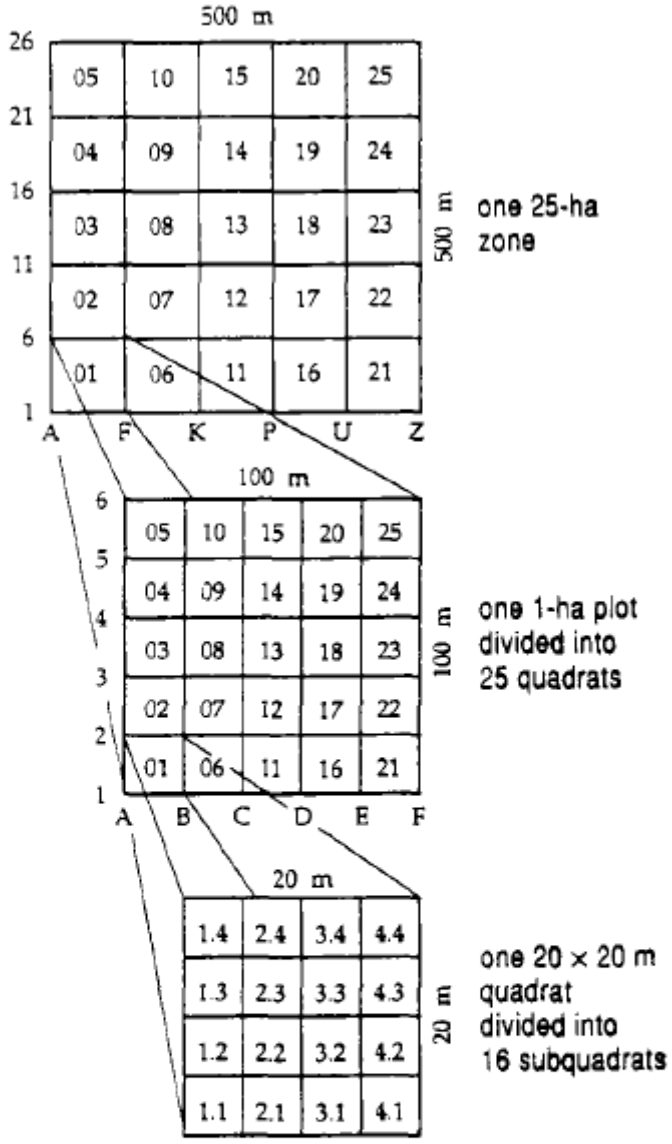


Figure 5. The 25-ha zone is divided into twenty-five 1-ha plots. Each plot is divided into 25 quadrats (each 20 × 20 m), and each quadrat into 16 subquadrats (each 5 × 5 m).

Fig.38: Figure 5 from Dallmeier (1992). A strongly nested design of multiple subplots. Even 20x20m quadrat was subdivided into 16 subquadrats, which was necessary for the dense tropical forest.

Literature

Dallmeier, F., ed. (1992). *Long Term Monitoring of Biological Diversity in Tropical Forest Areas - Methods for Establishment and Inventory of Permanent Plots*. MAB Digest 11. Paris, France: UNESCO.

Grabherr, G., Gottfried, M., & Pauli, H. (2000). GLORIA: a global observation research initiative in alpine environments. *Mountain Research and Development*, *20*(2), 190-191.

Jürgens, Norbert, et al. (2012). "The BIOTA Biodiversity Observatories in Africa—a standardized framework for large-scale environmental monitoring."*Environmental monitoring and assessment* 184.2: 655-678.

THANK YOU ALL FOR PARTICIPATION AND THE NICE TIME AT WONDO GENET!!!

Fig. 41: You and me taking this photo on our way to the Red Tower ☺

Hamburg, 22.05.2016