Solutions to exercises

Exercise I: Foliar litter fall

There are several ways to solve the problem and below we give two slightly different ones. One is to simply add the amounts collected in each litter trap that is not disturbed, which is 13, calculate an average value per litter trap which also is the average litter fall per 0.25m^2. We obtain a value of 71.08 grams (SD = 18.4), which means 284.32 grams per square meter or 2843.2 kg per hectare.

An alternative is to calculate an average value per sampling using n = 15 in sampling 1, and n=14 in samplings 2 and 3. The values we obtain for the separate samplings Nos 1, 2 and 3 are thus the average values for 0.25m^2, and in this case 71.4 grams per trap or 2856 kg per hectare. An advantage is that in this latter case we use all values:

																Average[1]
Litter trap No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Sampling 1	45	61	42	21	55	59	75	52	48	19	38	43	62	59	44	48.2
Sampling 2	18	15	19	9	11	9	16	14	13	5	22	--	13	14	12	13.6
Sampling 3	10	14	15	8	7	5	7	11	17	2	12	8	5	-	14	9.6
	73	90	76	38	73	73	98	77	78	26	72		80		70	**71.1 / 71.4**

[1] Average value per sampling including intact traps only

Exercise II: Comparing foliar litter fall of different tree species

The way to set up a study with measurements on litter fall like the present one is to arrange the stands in blocks. A not uncommon situation is that you may obtain values from experiments for which the design is less clear or not well described and the results of statistical tests may then become less clear. In the present case the stands were actually arranged in a block design with four blocks, each block having one stand of Sitka spruce and one stand of Austrian pine. Thus, we have four paired stands, each par consisting of the two species.

This is a typical "comparison problem", one of the most widely met problems in natural sciences. Not surprisingly, a broad range of methods have been developed to compare populations (in statistics, the term *population* has a somewhat different meaning than in biology and means simply a group of objects that are studied). Describing them all exceeds the scope of this book and below we give only examples how the problem can be approached.

Solution I. One of the simplest methods that can be used to compare two population, not necessarily blocked in pairs, is the Student's *t*-test. One can also use the simple analysis of variance (ANOVA), which with two groups being compared is equivalent to Student's *t*-test. This method can be used any time, even if stands were not paired. Remember however that without blocking (e.g., with stands randomly distributed over larger area) differences that you would detect between species might be actually caused by differences in local climate or soil rather then by species-specific characteristics. In each case care must be also taken of the assumptions of the method (normal distribution and homoscedascity, i.e. constant residual variances across treatments).

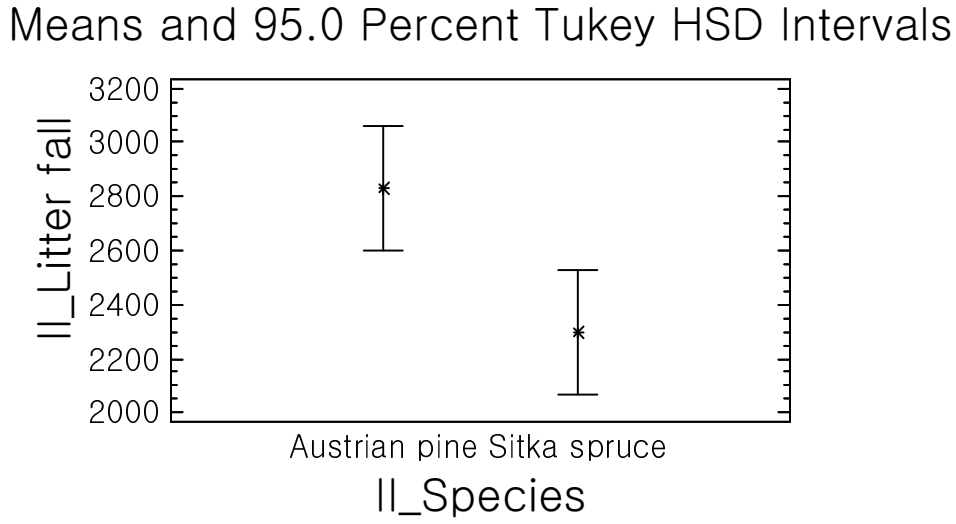
Below we give a printout from such an analysis:

One-Way ANOVA - II_Litter fall by II_Species

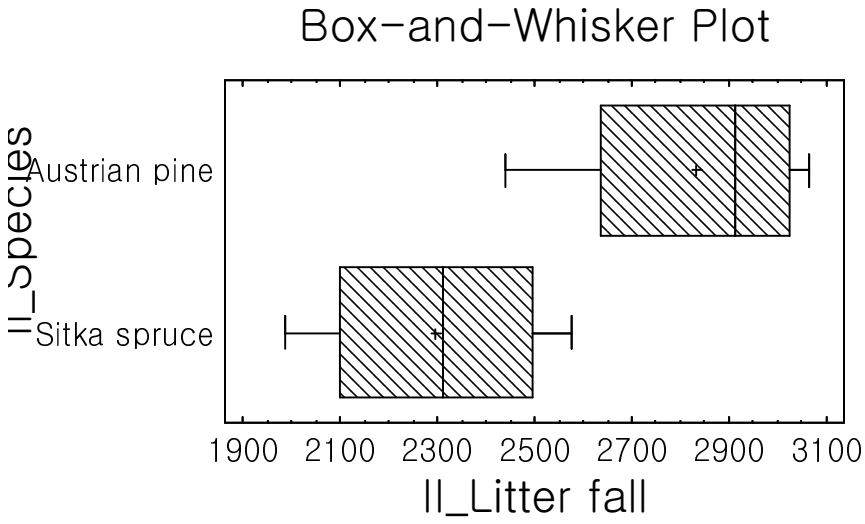
```
Analysis Summary

Dependent variable: II_Litter fall
Factor: II_Species

Number of observations: 8
Number of levels: 2

ANOVA Table for II_Litter fall by II_Species

                            Analysis of Variance
-------------------------------------------------------------------------------
Source              Sum of Squares     Df  Mean Square    F-Ratio      P-Value
-------------------------------------------------------------------------------
Between groups           568711.0      1     568711.0       7.97       0.0302
Within groups            428142.0      6      71357.0
-------------------------------------------------------------------------------
Total (Corr.)            996853.0      7
```

Comment: *The analysis of variance divides the variance of the variable studied (in this case litter fall) into two components: a between-group component and a within-group component. The F-ratio is a ratio of the between-group estimate to the within-group estimate. The p value indicates the probability of type I error and is called the*

significance level. In this particular case the significance level is ca. 0.03 meaning that the difference observed between the average litter fall values for the two species may result from pure chance rather then represent the real difference between the species only in 3 cases of 100. In natural and social studies it is commonly accepted that the difference is assumed to be true if p is lower or equal to 0.05.



Comment: *There is a number of methods to calculate confidence intervals around mean when comparing populations. In this case we used the so called "Tukey Honestly Significant Difference" (HSD) intervals. This method offers a good balance in protection against type I and type II errors.*



Comment: *As mentioned in Chapter 9, Box-and-Whisker plot gives very rich information about a data set. Here you can see medians (the central vertical lines inside the boxes), lower and upper quartiles (the boxes to the left and to the right of the median, respectively), means (small crosses inside the boxes) and minima and maxima (whiskers*

to the left and to the right of the boxes, respectively). The asymmetry of a box around the median value also gives some information about data distribution, i.e., if the data approximately follow the normal distribution or are, e.g., heavily skewed to the right or to the left.

Solution II. Although the method presented above is correct and very general, we did not make any use of the fact that the experiment was designed in paired stands. This actually may be an important advantage as we know that in each pair the two species grew in exactly the same climate and on similar soil. Some of the variance unexplained in ANOVA and thus adding to the error, may be explained by the variance between the stands which, however, should not affect differences between the species in litter fall. Thus, we will use now another comparison method – developed especially to compare paired samples:

Paired Samples - Ap litterfall & Sp litterfall

```
Analysis Summary

Data variable: Ap litterfall-Sp litterfall

4 values ranging from 449.0 to 627.0

Summary Statistics for Ap litterfall-Sp litterfall
```

Comment: *Note that this time all statistics are calculated not for each species separately but for the difference between the species on paired stands. Thus, the hypothesis tested is not that mean litter fall of species 1 = mean litter fall of species 2 but that the mean difference between the species =0.*

```
Count = 4
Average = 533.25
Median = 528.5
Variance = 6514.92
Standard deviation = 80.715
Minimum = 449.0
Maximum = 627.0
Range = 178.0
Stnd. skewness = 0.180395
Stnd. kurtosis = -1.19441


Hypothesis Tests for Ap litterfall-Sp litterfall

Sample mean = 533.25
Sample median = 528.5

t-test
------
Null hypothesis: mean = 0.0
Alternative: not equal

Computed t statistic = 13.2132
P-Value = 0.00093663
```

Comment: *Please note that when we used the information about paired stands, we obtained much higher significance level (that is, smaller p value = 0.000937). Thus, with exactly the same data as before, by performing the analysis that make use of additional information about pairing the stands, we obtained much stronger "confirmation" of the hypothesis that the species do differ in amount of litter fall.*

Exercise III: Foliar litter fall in a climatic transect after climate change

In the present problem the equation basically gives us the answer. First we calculate the new AET value which was 27% higher than the old one or 514 mm. This value is used in the relationship above and gives the value of 3148 kg ha^{-1}.

Exercise IV: Calculating litter mass loss

The litter that you originally incubated in the bags was air dried and contained 6.04% water. To obtain the real dry mass you need to subtract the 6.04% of water. When you have done that you will have a new set of values for litter mass dried at 85°C. Here we have organized those values in a new column giving that weight (original litter dry weight). To calculate litter mass loss you now simply use the data in columns 2 and 3 and obtain the mass loss values in column 4. A comment: when using this method the standard error normally is below 1.7 up to ca 60% mass loss. The reason for the higher SE value here may be that the litter was incubated in four blocks of which one block deviated as regards moisture and the litter decomposed somewhat faster there (last five values).

Original weight (g per bag)	Original litter dry weight (g per bag)	The same litter after 366 days incubation (g per bag)	Mass loss (%)
0.613	0.576	0.2783	51.7
0.615	0.578	0.2605	54.9
0.611	0.574	0.2802	51.2
0.611	0.574	0.1798	68.7
0.614	0.577	0.2733	52.6
0.616	0.579	0.2944	49.1
0.615	0.578	0.2449	57.6
0.612	0.575	0.1880	67.3
0.618	0.581	0.2551	56.0
0.614	0.577	0.3031	47.5
0.617	0.580	0.2049	64.7
0.610	0.573	0.1612	71.9
0.618	0.581	0.2443	58.0
0.619	0.582	0.2533	56.5
0.615	0.578	0.3037	47.5
0.613	0.576	0.1923	66.6
0.617	0.580	0.1650	71.5
0.619	0.582	0.1717	70.4
0.613	0.576	0.1422	75.3
0.613	0.576	0.1098	80.9
		Average	**61.0**
		Standard dev.	**9.8**
		Standard error	**2.2**

Exercise V: Calculating annual litter mass loss during decomposition

As a first step we suggest that you draw a graph showing accumulated mass loss against time as shown on Fig. V.I. In the (approximately) first year the mass loss was 27.3%, leaving 72.7% as remaining mass. For year 2, which is the period between day 376 and day 734, we simply consider the remaining substrate on day 376 and its chemical composition as a new starting point. Thus, the amount of substrate is the remaining mass, namely 72.7% of the original material that may be regarded as the initial substrate for the decomposition in the 2^{nd} year.

We have noted that many of us prefer not to think in the unit % but rather in an imaginary specific amount of litter, so let us say that we initially had samples with 1.0 gram in each. With 27.7 % mass loss in the first year the remaining amount was 1.0-0.273 g or 0.727 g. After two years' decomposition the accumulated mass loss was 45.8% and the remaining amount thus 0.542 g. The mass loss in the second year is thus the amount of the substrate at the beginning of the 2^{nd} year, minus what remained after 2 years (0.727 – 0.542 g). To obtain the percentage decomposition we divide by the initial amount at the start of the 2^{nd} year which gives the fraction. By multiplying by 100 we recalculate the fraction to %. The expression thus becomes $100\times(0.727 - 0.542)/0.727$, giving the mass loss of 25.4% of the amount still remaining after 1 year decomposition.

When we do the same operation for year 3 we obtain the expression $100\times(0.542 - 0.412)/0.542$ which gives a mass loss of 24.0%. For year 4 the expression is $100\times(0.412 - 0.335)/0.412$ which gives a mass loss of 18.7%, and for year 5 it is $100\times(0.335 – 0.250)/0.335$ or a mass loss of 25.4%.

We can object about this kind of calculation that some sampling times deviate from a year, which of course is a weakness that has been illustrated in the present example. However, in an example like the present one the average decomposition per day would be ca 0.07% which means that a few days difference are not that important. As the reader probably has noted about the data, the mainly three samplings per year are made in early summer, in September and in late autumn. With a data set like the present, it is of course possible to pick any one-year period. We have chosen one-year periods starting with the original incubation date which is not necessary. As the litter chemical composition and in part the weather is different among the samplings we may use all possible one-year periods without risk of using the same information twice. In the present data set there are ca 14 periods encompassing about one year and how many days the chosen periods should be allowed to deviate from 365 days can be decided upon for each data set and the purpose of the calculation.

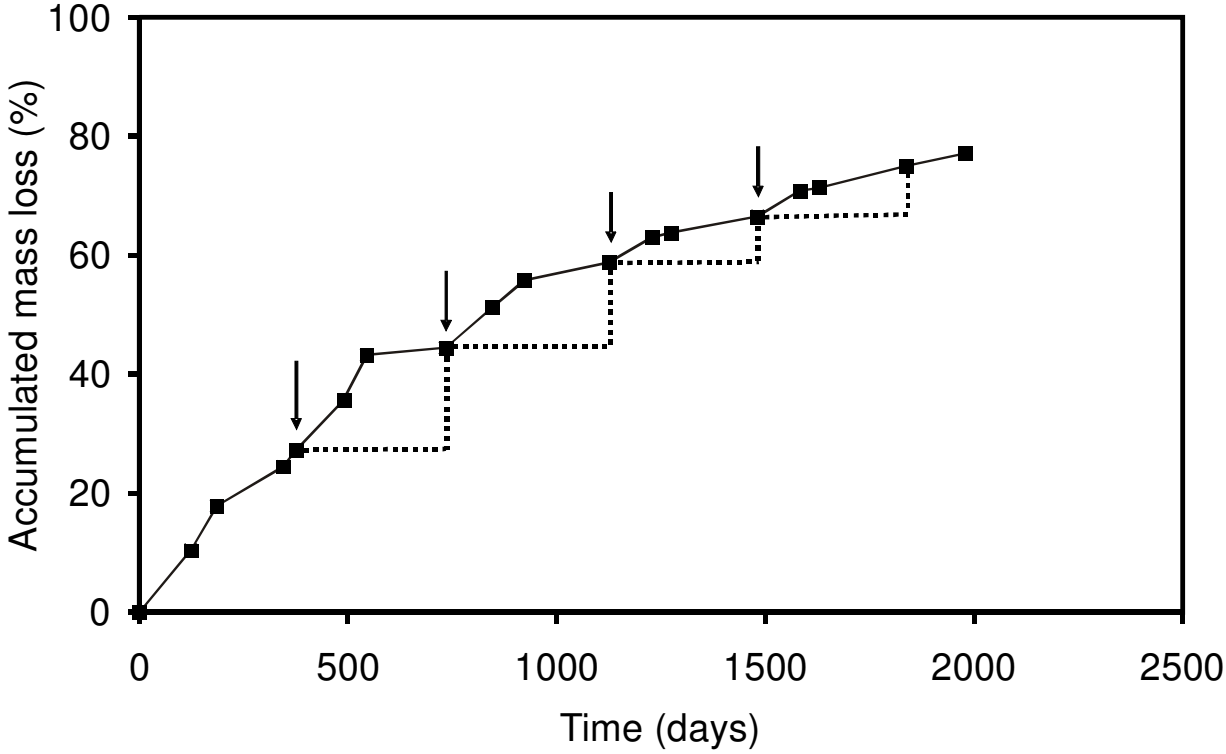


Figure V.I. Accumulated litter mass loss plotted versus time. Arrows indicate the samplings made with ca 1-yr intervals and the dotted horizontal and vertical lines show the period and the intervals for accumulated mass loss, respectively, that are used as basic units for calculating the annual mass loss.

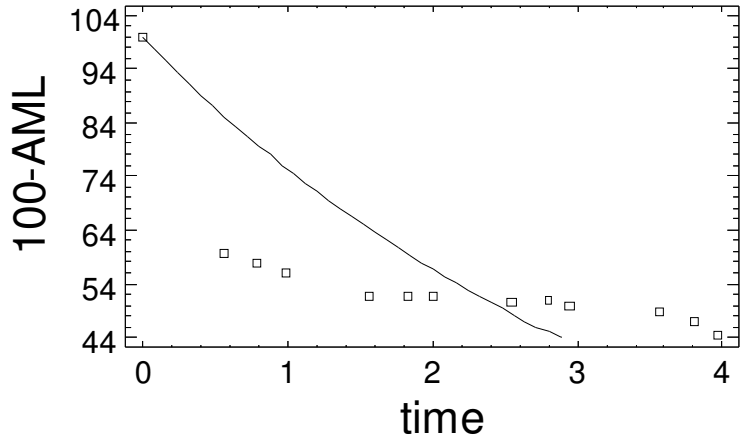
Exercise VI: Describing the accumulated litter mass loss dynamics by functions

The evident way of solving the problem is to fit the equations described earlier in the book, namely the one-compartment exponential function (first-order kinetics model), the two-compartment model and the asymptotic model. Below you can see printouts from such analyses with some comments to the results obtained. Considering that different software packages offer slightly different sets of information, only the most important information from the report has been retained.

Please note that to meet the requirements of the different models fitted, the data were used either as given above (accumulated mass loss in per cent, AML) or recalculated to remaining mass (100-AML). Also time has been expressed in years rather then in days as *k* values are usually reported per year, and when given per day the values become very small and not convenient for reporting.

Nonlinear Regression – alder leaves, one-compartment (Olson's) model

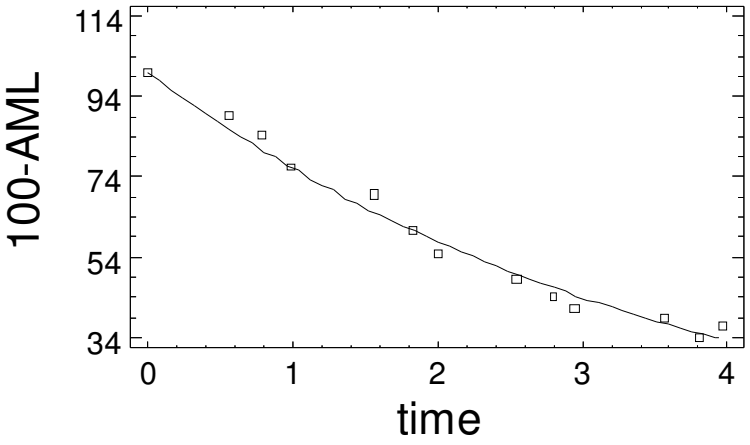
```
Dependent variable: 100-AML
Independent variables: time
Function to be estimated: 100*exp(k*time)

Estimation Results
-------------------------------------------------------------------------------
                                                      Asymptotic 95.0%
                                          Asymptotic  Confidence Interval
Parameter              Estimate  Standard Error       Lower         Upper
-------------------------------------------------------------------------------
k                     -0.284802       0.0368065    -0.364997     -0.204607
-------------------------------------------------------------------------------

R-Squared = 1.47508 percent
R-Squared (adjusted for d.f.) = 1.47508 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between 100-AML and 1
independent variable. The equation of the fitted model is
100*exp(-0.284802*time)
```



Comment: *Please note that although the estimated k value is significant (i.e., differs significantly from 0 at 95% confidence level as indicated by the estimated 95% confidence intervals reported in the table), the fit is actually very poor. The R^2 is less then 1.5%, and the fitted line obviously does not describe the decomposition of alder leaves well. It can be clearly seen from the plot above that at the early decomposition stage the actual decomposition rate is substantially higher then predicted by the model, while at the late stage the litter decomposes slower than the model would predict. Thus, we should conclude that the Olson's model, even if significant, is inadequate for describing decomposition of grey alder leaves.*

Nonlinear Regression – lodgepole pine needles, one-compartment (Olson's) model

```
Dependent variable: 100-AML
Independent variables: time
Function to be estimated: 100*exp(k*time)

Estimation Results
------------------------------------------------------------------------------
                                                      Asymptotic 95.0%
                                    Asymptotic        Confidence Interval
Parameter              Estimate  Standard Error        Lower         Upper
------------------------------------------------------------------------------
k                     -0.273737      0.00695995     -0.288902     -0.258573
------------------------------------------------------------------------------

R-Squared = 98.4866 percent
R-Squared (adjusted for d.f.) = 98.4866 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between 100-Lp aml and 1
independent variables.  The equation of the fitted model is

100*exp(-0.273737*time)
```



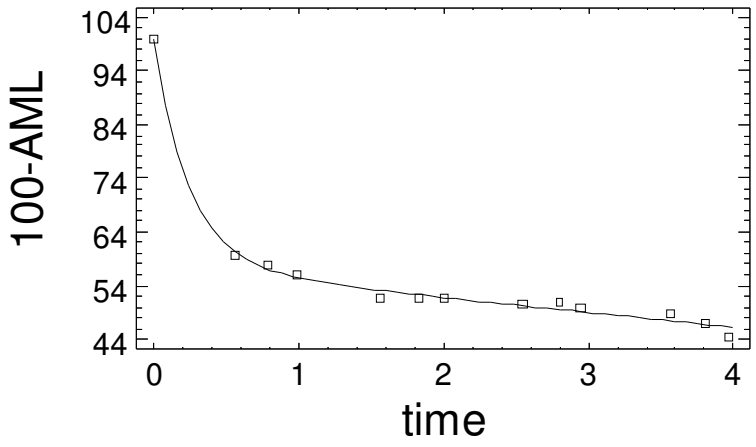
Comment: *In contrast to grey alder leaves, the decomposition of lodgepole pine needles seems to be described well by the Olson's model. Note that as much 98.5% of the variability in mass loss is described by the model. We could thus conclude that lodgepole pine needles decompose following the simple, one-compartment model at least within the*

investigated interval for accumulated mass loss . However, before accepting this conclusion we should yet check if the other two models do not explain the decomposition of lodgepole pine needles still better.

Nonlinear Regression – grey alder leaves, two-compartment model

Comment: *Note that in this model we have two decomposition constants, k1 and k2. We also have two compartments, w1 and w2, which represent two different groups of organic matter, namely 'easy-decomposable' and 'resistant' parts of organic matter expressed as percentages in the initial material.*

```
Dependent variable: 100-AML
Independent variables: time
Function to be estimated: w1*exp(k1*time)+w2*exp(k2*time)
Initial parameter estimates:
   w1 = 20.0
   k1 = -1.0
   w2 = 80.0
   k2 = -0.0001

Estimation Results
------------------------------------------------------------------------------
                                                    Asymptotic 95.0%
                                        Asymptotic  Confidence Interval
Parameter              Estimate  Standard Error      Lower         Upper
------------------------------------------------------------------------------
w1                      42.1254         1.73477      38.201       46.0497
k1                     -4.15049         0.66995     -5.66603     -2.63496
w2                      57.8601         1.33276      54.8451       60.875
k2                   -0.0552087      0.00831569    -0.0740201   -0.0363973
------------------------------------------------------------------------------

R-Squared = 99.5194 percent
R-Squared (adjusted for d.f.) = 99.3592 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between 100-Alder aml and 1
independent variables.  The equation of the fitted model is
42.1254*exp(-4.15049*time)+57.8601*exp(-0.0552087*time)
```



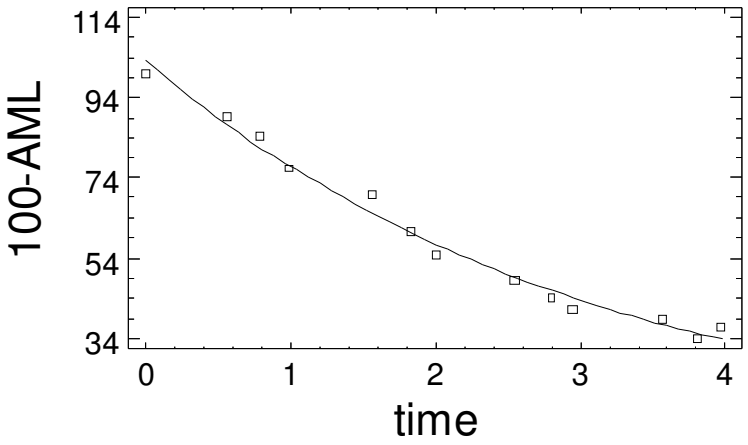
Comment: *Note how much better the two-compartment model fits the data for grey alder leaves, explaining almost 100% of the variability in mass loss. We would conclude now*

that grey alder leaves apparently contain two very different compartments of organic matter: approximately 42% of easily decomposed matter with a k *value of -4.2, and ca. 58% of resistant substrate decomposing at a* k *value as low as 0.055. The latter k value, although low, is still significantly different from 0, indicating that indeed this part of litter is not completely resistant to decomposition although it decomposes at a very low rate as seen in the figure above.*

Nonlinear Regression – lodgepole pine needles, two-compartment model

Comment: *As we mentioned before, although the Olson's model fit well the decomposition data for lodgepole litter, we will still use the two-compartment model to check for possible distinction between resistant and easily-decomposable fractions in this litter.*

```
Dependent variable: 100-AML
Independent variables: time
Function to be estimated: w1*exp(k1*time)+w2*exp(k2*time)
Initial parameter estimates:
   w1 = 80.0
   k1 = -1.0
   w2 = 20.0
   k2 = -0.0001

Estimation Results
------------------------------------------------------------------------------
                                                    Asymptotic 95.0%
                                        Asymptotic  Confidence Interval
Parameter              Estimate  Standard Error      Lower         Upper
------------------------------------------------------------------------------
w1                      102.398          16.257      65.6223       139.174
k1                    -0.303766        0.129616    -0.596979    -0.0105539
w2                     0.768211          17.432     -38.6659       40.2023
k2                     0.383385         4.13055     -8.96058       9.72736
------------------------------------------------------------------------------

R-Squared = 98.7407 percent
R-Squared (adjusted for d.f.) = 98.321 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between 100-Lp aml and 1
independent variables.  The equation of the fitted model is
102.398*exp(-0.303766*time)+0.768211*exp(0.383385*time)
```



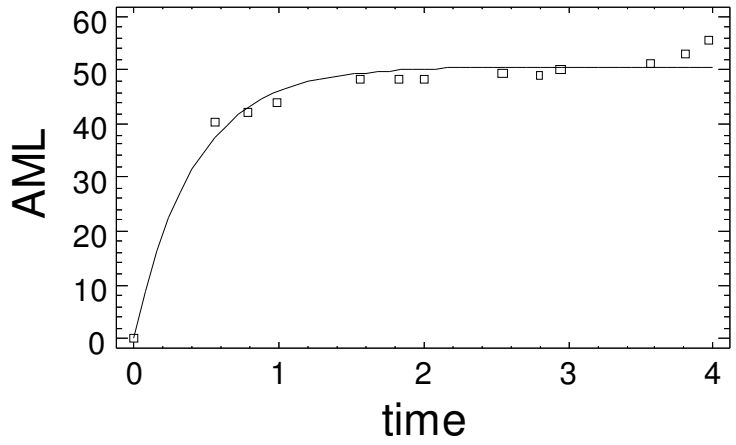
Comment: *The two-compartment model also seems to fit the data for lodgepole pine needles pretty well with R^2_{adj} = 98.3% which is only marginally lower than R^2 obtained with the Olson's model. To solve the question whether there is one or two compartments in lodgepole needle litter look closely at the results table. You will notice there that the estimate for the first compartment is 102% and does not differ significantly from 100% and that both parameters describing the second compartment, k1 and w2, are not significant (i.e., their 95% confidence intervals cover 0). Thus, we may reject the hypothesis that the lodgepole pine needle litter consists of two compartments with different decomposition rates.*

Nonlinear Regression – alder leaves, asymptotic model

Comment: *Note that in this is a two-parameter model: besides the* k *value (which is not equivalent to the k values from Olson's and two-compartment models as described earlier in the book) also the asymptote* m *is estimated.*

```
Dependent variable: AML
Independent variables: time
Function to be estimated: m*(1-exp((k*tyrs)/m))
Initial parameter estimates:
   m = 60.0
   k = -100.0

Estimation Results
------------------------------------------------------------------------------
                                                      Asymptotic 95.0%
                                    Asymptotic        Confidence Interval
Parameter              Estimate  Standard Error        Lower         Upper
------------------------------------------------------------------------------
m                       50.6259        0.786011       48.8959       52.3559
k                      -122.466         11.4297      -147.623      -97.3095
------------------------------------------------------------------------------

R-Squared = 97.7356 percent
R-Squared (adjusted for d.f.) = 97.5298 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between Alder aml and 1 independent
variables.  The equation of the fitted model is
```

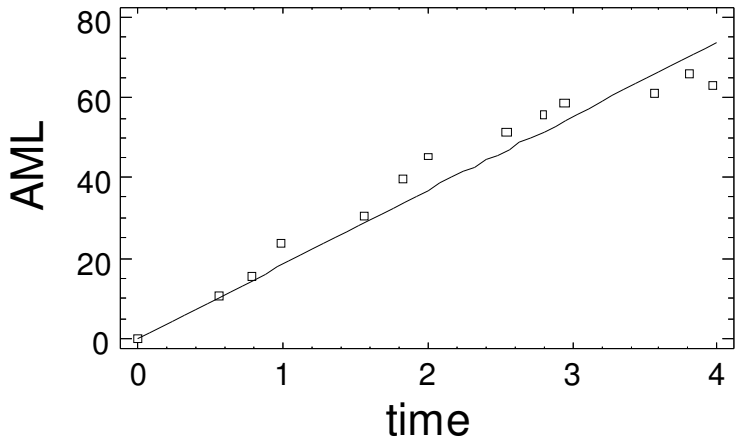
```
50.6259*(1-exp((-122.466*time)/50.6259))
```



Comment: *The asymptotic model fits well the decomposition dynamics of the grey alder leaves with both estimated parameters,* k *and* m*, significant. Thus, we cannot reject the hypothesis that the decomposition of alder leaves stops after ca 2.5 years of decomposition. This undecomposable fraction has been estimated to 50.6%. Notice however that the* R^2_{adj} *value is lower in this model than in two-compartment one (97.5% vs. 99.4%). Thus, although both regressions are significant, the two-compartment model gives a better fit and explains the decomposition dynamics better.*

Nonlinear Regression – lodgepole pine needles, asymptotic model

```
Dependent variable: AML
Independent variables: time
Function to be estimated: m*(1-exp((k*time)/m))
Initial parameter estimates:
   m = 80.0
   k = -10.0

Estimation Results
------------------------------------------------------------------------------
                                                    Asymptotic 95.0%
                                        Asymptotic  Confidence Interval
Parameter              Estimate  Standard Error         Lower         Upper
------------------------------------------------------------------------------
m                     5.10074E8       2.88789E8    -1.25548E8      1.1457E9
k                      -18.4271        0.633325      -19.8211      -17.0332
------------------------------------------------------------------------------

R-Squared = 94.1361 percent
R-Squared (adjusted for d.f.) = 93.603 percent

   The output shows the results of fitting a nonlinear regression
model to describe the relationship between Lp aml and 1 independent
variables.  The equation of the fitted model is
5.10074E8*(1-exp((-18.4271*time)/5.10074E8))
```



Comment: *Although the asymptotic model explains as much as 93.6% of the variability in the decomposition dynamics of the lodgepole pine needles, the asymptote m is apparently not significant. Thus, we may reject the hypothesis that the lodgepole needles do not decompose completely.*

Final conclusion:

After analyzing the three different models of litter decomposition for the grey alder leaves and the lodgepole pine needles, we may conclude that the two litter types differ substantially in their decomposition patterns and rates. The lodgepole pine needles follow the simple, one-compartment (Olson's) decay model described by one decomposition constant k *with the asymptote giving 0% remaining material (that is, asymptotically 100% decomposition). In contrast, the grey alder leaf litter consists of two markedly different fractions, one being easily decomposable and comprising ca 42% of the organic matter, and the other decomposing very slowly and forming the remaining 58% of the matter, which alternatively may be called "undecomposable".*

Exercise VII: Regulating factors for decomposition rates

One way of determining the decomposition rate is to use the mass loss over a certain period, e.g., one year. We discussed in the Exercise V how to do this and that we may consider the remaining litter as a new substrate with a new chemical composition at the start of each such one-year period. In the present exercise we use the same data set as in exercise VI but have already calculated the one-year mass loss values and listed them in the table below. In principle we can take any period that covers 365 days, but since we want to determine the substrate quality factors that influence litter mass loss rate, we want to avoid the influence of climate and we do that by selecting and comparing periods for which the climate (or weather) is constant for all five litter types.

So after some calculation you will have a new data base with 25 numbers:

Litter type	Yearly mass loss			
	yr 1	yr2	yr 3	yr 4
Ih	26.5	29.4	22.8	19.0
N0	32.7	27.4	22.1	18.0
N1	31.3	26.6	19.3	20.4
N2	32.2	27.9	17.3	26.7
N3	36.3	26.3	15.7	18.2

In that way, we may find which factors determine the decomposition rate during the consecutive years of decomposition and, thus, how they change in the course of decomposition.

Let us start with the first year to see what regulated the mass-loss rate in that period. We obtained R = 0.99 for P, 0.76 for N and R = 0.03 for lignin (n=5). Of these relationships only that to P is significant at $p<0.05$.

We continue with year 2. For N we obtain R = -0.580, for P, R becomes = -0.762 and for lignin R is = -0.815. Of these relationships the best one is that to lignin although not quite significant at $p<0.1$.

For year 3 we obtain: for N an R value of -0.926, for P an R value of -0.898, and for lignin an R value of -0.917.

For year 4 we obtain for N an R value of 0.663, for P an R value of -0.000 and for lignin an R value of 0.338. None was significant at $p<0.1$.

An overview of the R-values gives us the following table:

	N	*P*	Lignin
Year 1	+0.76	+0.99	+0.03
Year 2	-0.580	-0.762	-0.815
Year 3	-0.926	-0.898	-0.917
Year 4	0.663	-0.000	0.338

The R values in the table may be interpreted as follows:

In the first year the concentration of P has a significant and stimulating effect on the decomposition process. Although no really significant effect of N is seen, the high R value gives a certain support to the hypothesis that there is a stimulating effect of the main nutrients in the first year of decomposition. We have seen (chapter 4) that the components that are decomposed in the first year for Scots pine needles are mainly water solubles and hemicelluloses and according to basic physiology their degradation should be stimulated by higher levels of the main nutrients. It also appears that there is no effect of lignin. According to the existing information lignin should be degraded slowly, at least in the presence of N of the levels found in foliar litter.

In the second year the relationships to N and P are negative, suggesting a suppressing effect of the two main nutrients on decomposition. The concentrations of both of these nutrients increase during the decomposition process so had there been a stimulating effect of one of them or of both that should have been seen not only as positive R values but also as a generally higher rate in the second year. The mass loss data for year 2 show that the most N-and P-poor litter has the highest mass loss and the litter being the most nutrient rich has the lowest rate. We may look at the relationship to lignin, which is negative. Although not really significant we may say that $p<0.1$ suggests some effect. Lignin has been suggested as a compound that is resistant to decomposition and we can see, e.g. in chapter 4, that its degradation starts late and that its concentration increases as decomposition of the whole litter proceeds, or expressed in another way – lignin has a slower decomposition than other litter components. A reasonable conclusion is that there is a suppressing effect of lignin on the decomposition rate. Thus, in the second year there may be a change in factors that regulate litter mass loss rate. Judging from the R values lignin concentration may have a strong negative influence. We have seen in chapter 4 that litter N concentration may have a suppressing effect on lignin degradation rate but the R value is rather low to allow us to suggest such an effect. See also figure VI.

In the third year the negative effect of lignin is statistically significant as is a negative relationship to N. The negative relationship to P may not necessarily be interpreted biologically as there is no known such suppressing effect of P on, e.g., lignin degradation. The high R value may simply be due to the fact that the concentrations of N and P both increase with accumulated mass loss. These relationships support what we found for year 2. See also figure VI.

The R values for the fourth year do not give any clear picture of regulating factors and we cannot exclude that lignin concentration as a regulating factor has been replaced by another one. See also figure VI.

Years 2 and 3 combined. We may combine the values for, e.g., years 2 and 3 and investigate a relationship with n=10. We can see that the negative relationship between annual mass loss and lignin concentration was improved (Fig. VI). A combination of N and lignin in a multiple regression did not add any further explanation ($R^2 = 0.866$ for lignin and $R^2 = 0.868$ for lignin and N). We should be aware that we now used two different years and that a difference in climate between years may influence the result.

A general conclusion of this investigation is that we may see an early stage illustrated by the mass loss in year 1. In years 2 and 3 the mass losses appear regulated by lignin degradation which may constitute another (later) stage. Finally in the last year it appears that the regulating effect of lignin disappears. Still we can only observe this, and understand that a next stage appears but in this investigation we do not have any regulating factor.

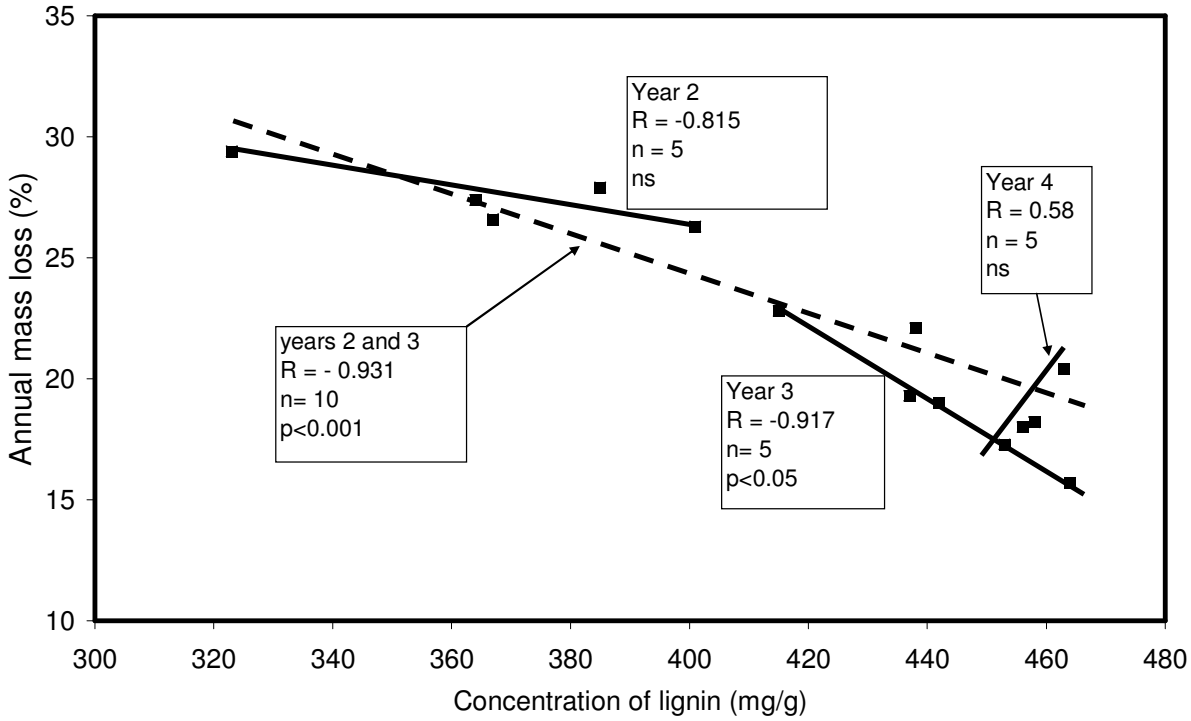


Figure VII.I. Linear relationships between concentration of lignin and annual mass loss. Full lines give mass losses for the single years 2, 3 and 4 and the dashed line gives the regression for years 2 and 3 combined.

Exercise VIII: Nitrogen dynamics – concentrations and amounts

Solution I. To plot N concentration versus time is relatively simple as all information is already there. To plot the changes in absolute amount you need to calculate the values for absolute amount. By absolute amount we mean of course the remaining amount as related to the initial amount. For example, in the initial litter 1.0 g contains 4.8 mg N. After 15.6 % decomposition 0.844 grams remain with a concentration of 5.1 mg/g. By multiplying 0.844 by 5.1 we obtain the remaining amount of N, which is 4.3 mg. A key question to do this is to ask "The given N concentration is the concentration in what amount of litter?" The obvious answer is in the remaining amount of the litter. Doing these calculations we obtain the data set below. As some of us find it easier to imagine remaining amounts of a certain given original mass we have chosen to use the unit 1.0 grams as an imaginary initial amount.

Table VIII.II.

Time (days)	*litter mass loss (%)*	*remaining amount of litter (g)*	*N concentration (mg/g)*	*N abs. amount* (mg)
0	0	1.000	4.8	4.8
204	15.6	0.844	5.1	4.3
286	22.4	0.776	5.4	4.2
358	29.9	0.701	5.4	3.8
567	38.5	0.615	8.3	5.1
665	45.6	0.544	9.2	5.0
728	47.5	0.525	8.8	4.6
931	54.1	0.459	9.8	4.5
1021	58.4	0.416	11.1	4.6
1077	62.5	0.375	11.5	4.3
1302	66.0	0.340	12.2	4.1
1393	67.4	0.326	12.5	4.1

With this data set we may plot the data. As we may see (Fig. VII.I) the concentration increases as far as the litter decomposition process was followed. We can also see that for this litter type there are just small fluctuations in amount, and at the end of the measurements most of the N is still bound to the litter structure.

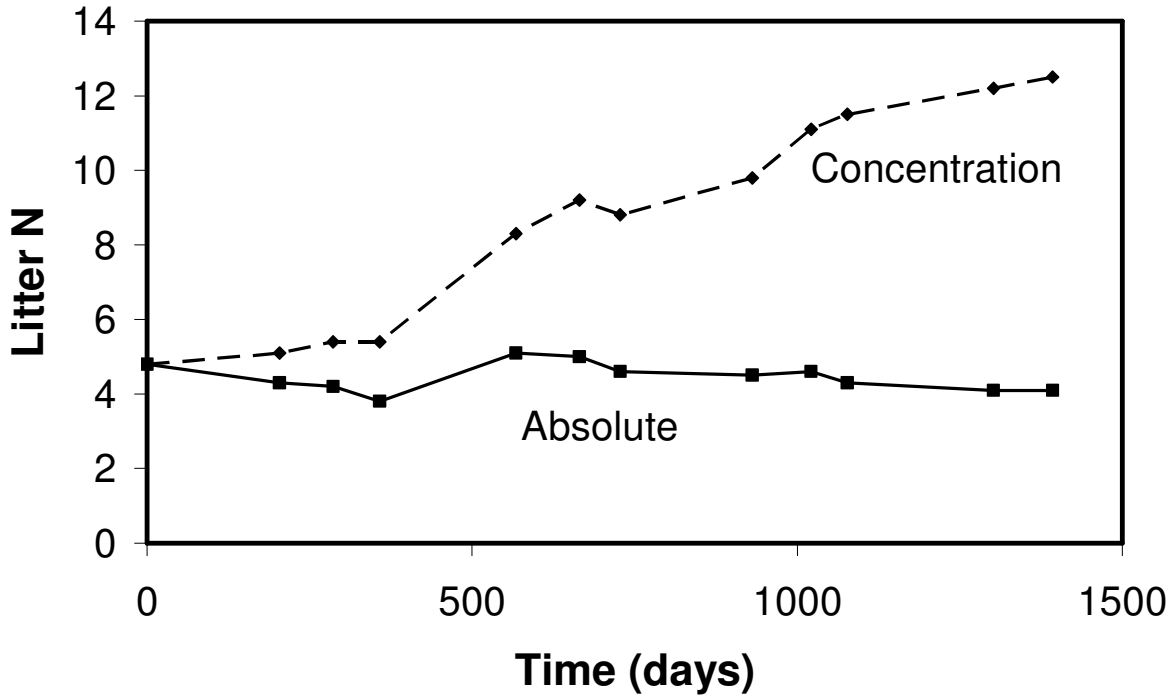


Fig. VIII.I. Plot of the dynamics in N concentration and N amounts in decomposing litter.

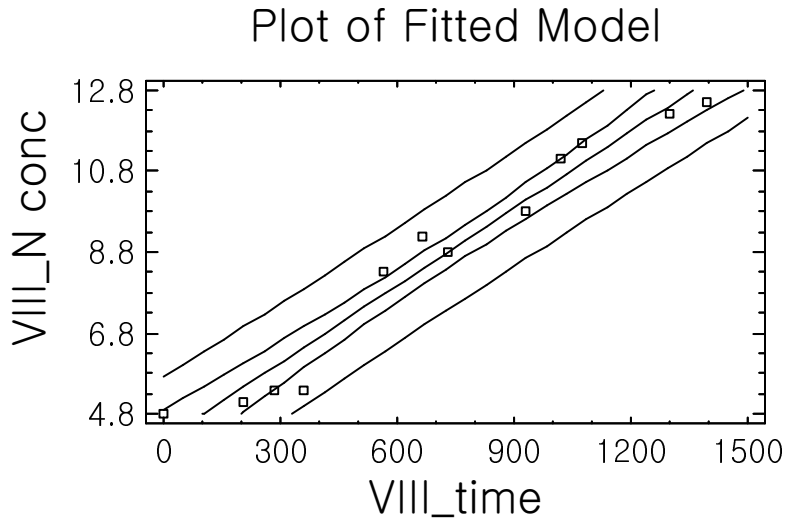
Solution II. If we need to test formally whether the concentration or amount changes significantly with time (that is, can we really say that the concentration or amount indeed increases/decreases or the changes can be considered a random variance) we have to perform a little more complicated task, namely the regression analysis. In this particular case the increase in concentration seems approximately linear for the time span used in the investigation so we will apply the linear regression. As in earlier exercises, you will find below a printout form a statistical program with some comments.

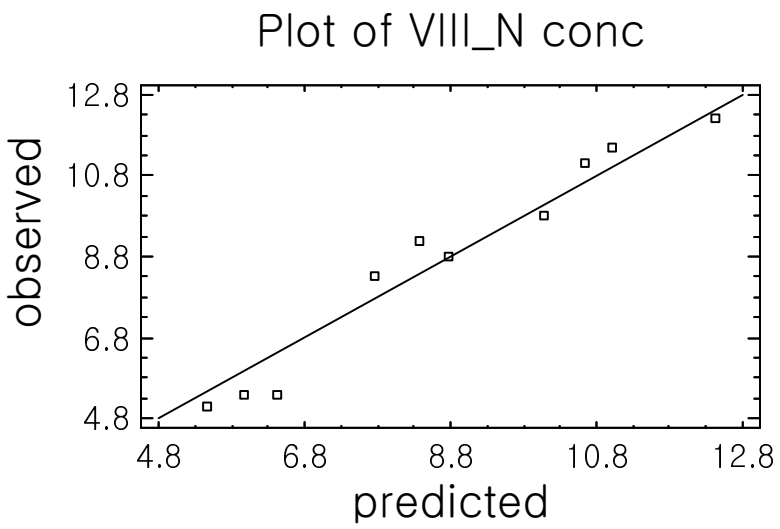
Simple Regression - VIII_N conc vs. VIII_time

```
Regression Analysis - Linear model: Y = a + b*X
------------------------------------------------------------------------------
Dependent variable: VIII_N conc
Independent variable: VIII_time
------------------------------------------------------------------------------
                             Standard          T
Parameter       Estimate         Error       Statistic        P-Value
------------------------------------------------------------------------------
Intercept        4.15835        0.34294        12.1256         0.0000
Slope         0.00635253    0.000413599        15.3592         0.0000
------------------------------------------------------------------------------


                           Analysis of Variance
------------------------------------------------------------------------------
Source              Sum of Squares     Df  Mean Square    F-Ratio      P-Value
------------------------------------------------------------------------------
Model                     88.1268      1      88.1268     235.90       0.0000
Residual                  3.73571     10     0.373571
------------------------------------------------------------------------------
Total (Corr.)             91.8625     11

Correlation Coefficient = 0.979456
R-squared = 95.9334 percent
```

```
R-squared (adjusted for d.f.) = 95.5267 percent

   The output shows the results of fitting a linear model to describe
the relationship between VIII_N conc and VIII_time.  The equation of
the fitted model is

   VIII_N conc = 4.15835 + 0.00635253*VIII_time

Since the P-value in the ANOVA table is less than 0.01, there is a
statistically significant relationship between VIII_N conc and
VIII_time at the 99% confidence level.
```

Comment: *As could be expected from the simple X-Y plot (Fig. VIII.I), the relationship between time and N concentration appeared highly significant. The relationship itself can be seen below as a plot of the fitted model, including the original data points as well as 95% confidence limits (inner bounds) and 95% prediction limits (outer bounds). The latter indicate the area around the regression line where 95% of real observations should fall. Before we are satisfied with the regression, we should check whether we have selected a proper model. It may happen that although the model is significant, it is not really a good model for a particular data set. For example, a linear regression would be significant when used to describe the relationship between litter mass loss and time, but it is certainly not a good model as the relationship is non linear. Whether the model is proper can be checked simply by looking at the "observed vs. predicted" plot (below). If the model fits the data set well, then the points should be randomly distributed around the 1:1 line. Any clear deviation from this random distribution (e.g., points drop down off the 1:1 line at the upper end) suggests that we should look for a better model. In this particular case there are no indications of bad fit of the model so we may accept the idea that N concentration increases approximately linearly in the litter studied throughout the whole incubation time. There is also a more formal test for the goodness of fit, but it requires that the data are replicated at least at some points. Thus, form that point of view it would be better to use the original data points rather then averages.*





Simple Regression – VIII_N amount vs. VIII_time

```
Regression Analysis - Linear model: Y = a + b*X
------------------------------------------------------------------------------
Dependent variable: VIII_N amount
Independent variable: VIII_time
------------------------------------------------------------------------------
                              Standard          T
Parameter       Estimate         Error       Statistic        P-Value
------------------------------------------------------------------------------
Intercept        4.57906       0.224298        20.4151         0.0000
Slope       -0.000181518    0.000270513      -0.671015         0.5174
------------------------------------------------------------------------------


                           Analysis of Variance
------------------------------------------------------------------------------
Source              Sum of Squares     Df  Mean Square    F-Ratio      P-Value
------------------------------------------------------------------------------
Model                   0.0719537      1    0.0719537       0.45       0.5174
Residual                  1.59805     10     0.159805
------------------------------------------------------------------------------
Total (Corr.)                1.67     11

Correlation Coefficient = -0.207572
R-squared = 4.30861 percent
```

Comment: *As you can see from the ANOVA table, the regression is highly nonsignificant. Thus, there is no point in showing the regression plot. The nonsignificance of a regression means that the slope coefficient does not differ from zero. In this particular case, it means that the N amount was approximately constant during the 1400 days of incubation (there was no net release or accumulation of nitrogen). This also explains the increase in concentration during the decomposition because as much as 67% of organic matter has been mineralized.*

Exercise IX: Increase rate in litter N concentration

Refer to the discussion in chapter 5 about N concentration increase rate (NCIR). We use the linearity in the relationship between the accumulated litter mass loss and N concentration. The use of this linear relationship may be a tool for your own use and for your own study. What this measure gives is the increase relative to the mass loss and time is ignored. See also Fig. IX.I.

We obtain a highly significant linear relationship:

$$\text{N concentration} = 3.219 + 0.1289 \times \text{Acc. ml.}$$

The standard error for the intercept is 0.839 and for the slope 0.0117.

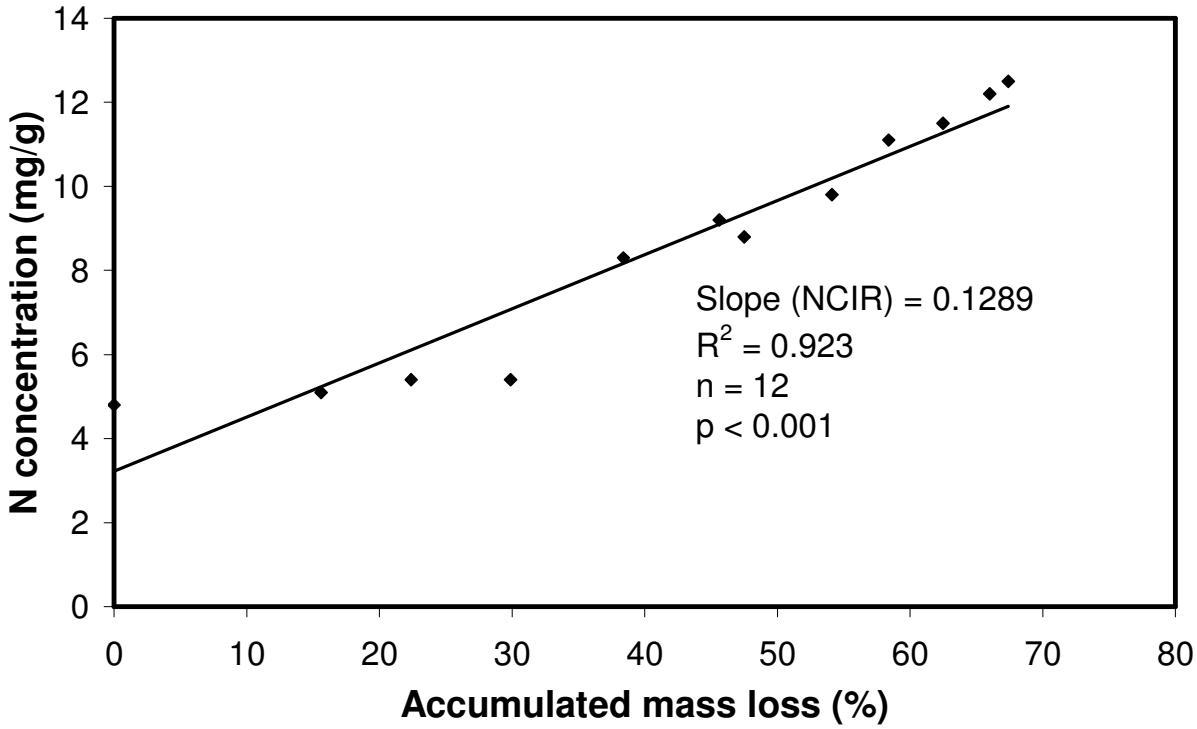


Fig. IX.I. The linear relationship between accumulated mass loss and litter N concentration.

Exercise X: Differences in nitrogen increase rates.

This is a typical regression analysis problem where two or more regression lines are to be compared. As described earlier in the book, the solution to this problem is a regression with 'dummy' (or indicator) variables. Many statistical packages offer either directly an options of comparing regression lines or automatic creation of dummy variables. If this is not the case, one can still easily perform the analysis by adding a dummy variable himself. In our example, the analysis requires to add just one column consisting of zeros and ones so that the data appear as follows:

Table X.II. Accumulated mass loss and N concentration in two decomposing litter types with an additionally created dummy variable necessary to compare two calculated regressions.

Mass loss (%)	*N (mg g^{-1})*	*litter type*	*dummy variable*
0.0	15.1	green	1
23.3	19.0	green	1
28.8	20.8	green	1
38	23.8	green	1
44.9	27.3	green	1
48.8	30.4	green	1
52.1	30.8	green	1
54.2	30.7	green	1
58	31.7	green	1
60.5	29.5	green	1
63.4	31.6	green	1
65.9	31.6	green	1
0	4.8	brown	0
15.6	5.1	brown	0
22.4	5.4	brown	0
29.9	5.4	brown	0
38.4	8.3	brown	0
45.6	9.2	brown	0
47.5	8.8	brown	0
54.1	9.8	brown	0
58.4	11.1	brown	0
62.5	11.5	brown	0
66	12.2	brown	0
67.4	12.5	brown	0

As you can see, the only purpose of the dummy variable (D) is to distinguish between the two types of litter. Now we can formulate the full model including the information about the litter type:

$$N = a1 + b1 \times MassLoss + a2 \times D + b2 \times D \times MassLoss$$

Analyze this model closely and you will see that for brown needles the models simplifies to

$N = a1 + b1 \times MassLoss$

because for brown needles $D = 0$ so both $a2 \times D$ and $b2 \times D \times MassLoss$ become also 0. Thus, the regression coefficients for brown needles are *a1* and *b1*. However, for green needles $D = 1$ so $a2 \times D$ and $b2 \times D \times MassLoss$ become meaningful (non-zero). If, e.g., the slope of the regressions for brown and green needles are the same then almost all of the variability will be explained by the first part of the model ($N = a1 + b1 \times MassLoss$) anyway and adding the term $b2 \times D \times MassLoss$ will not change the fit significantly – the *b2* term will be nonsignificant. Turning that reasoning around, if regression analysis results in significant *b2*, it means that the regressions do differ significantly in their slopes. By analogy, the significance of the *a2* term means significant difference in intercepts. Now let us have a look at the computer printout from such an analysis:

Comparison of Regression Lines - X_N versus X_AML by X_type

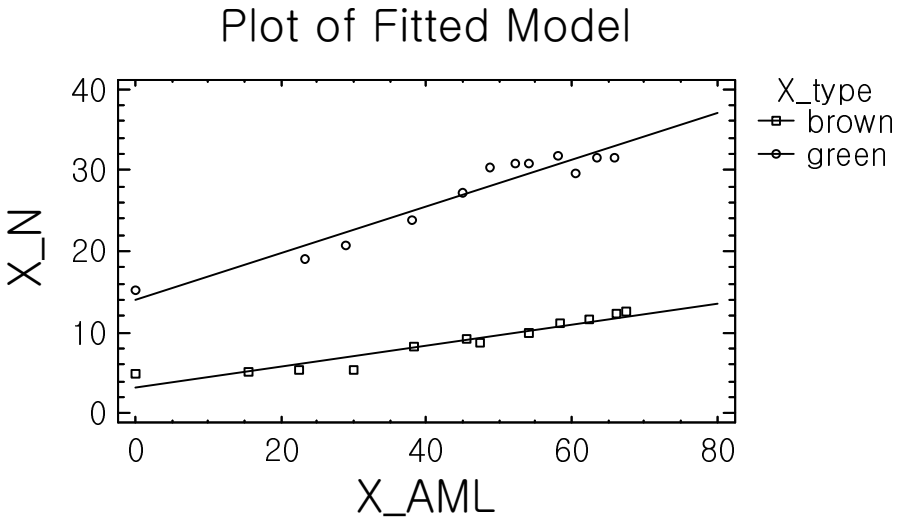
```
Dependent variable: X_N
Independent variable: X_AML
Level codes: X_type
```

Comment: *The variable names stand for: X_N – N concentration; X_AML – accumulated mass loss; X_type – litter type (this variable is automatically recoded to dummy variable).*

```
Number of complete cases: 24
Number of regression lines: 2

Multiple Regression Analysis
-----------------------------------------------------------------------------
                                        Standard          T
Parameter                 Estimate         Error       Statistic        P-Value
-----------------------------------------------------------------------------
CONSTANT                   3.21945       0.830358        3.87718         0.0009
X_AML                     0.128922      0.0176394        7.30877         0.0000
X_type=green               10.7991        1.26185        8.55816         0.0000
X_AML*X_type=green        0.157521      0.0263551        5.97686         0.0000
-----------------------------------------------------------------------------

                          Analysis of Variance
-------------------------------------------------------------------------------
Source             Sum of Squares     Df  Mean Square    F-Ratio      P-Value
-------------------------------------------------------------------------------
Model                      2408.4      3      802.799     505.58       0.0000
Residual                  31.7574     20      1.58787
-------------------------------------------------------------------------------
Total (Corr.)             2440.15     23

R-Squared = 98.6985 percent
R-Squared (adjusted for d.f.) = 98.5033 percent

The output shows the results of fitting a linear regression model
to describe the relationship between X_N, X_AML and X_type.  The
equation of the fitted model is

   X_N = 3.21945 + 0.128922*X_AML
      + 10.7991*(X_type=green)
      + 0.157521*X_AML*(X_type=green)

where the terms similar to X_type=green are indicator variables which
take the value 1 if true and 0 if false.  This corresponds to 2
```

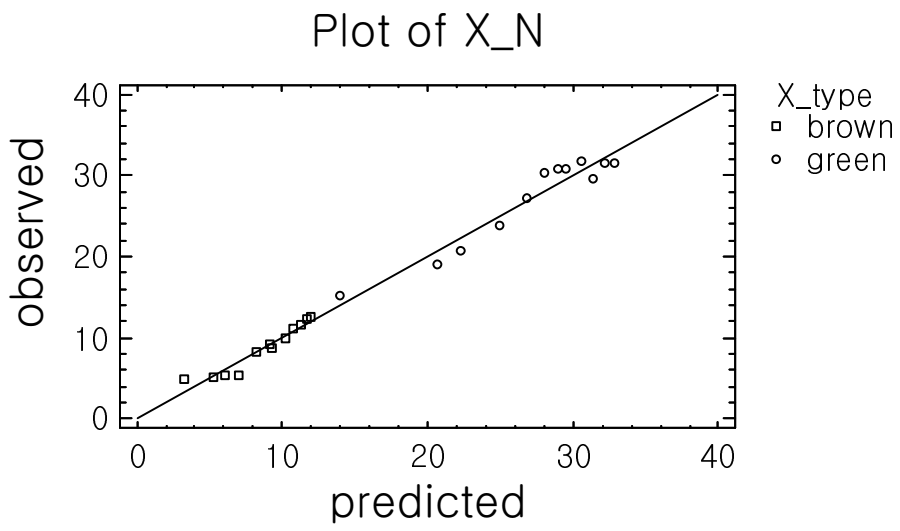
```
separate lines, one for each value of X_type.  For example, when
X_type=brown, the model reduces to

   X_N = 3.21945 + 0.128922*X_AML

When X_type=green, the model reduces to

   X_N = 14.0185 + 0.286443*X_AML

  Because the P-value in the ANOVA table is less than 0.01, there is a
statistically significant relationship between the variables at the
99% confidence level.
```

Comment: *As you can see, the regression is highly significant (cf. Analysis of Variance table) as are all the variables (Multiple Regression Analysis table). The latter table suggests also that both the intercepts and the slopes do differ significantly. However, we will still perform the formal test by checking the significance of the all variables (below) in the order they are fitted. The plot below shows the two regression lines fitted and, indeed, the two litter types appear quite different both in their initial N concentrations and in N increase rates.*




```
Further ANOVA for Variables in the Order Fitted
-------------------------------------------------------------------------------
Source              Sum of Squares     Df  Mean Square    F-Ratio      P-Value
-------------------------------------------------------------------------------
X_AML                      483.181      1      483.181     304.29       0.0000
Intercepts                 1868.49      1      1868.49    1176.73       0.0000
Slopes                     56.7232      1      56.7232      35.72       0.0000
-------------------------------------------------------------------------------
Model                       2408.4      3
```

```
   This table allows you to test the statistical significance of the
terms in the model.  Because the P-value for the slopes is less than
0.01, there are statistically significant differences among the slopes
for the various values of X_type at the 99% confidence level. Because
the P-value for the intercepts is less than 0.01, there are
statistically significant differences among the intercepts for the
various values of X_type at the 99% confidence level.
```



Comment: *The analysis is finished and now we can tell that: (1) in both litter types N concentration increases significantly with litter mass loss (model significant as indicated in the ANOVA table); (2) the litters differ in their initial N concentrations (significant difference in intercepts); (3) the litters differ in N concentration increase rates (significant difference in slopes); (4) the linear model fits the data well (no major trends in the "observed vs. predicted" plot).*

Exercise XI: Calculating the sequestered fraction of litter N

The basic information necessary to solve this problem is given in chapters 4 and 5. The recalcitrant part of the litter we find as the remains when the litter has decomposed to the limit value. So a first step would be to calculate the limit value and we obtained 88.5%. Please note that the estimated asymptote may vary slightly depending on the estimation procedure used. Here we used the Marquardt procedure (see the printout below).

In a next step we calculate the concentration of N at the limit value as described in chapter 5. We obtain the equation N = 0.1289×(mass loss) + 3.218.

We substitute mass loss for 88.5 as the limit value also is a value for accumulated mass loss and obtain an N concentration of 14.6 mg g^{-1}. That is the N concentration in the remaining amount which is 11% of the original amount.

If we imagine an initial amount of 1.0 gram with N concentration of 4.8 mg g^{-1} this means that in 1 gram there was 4.8 mg. The same amount has now decomposed and only 11% remain which means 0.11 grams which have an N concentration of 14.6 mg g^{-1}. Thus 0.11×14.6 mg g^{-1}, or 1.61 mg, which is the amount of N that remains in the litter. The fraction that remains is thus 1.61/4.8 or 0.335 which also can be written as 33.5% of the N initially present.

Step 1 – estimating the decomposition limit value (the asymptote)

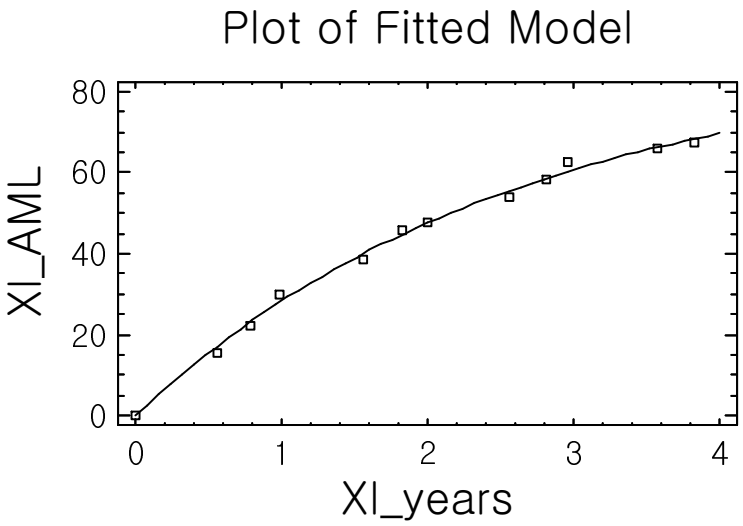
Nonlinear Regression - XI_AML

```
Dependent variable: XI_AML
Independent variables: XI_years
Function to be estimated: m*(1-exp((k*XI_years)/m))
Initial parameter estimates:
   m = 100.0
   k = -10.0

Estimation method: Marquardt
Estimation stopped due to convergence of residual sum of squares.
Number of iterations: 9
Number of function calls: 35

Estimation Results
------------------------------------------------------------------------------
                                                    Asymptotic 95.0%
                                        Asymptotic  Confidence Interval
Parameter              Estimate  Standard Error      Lower         Upper
------------------------------------------------------------------------------
m                       88.5262         3.67862    80.3297       96.7227
k                      -34.1105         1.08391   -36.5256      -31.6953
------------------------------------------------------------------------------

Analysis of Variance
-----------------------------------------------------
Source             Sum of Squares     Df  Mean Square
-----------------------------------------------------
Model                     26581.7      2      13290.8
Residual                  17.7024     10      1.77024
-----------------------------------------------------
Total                     26599.4     12
Total (Corr.)              5102.5     11

R-Squared = 99.6531 percent
```

R-Squared (adjusted for d.f.) = 99.6184 percent

The output shows the results of fitting a nonlinear regression model to describe the relationship between XI_AML and 1 independent variables. The equation of the fitted model is

88.5262*(1-exp((-34.1105*XI_years)/88.5262))



Exercise XII: Nitrogen stored in litter at the limit value

In the presentation of the problem you obtained the information about the limit values and thus about how much recalcitrant remains there are from each litter species. You also know the N concentration in these remains. We can apply here the same method as we used in exercise XI.

Our table (XII.II) thus has obtained two further columns, one giving N_{capac} as mg of N that is stored in the remains of originally 1.0 grams of litter. This is simply the amount of N given in milligrams per gram litter.

The last column gives the fraction as the remaining N/initial N, for example 0.68/4.0. By multiplying by 100 we obtain the percentage of N remaining, in the given example 17%.

As a final step – why not plot the calculated data in the two last columns, e.g. versus initial N concentration. What is your conclusion?

Table XII.II. The same data as in Table XII.I supplemented with two columns giving the calculated capacities of litters to store N (N_{capac}) and the percentage of initial N sequestered.

Litter type	*Initial N conc.* ($mg\ g^{-1}$)	*Limit value* (%)	*N conc. at limit value* ($mg\ g^{-1}$)	N_{capac} ($mg\ g^{-1}$)	Sequestered part of the N (%)
Lodgepole pine	4.0	94.9	13.6	0.68	17
Scots pine	4.2	81.3	12.76	2.39	57
Scots pine	4.8	89.0	14.7	1.62	34
Norway spruce	5.44	74.1	14.46	3.74	69
Silver birch	9.55	77.7	22.71	7.34	77
Common beech	11.9	59.1	24.05	9.84	83
Silver fir	12.85	51.5	21.93	10.86	85

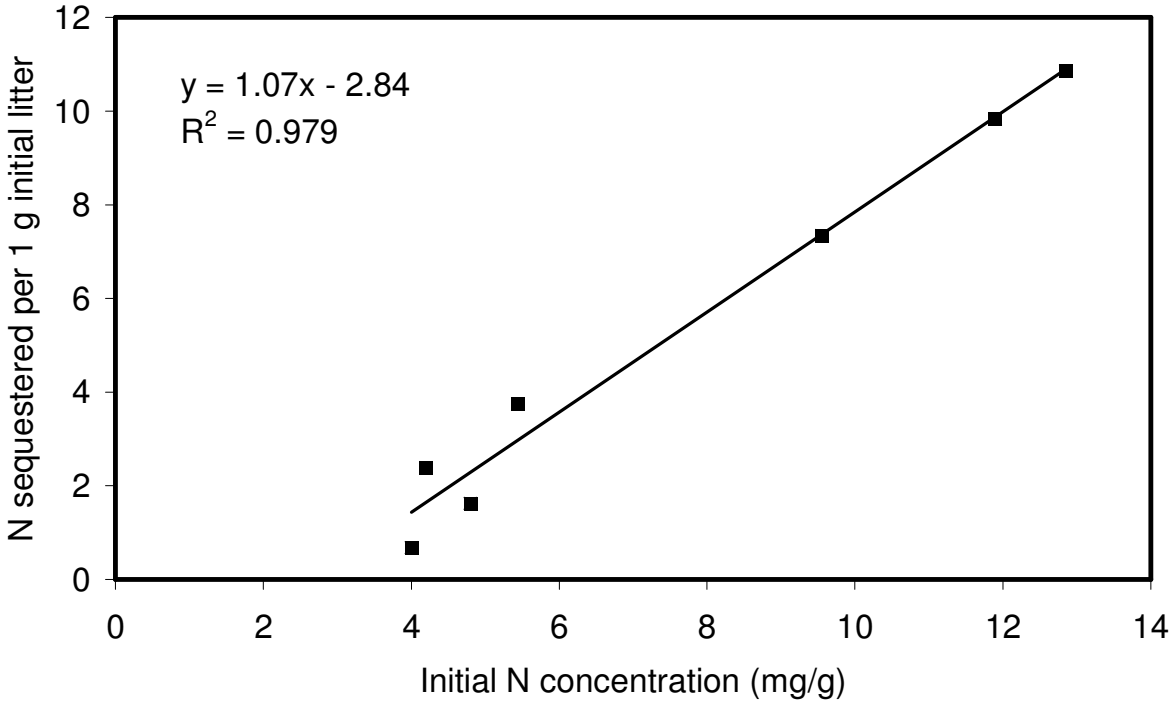


Figure XII.I. Sequestered N in litter that has decomposed to the limit value. The amount of N stored in different litter species is related to the initial litter concentration.