Analysis of species richness

Analysis of species richness

Species richness is the number of species that has been recorded for a specific group of organisms during a specific time period. It also refers to a specific area. That may be the study area, it could be a plot or sampling unit, or an assemblage of sampling units.

Although it is relatively easy to calculate the number of species identified in a set of sample plots, it is trickier to find estimates that can be used to compare the species richness between various subsets in a particular dataset.

How can species richness be calculated?

It is relatively easy to calculate the number of species if the species identity of every individual tree is known. It is good practice to document how species were identified, and ideally where herbarium specimens were deposited. If you do not know the identities of each and every species, then you could opt to only base your analysis on the species with confirmed identities. Some researchers include morphospecies in the species matrix that they analyse: these are species that can be confidently categorized as being different from the other species in the species matrix, but can not be identified.
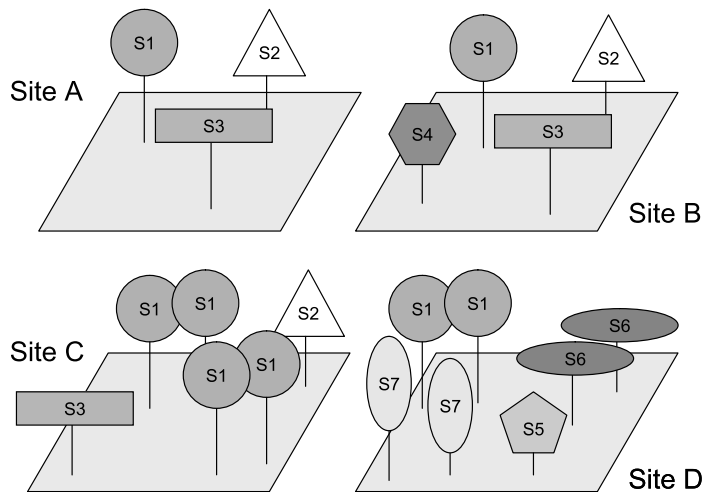

Figure 4.1 Species richness depends on the scale at which it is measured in a landscape. In this example, the number of species on site A is 3, the average number of species per site is 3.5 and the total number of species in the survey area (equaling 4 sites since this is a simplified example) is 7.

Always mention where (and possibly when) you counted the species. Figure 4.1 shows that you can count 3 or 4 species within a single site and 7 species in the total survey. You should always mention the sample size for which you counted the species.

Calculating the total number of species or total species richness

The total number of species can be calculated relatively easy. The total number of species for the dune dataset is 30. If you prepared your species matrix correctly, then the total species richness is the same as the number of columns of your species matrix. In Figure 4.1, the total number of species is 7.

Calculating the total number of species for various subsets in the data

You may be interested in finding differences in the total number of species in various subsets in your data. For example, you could calculate the total number of species for each site. You could also be interested in differences in the species richness for subsets based on the type of management for the dune datasets (see chapter on data preparation). You could for instance hypothesize that the management will influence the species richness. You will obtain the following result:

```
Management n richness
       BF 3       16
       HF 5       21
       NM 6       21
       SF 6       21
```

You could conclude that species richness is lowest for biological farming (BF) since the species

richness calculated was 16, and it was 21 for the other categories of management. The n in the output stands for the number of sample units of each category. When you check for differences in n between the various categories, then you also notice that BF has the lowest value. Since both species richness and the sample size are different, we are not in a good position to compare management classes. The difference could be caused either by an actual difference in species richness, or a perceived difference because of sample size differences.

The reason is that species richness is dependent on the sample size. Study Figure 4.1, for example. You can see that the entire area portrayed contains 7 different species, or a species richness of 7. The 4 sites separately each have a species richness of 3 or 4 species. The average species richness of a site is 3.5.

Calculating the average number of species for various subsets in the data

Instead of being interested in the **total** number of species in each subset of the data, you may be interested in finding out differences in the **average** number of species per site in various subsets in your data. For example, you could compare the average number of species on farms located next to forests with the average number of species on farms that are not adjacent to forests. In this case, the research hypothesis specified that differences were expected in the average number of species per farm and not necessarily in the total number of species.

To investigate differences in the average number of species, regression techniques as described in Chapter 6 offer the best approach to analysis. This approach does not require that sample sizes are equal, as is required to compare the total number of species. Note however that the size of the sample unit should also be the same, and that the size of

the sample unit can be measured in different ways as shown below (section: changing the scale of the horizontal axis for species accumulation curves). To investigate whether differences in species richness between farms close and far away from the forest could be caused by differences in farm size, you could construct species accumulation curves for the farms that are close and farms that are far and compare the curves (see below: using species accumulation curves to compare total species richness of various subsets of data).

Constructing species accumulation curves

It is important to realize that the total number of species of two sites combined is not always equal to the sum of the species on site 1 plus the species on site 2. This will only be the case if site 1 and site 2 do not share any species. If they share some species, then the total number of species on the two sites combined will be smaller than the sum of the richness of each site. Because of this difficulty, we need special types of calculation to find the number of species for combinations of sites.

Species accumulation curves show the species richness for combinations of sites. These curves portray the **average pooled species richness** when 1, 2, … , all sites are combined together.

The reason that the **average** pooled species

richness is calculated is that different combinations will have different species richness. For example, for the dune dataset, the species richness of site X1 is 5, for sites X2 and X3 it is 10. In other words, there is not a single value for the species richness of 1 site. For this reason, we calculate the average value. The average species richness for 1 site is 9.85.

When we combine sites X1 and X2, then we obtain a total combined species richness of 10. When we combine sites X3 and X4, then we obtain a total combined species richness of 13. The average species richness for combinations of two sites is 15.11. Note that it not so easy to calculate the average pooled species richness for 2 sites because many different pairs can be formed (20 * 19 / 2 = 190 actually).

Species accumulation curves calculate the average species richness for larger combinations of sites, such as combinations of 3 or combinations of 16 sites, until all possible sizes of combinations were investigated. The result for the dune dataset are shown below.

The output shows that the average richness for all possible combinations of 14 sites is 28.85, whereas for all possible combinations of 17 sites it is 29.51. The `sd` indicates the standard deviation observed as, by chance, some combinations of sites contain small numbers of species, and other combinations contain large numbers of species. Species accumulation patterns are usually

```
Sites     1.000000  2.000000  3.000000  4.000000  5.000000  6.000000  7.000000
Richness 9.850000 15.110526 18.510526 20.937461 22.754321 24.149587 25.239564
sd        2.351064  1.876385  1.572271  1.446958  1.390159  1.353035  1.316480


Sites     8.000000  9.000000 10.000000 11.000000 12.000000 13.0000000
Richness 26.103548 26.798244 27.364962 27.833984 28.227546 28.5620356
sd        1.274903  1.228201  1.176341  1.119344  1.056454  0.9874094


Sites    14.0000000 15.000000 16.000000 17.0000000 18.000000 19.0000000 20
Richness 28.8496388 29.099587 29.319092 29.5140351 29.689474 29.8500000 30
sd        0.9115998  0.828689  0.738092  0.6333903  0.513971  0.3570714   0
```

shown graphically by species accumulation curves. Figure 4.2 shows the species accumulation curve for the dune dataset. It is important to realize that both horizontal and vertical axes show results of pooling or combination: on the horizontal axis the sites are combined (10 = 10 sites combined), whereas on the vertical axis the species are combined (20 = 20 species combined).

The results below are based on exact calculations of the average species richness for combinations of sites. Another approach to approximate this average species richness is a randomisation approach. For example, to find an estimate of the average richness for 3 sites, select 3 sites at random from all 30 sites. Calculate the species richness. Then select another 3 sites at random, calculating a second richness. Repeat maybe 1000 times. Then the overall estimate is the average of the 1000 values. Although this randomisation approach is the classic approach to calculate species accumulation curves and it is based directly on the idea of calculating the average species richness of a series of randomly combined sites, it is better to use the exact method which will usually be faster and more precise.

The results for species accumulation curve based on randomisation (calculated from 1,000 randomisations) for the dune dataset are shown below.

Note that you will obtain slightly different results when you do this analysis again, since selections

```
Sites    1.00000  2.000000  3.000  4.000000  5.000000  6.000000  7.000000
Richness 9.85100 15.163000 18.654 21.012000 22.829000 24.181000 25.276000
sd       2.42088  2.227868  2.271  2.188297  2.053771  1.911516  1.762081

Sites     8.000000  9.000000 10.000000 11.000000 12.000000 13.000000 14.0000000
Richness 26.096000 26.754000 27.323000 27.788000 28.196000 28.509000 28.7980000
sd        1.587862  1.450375  1.358866  1.246840  1.112124  1.015357  0.9219794

Sites     15.0000000 16.0000000 17.0000000 18.0000000 19.0000000 20
Richness 29.0630000 29.2890000 29.4860000 29.6820000 29.8420000 30
sd        0.8376949  0.7470188  0.6404797  0.5149186  0.3649235  0
```
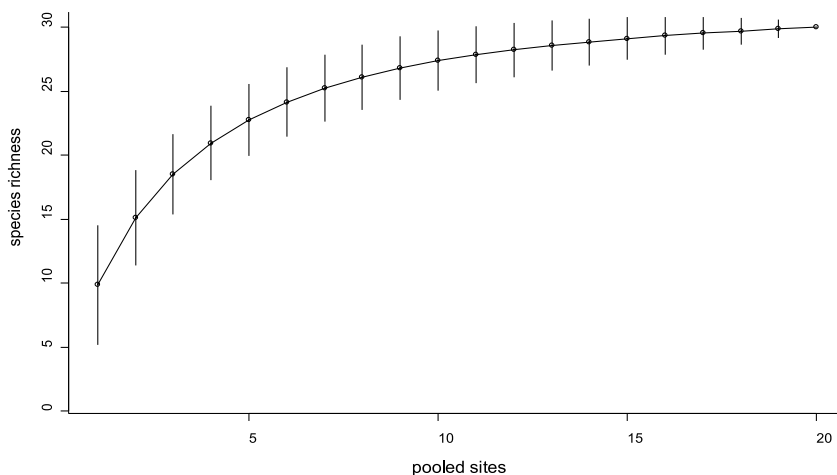

Figure 4.2 Species accumulation curve for the dune meadow dataset. The bars indicate +2 and -2 standard deviations.

are done at random. The `sd` again indicates the standard deviation observed. You can see that the results are similar to the exact results provided earlier. Many randomisations are required to obtain results that will be very close to the exact results. It would not be easy to spot differences between plots of both results, however.

Using species accumulation curves to compare total species richness of various subsets of data

Species accumulation curves are especially useful when comparing species richness for subsets in the data when sample sizes of subsets are different.

When you calculate species accumulation curves for each category of management of the dune dataset separately, then you obtain Figure 4.3. You can observe the total species richness of 16 for a sample size of 3 sites for biological farming, and the total species richness of 21 for a sample size of 5 sites for hobby farming. The results now allow comparing species richness at the same sample size. For example, we can compare the 4

categories at sample size 3. We can observe that hobby farming has substantially larger species richness (19.10) than the three other categories (16, 16.2 and 16.25). More importantly, we can see that there is little evidence now that nature management and standard farming have more species than biological farming, although the total number of species that was observed for the latter categories was larger.

These results emphasize that species richness depends on – among other factors - sample size. Therefore, a value of species richness without indication of sample size is meaningless. You need to provide both sample size and species richness. For example, you could state that 21 species were recorded for nature management for a sample size of 6 sites of 4 m². A shorter way of communicating this information would be: "21 species were recorded for nature management *(n=6)*". The same goes for the entire survey. You should indicate that the total species richness of 30 was recorded for 20 sites. You should definitely indicate your sample size when you describe your methodology.

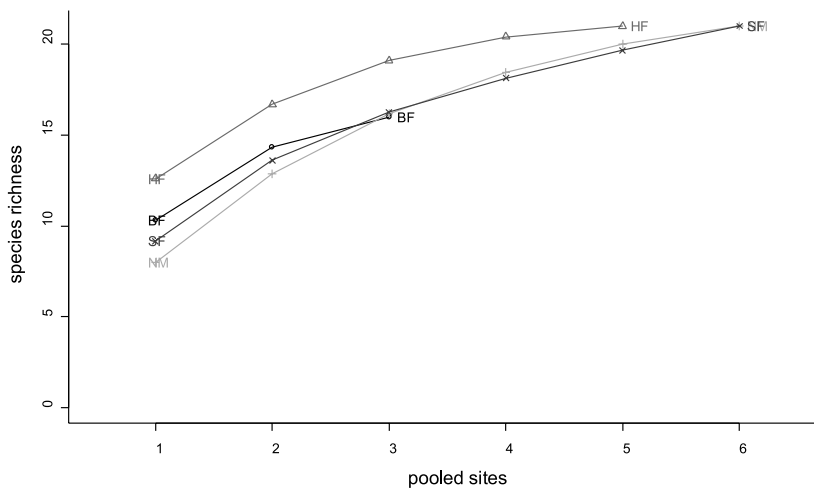Note that we did not make any comparisons

Figure 4.3 Species accumulation curves for various subsets of the dune meadow dataset based on management.

of species richness at the scale of the entire landscape, but only for areas of equal sample size. It is possible that patterns are different in the entire landscape, since we only have data from a fraction of the total landscape. For example, it could be possible that more species occur in the entire landscape in standard farming if 95% of the total area is under this type of management. Although species accumulate more quickly in hobby farming (Figure 4.3), a **smaller total area** of farmland under hobby farming could potentially result in smaller **total richness** for hobby farming for the entire landscape. It is difficult to compare different subsets at the level of the total landscape since it is extremely difficult to extrapolate species richness beyond the area that was sampled (see below: estimating the total number of species of the survey area). You could mention important differences in coverage of different subsets of data that could potentially influence patterns at the scale of the entire landscapes that you are studying.

It is also important to realize that similarity or difference in species richness does not mean that the identities of the species are the same: in the example above, it could be that standard farming has a subset of the species that occur in hobby farming, or it could be that most species under standard farming are different from those that occur in hobby farming. The species accumulation curves do not provide information on overlap in species composition. If the objectives of your study require understanding differences in species composition of the various categories of landuse then look at the methods of chapter 8 and beyond.

Species accumulation curves based on the number of plants surveyed

The species accumulation curves that were calculated earlier gave values for combinations of 1, 2 … ,all sites. This is only one method by which species accumulation curves can be calculated.

An alternative method of calculating a species accumulation curve is not to calculate the average number of species for 1, 2, …, all **sites**, but for 1, 2, …, all **sampled plants** (individuals). If you check Figure 4.1 again, you could notice that some sites have 3 trees whereas other sites have 6 trees. As all the sites have the same number of species, you can deduct that some sites have fewer species per tree.

For example, assume that the values of the species matrix of the dune dataset indicate the number of plants observed for every site and for each species. The total number of plants is now 685. A species accumulation curve can now be calculated by selecting the first plant at random from the 685, the second plant at random from the remaining 684 plants, …, until all plants were selected. An alternative species accumulation curve will be obtained.

This approach has been described as **individual-based species accumulation**. The approach where sites are combined can be referred to as **sample-based species accumulation**.

For some datasets, information is not collected from sites, but from observed individuals. Often surveys on vertebrates are obtained in this manner, recording the species identity for the first observed animal, then the identity for the second observed animal and so on. In such situations, the only accumulation curve that can be calculated is an individual-based species accumulation. The individual-based species accumulation results for the dune dataset are shown on the next page.

```
Indiv     34.000000 68.000000 103.000000 137.000000 171.000000 206.000000 240.000000
Richness 17.111627 22.239417  24.702790  26.049452  26.907559  27.520440  27.963881
sd        1.797432  1.733433   1.535193   1.374937   1.253959   1.157158   1.078629


Indiv    274.000000 308.0000000 342.0000000 377.0000000 411.0000000 445.000000
Richness  28.313305  28.5986770  28.8374254  29.0458951  29.2195268  29.369823
sd         1.008308   0.9423203   0.8785947   0.8141377   0.7518479   0.689217


Indiv    480.0000000 514.0000000 548.0000000 582.0000000 616.0000000 651.0000000 685
Richness  29.5041434  29.6177792  29.7170766  29.8037462  29.8790427  29.9455652  30
sd         0.6236441   0.5579764   0.4891322   0.4151685   0.3324024   0.2278851   0
```

Figure 4.4 shows the results. You could have noticed that the results do not indicate all 685 possible combinations of individuals, although the species richness can be calculated for any number of individuals. The results were only reported for multiples of the average number of individuals of a site. In the case of the dune meadow dataset, the average number of individuals per site is 685/20 = 34.25. The number of accumulated individuals **closest** to multiples of the average number of individuals are listed. These numbers are the 34, 68, 103, …, 651, 685 printed above for Indiv. An

integer number is chosen as the individual-based species accumulation curve can not be calculated for fractional numbers of combined individuals.

Since 34.25 individuals occur on a site on average, the results of an individual-based species accumulation curve can also be expressed at the scale of the expected number of combined sites rather than the number of combined individuals. The scale can easily be changed by dividing the number of combined individuals by the average number of individuals per site.   For the dune meadow dataset, these numbers of sites equal
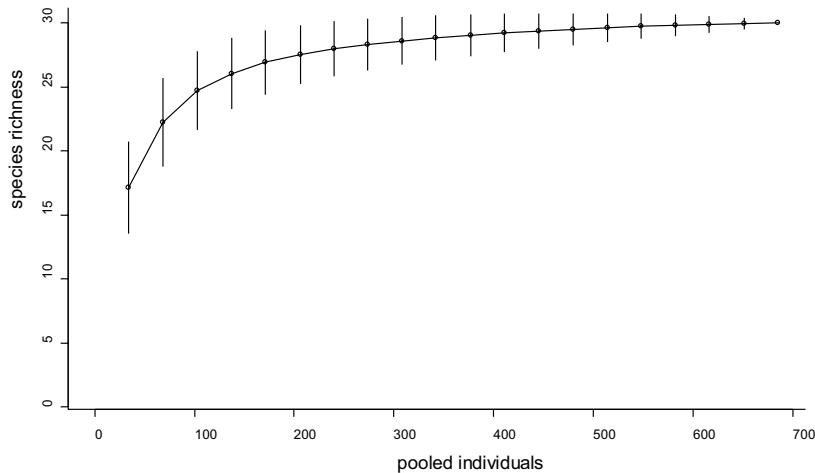

Figure 4.4 Species accumulation curve for the dune meadow dataset, based on individual-based species accumulation.

0.9927007 (= 34/34.25), 1.985401 (= 68/34.25), 3.007299 (= 103/34.25), …, 19.0072993 (= 651/34.25) and 20 (= 685/34.25). Since the average number of individuals has decimals, it is not possible to calculate the individual-based species accumulation for exact (integer) numbers of accumulated sites.

One of the reasons for only reporting individual-based species accumulation curves at the scale of the expected number of sites is that results become very lengthy for large numbers of individuals otherwise (in our example reporting the average number of species for 1, 2, 3, 4, …, 683, 684, 685 individuals – many surveys record substantially larger numbers of individuals and would require lengthier outputs). The most important reason for reporting only for multiples of the average

number of individuals is that the sample-based and individual-based species accumulation curves can then be plotted on the same graph. Figure 4.5 gives an example for the dune meadow dataset. The reason that the curves can be plotted together is that we obtain multiples of the average number of individuals when we combine sites at random in sample-based species accumulation curves, so that both curves can be plotted at the same scale (Gotelli and Colwell 2001).

Since we expect multiples of the average number of individuals per site, we can also choose to scale the horizontal axis by the average number of individuals. Figure 4.6 gives an example. Note that the shapes of the curves of Figures 4.5 and 4.6 are the same, as it is only the scale of the horizontal axis that is different.
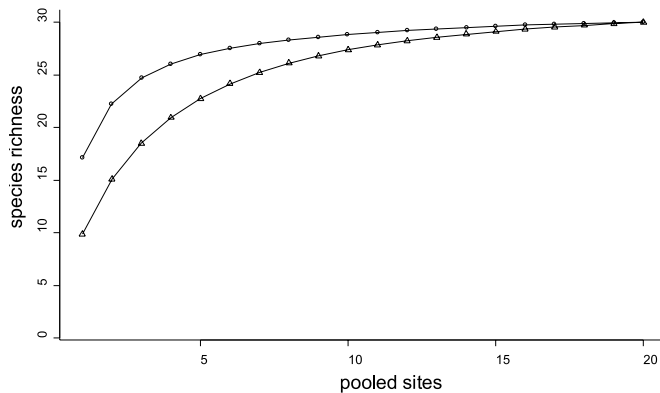

Figure 4.5 Plot of sample-based (Δ) and individual-based (O) species accumulation for the dune meadow dataset.
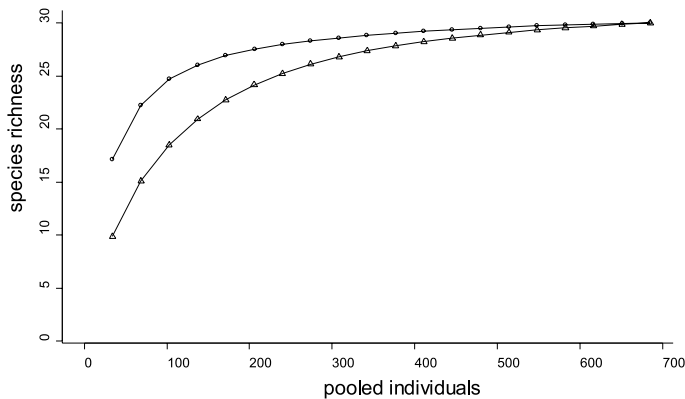

Figure 4.6 Plot of sample-based (Δ) and individual-based (O) species accumulation for the dune meadow dataset. The horizontal axis is scaled by the average number of combined individuals.

The difference between the individual-based and sample-based species accumulation (as can be seen in figures 4.5 and 4.6) is a result of the fact that species are not distributed randomly over the sites. Remember that individual-based species accumulation is based on randomly selecting individuals from the entire set of individuals not taking site information into account. If both curves have similar patterns, you can conclude that species are distributed at random over the sites.

The difference is especially big for the first site of the species accumulation curves. Figure 4.6 shows that you find more species when taking 34 individuals at random (average species richness = 17.1) than found on one site selected at random (average species richness = 9.8, average number of individuals = 34.25). This means that a single site is more likely to contain individuals of the same species than expected from the frequencies of species in the whole survey. These results thus show that plants of the same species tend to be clustered.

Changing the scale of the horizontal axis for species accumulation curves

We can change the scale of the horizontal axis, by multiplying or dividing the number of sites by a specific number. We can make this choice of scaling for the horizontal axis both for the sample-based and individual-based species accumulation curve. The shapes of the curves will remain the same.

However, when comparing different subsets in the data, then different patterns will be obtained by changing between number of sites and the number of individuals on the horizontal axis. Figure 4.7 gives an example. The subsets based on management of the dune meadow datasets were used again, and a sample-based species accumulation pattern was calculated, but in contrast with Figure 4.3, the scale of the horizontal axis was now based on the number of individuals. Because the total number of individuals is different, the relative positions of the curves will change.
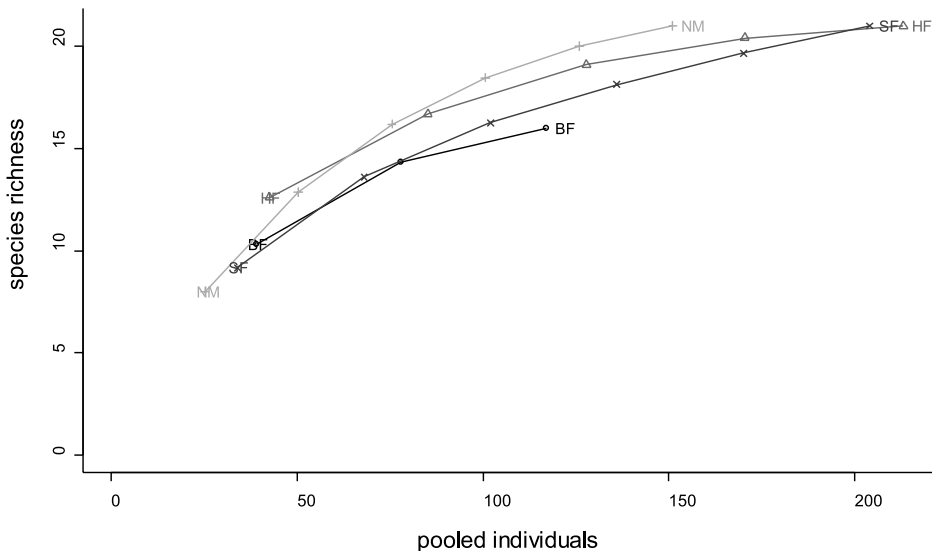

Figure 4.7 Sample-based species accumulation curves for various subsets of the dune meadow dataset based on management. The horizontal axis is scaled by the average number of combined individuals.

You can see now that hobby farming does not have a curve above the other curves as was seen in Figure 4.3. The reason for this pattern is that hobby farming had the largest number of individuals of the 4 categories. In this situation, we can conclude that the pattern observed for sample-based species accumulation curves (Figure 4.3) are mainly a result from differences in density between management systems. It will often be a good idea to compare sample-based with individual-based species accumulation to get a feel of the influence of density on sample-based results. And of course it is also important to summarize what these differences in plant density are. They may turn out to be a key aspect of the differences.

You can choose for other methods of scaling the horizontal axis. You could for instance scale the horizontal axis by the average size of the site. This would result in a different pattern in case sizes of sites are not constant. Since different patterns can be obtained, it is important that you started your study with clear objectives on how the results will be compared.

To summarize, you have two options to construct species accumulation curves. The first option is to either accumulate sites or to accumulate individuals. The second option concerns how you scale the horizontal axis of the species accumulation curve. The horizontal axis needs to be scaled by an average for all the sites. This average could be the average number of sites per site (=1 site/site), the average number of individuals per site, the average area per site, or you may think of others!

The important message here is that since species richness depends on sample size, and since sample size can be measured by different methods (number of sites, size of sample area, number of individuals, …), then different results can be obtained for different sample sizes. You should always mention the method that you used to determine sample size, and if possible compare results for different sample sizes. You should make sure that the method that you use meets your initial objectives for conducting the survey, since calculation of species accumulation curves should never be an end in itself.

Estimating the total number of species of the survey area

In most situations, we record the number of species for a number of sites. Normally we will not cover the entire area that we are interested in. We will not know the species composition of the area that we did not sample. However, since species richness depends on sample size, we can expect that we will not have recorded all the species that occur in the survey area.

Some formulae were developed to estimate the total number of species of a survey area (e.g. Longino et al. 2002). In terms of species accumulation curves, we need to extrapolate the curve until the point on the x axis corresponding to the whole study area. The estimation methods are based on different mathematical methods for extrapolation. These formulae depend on the fact that the sites were selected at random from the entire survey area. Different formulae make different assumptions of how species accumulate beyond the sampled sites. Probably one method will be more precise in one situation, and another method will be better for a second situation. Since you have no data to check for the accuracy of an extrapolation, you can not determine the best extrapolation method for your data. Any results from these methods have to be treated with caution.

If you want to extrapolate, then the best approach is to report the range in values obtained with the different methods and expect that the correct total richness lays somewhere within that range. For the dune meadow dataset, some predictions of total species richness (using the first- and second-order Jackknife, Chao and bootstrap formulae) are:

```
     Jack.1
all  32.85
     Jack.2
all  33.84
     Chao
all 32.25
     Boot
all 31.54
```

You can see that these estimates are quite similar, giving a range of 31-34 species. This is not surprising given the shape of the species accumulation curves, which flatten off for large numbers of sites, suggesting that the sampling has captured nearly all the species in the study area. In other situations, the estimates will differ more strongly. This will be the case if the species accumulation curves are still climbing at the right hand end. For such curves there are many ways they can be extrapolated, hence a wide range of answers. The specialist software *EstimateS* (http://viceroy.eeb.uconn.edu/EstimateS) was developed especially to calculate estimations of total diversity.

Calculating genus richness or family richness

You can calculate accumulation curves for the number of genera, number of families, or any other taxonomic level for which you have appropriate data. You could also calculate accumulation curves at intraspecific levels, such as for specific alleles. To be able to calculate those curves, you need to modify the species matrix so that the columns will refer to genera or families. This will be easy with a good database where you keep your data (see Chapter 2). The rest of the procedure is the same as for the calculation of species accumulation curves.

Some other methods of species accumulation

The methods described earlier were based on random accumulation of sample plots. Some other methods use the spatial configuration of sample plots.

One spatial method constructs a species accumulation curve based on a complete inventory of an area. This means that there are no unsampled areas within the area. This method has been used for 50-ha forest surveys, for instance (Plotkin et al. 2000). The total area is then subdivided, and the average species richness for half the area is estimated. The average species richness is then calculated for further subdivisions. For example, the average species richness is calculated for the two 25-ha halves, for the four 12.5-ha quarters and for the eight 6.25-ha subdivisions.

Another spatial method is also based on the species richness for sample plots of different sizes. Randomly in an area, sample plots of different size classes are placed. Afterwards, the average species richness for each size class is calculated.

Yet another method is to select the first sample plot at random, then add the second sample plot that is nearest, then the third that is second nearest and so on.

You can use these methods when you are specifically interested in the influence of spatial proximity of sites on the species accumulation curves. You could compare the curve that uses the spatial configuration with the curve that is based on random combination of sites to get an idea of the influence of spatial proximity. The approach is similar to the comparison of the sample-based and individual-based species accumulation curves. As always, you should only conduct this type of analysis if you have a clear objective for doing so.

References

Brose U, Martinez ND and Williams RJ. 2003. Estimating species richness: sensitivity to sample coverage and insensitivity to spatial patterns. *Ecology* 84: 2364-2377.

Fonseca CR and Ganade G. 2001. Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology* 89: 118-125.

Gotelli NJ and Colwell RK. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379-391 (recommended as first priority for reading)

Griffiths D. 1999. On investigating local-regional species richness relationships. *Journal of Animal Ecology* 68: 1051-1055.

Harte J, Kinzig A and Green J. 1999. Self-similarity in the distribution and abundance of species. *Science* 284: 334-336.

Hayek L-AC and Buzas MA. 1997. *Surveying natural populations.* New York: Columbia University Press.

He F and Legendre P. 2002. Species diversity patterns derived from species-area models. *Ecology* 83: 1185-1198.

Hurlbert SH. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 52: 577-586.

Kindt R. 2002. *Methodology for tree species diversification planning for African agroecosystems.* Ph. D. thesis. Ghent: University of Ghent, Belgium.

Kindt R, Van Damme P and Simons AJ. in press. Patterns of species richness at varying scales in western Kenya: planning for agroecosystem diversification. Biodiversity and Conservation.

Krebs CJ. 1994. *Ecological methodology*. Second edition. Menlo Park: Benjamin Kummings.

Legendre P and Legendre L. 1998. *Numerical ecology.* Amsterdam: Elsevier Science BV.

Lomolino MV. 2000. Ecology's most general, yet protean pattern: the species-area relationship. *Journal of Biogeography* 27: 17-26.

Longino JT, Coddington JA and Colwell RK. 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. *Ecology* 83: 689-702.

Loreau M. 2000. Are communities saturated? On the relationship between α, ß and γ diversity. *Ecology Letters* 3: 73-76.

Magurran AE. 1988. *Ecological diversity and its measurement.* Princeton, N.J.: Princeton University Press.

Moreno CE and Halffter G. 2001. On the measure of sampling effort used in species accumulation curves. *Journal of Applied Ecology* 38: 487-490.

Pielou EC. 1969. *An introduction to mathematical ecology.* New York: Wiley - Interscience.

Plotkin JB, Potts MD, Yu DW, Bunyavejchewin S, Condit R, Foster R, Hubbell S, LaFrankie J, Manokaran N, Seng LH, Sukumar R, Nowak MA and Ashton PS. 2000. Predicting species diversity in tropical forests. *Proceedings of the National Academy of Sciences* 97 (20), 10850-10854

Rosenzweig ML. 1995. *Species diversity in space and time.* Cambridge: Cambridge University Press.

Rosenzweig ML. 1999. Heeding the warning of biodiversity's basic law. *Science* 284: 276-277.

Rosenzweig ML, Turner WR, Cox JG and Ricketts TH. 2003. Estimating diversity in unsampled habitats of a biogeographical province. *Conservation Biology* 17: 964-874.

Ugland K I, Gray JS and Ellingsen KE. 2003. The species-accumulation curve and estimation of species richness. *Journal of Animal Ecology* 72: 888-897.

Whittaker RJ, Willis KJ and Field R. 2001. Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography* 28: 453-470.

Doing the analyses with the menu options of Biodiversity.R

Select the species and environmental matrices:

> Biodiversity > Environmental Matrix > Select environmental matrix
> → Select the dune.env dataset
> Biodiversity > Community Matrix > Select community matrix
> → Select the dune dataset

To calculate the total number of species:

> Biodiversity > Analysis of diversity > Diversity indices…
> → Diversity index: richness
> → Calculation method: all

To calculate the total species richness for separate sites:

> Biodiversity > Analysis of diversity > Diversity indices…
> → Diversity index: richness
> → Calculation method: separate per site

To compare the total number of species for various subsets of data:

> Biodiversity > Analysis of diversity > Diversity indices…
> → Diversity index: richness
> → Calculation method: all
> → Subset options: Management
> → Subset: .

To calculate a sample-based species accumulation curve

> Biodiversity > Analysis of diversity > Species accumulation curves…
> → Accumulation method: exact
> → Accumulation method: random (alternative to the exact method)

To calculate a sample-based species accumulation curve scaled by the number of accumulated individual plants:

> Biodiversity > Analysis of diversity > Diversity indices…
> → Diversity index: abundance
> → Calculation method: separate per site
> → Output options: save results
> Biodiversity > Analysis of diversity > Species accumulation curves…
> → Accumulation method: exact
> → Scale of x axis: abundance

To calculate an individual-based species accumulation curve scaled by the number of accumulated individual plants:

>Biodiversity > Analysis of diversity > Diversity indices…
>→ Diversity index: abundance
>→ Calculation method: separate per site
>→ Output options: save results
>Biodiversity > Analysis of diversity > Species accumulation curves…
>→ Accumulation method: rarefaction
>→ Scale of x axis: abundance

To compare species richness between various subsets in the data using species accumulation curves

>Biodiversity > Analysis of diversity > Species accumulation curves…
>→ Accumulation method: exact
>→ Subset options: Management
>→ Subset: .

Calculating the expected species richness for the entire survey area:

>Biodiversity > Analysis of diversity > Diversity indices…
>→ Diversity index: Jack.1
>→ Calculation method: all

Doing the analyses with the command options of Biodiversity.R

To calculate the total number of species:

```
Diversity.1 <- diversityresult(dune, index='richness')
Diversity.1
```

To calculate the total species richness for separate sites:

```
Diversity.2 <-  diversityresult(dune, index='richness',
  method='s')
Diversity.2
summary(Diversity.2)
Diversity.3 <- diversityresult(dune[1:2,], index='richness')
Diversity.3
```

To compare the total number of species for various subsets of data:

```
Diversity.4 <- diversitycomp(dune, y=dune.env,
  factor1='Management', index='richness' ,method='all')
Diversity.4
```

To calculate a sample-based species accumulation curve

```
Accum.1 <- accumresult(dune, method='exact')
Accum.1
accumplot(Accum.1)
Accum.2 <- accumresult(dune, method='random',
  permutations=1000)
Accum.2
accumplot(Accum.2)
```

To calculate a sample-based species accumulation curve scaled by the number of accumulated individual plants

```
dune.env$site.totals <- apply(dune,1,sum)
Accum.3 <- accumresult(dune, y=dune.env, scale='site.totals',
  method='exact')
Accum.3
accumplot(Accum.3, xlab='pooled individuals')
```

To calculate an individual-based species accumulation curve (first scaled by the number of sites in Accum.4, then by the number of accumulated plants in Accum.5)

```
Accum.4 <- accumresult(dune, method='rarefaction')
Accum.4
accumplot(Accum.4)
dune.env$site.totals <- apply(dune,1,sum)
Accum.5 <- Accum.5 <- accumresult(dune, y=dune.env,
   scale='site.totals', method='rarefaction')
Accum.5
accumplot(Accum.5, xlab='pooled individuals')
```

To compare species richness between various subsets in the data using species accumulation curves

```
Accum.6 <- accumcomp(dune, y=dune.env, factor='Management',
method='exact')
Accum.6
dune.env$site.totals <- apply(dune,1,sum)
Accum.7 <- accumcomp(dune, y=dune.env, factor='Management',
   scale='site.totals', method='exact', xlab='pooled
   individuals')
Accum.7
```

To calculate a collector's curve

```
Accum.8 <- accumresult(dune, method='collector')
Accum.8
accumplot(Accum.8)
```

Calculating the expected species richness for the entire survey area

```
Diversity.5 <- diversityresult(dune, index='Jack.1')
Diversity.5
Diversity.6 <- diversityresult(dune, index='Jack.2')
Diversity.6
Diversity.7 <- diversityresult(dune, index='Chao')
Diversity.7
Diversity.8 <- diversityresult(dune, index='Boot')
Diversity.8
```