United Nations Economic Commission for Europe (UNECE)

Convention on Long-range Transboundary Air Pollution (CLRTAP)

International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests)

MANUAL

on

methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests

Part VII.2

Assessment of Epiphytic Lichen Diversity

Version 05/2016

Prepared by:

Silvia Stofer, Vicent Calatayud, Paolo Giordani, Pat Neville

Stofer S, Calatayud V, Giordani P, Neville P, 2016: Part VII.2: Assessment of Epiphytic Lichen diversity. In: UNECE ICP Forests Programme Co-ordinating Centre (ed.): Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Thünen Institute of Forest Ecosystems, Eberswalde, Germany, 13 p. + Annex [http://www.icp-forests.org/manual.htm]

ISBN: 978-3-86576-162-0

Programme Co-ordinating Centre of ICP Forests
Thünen Institute of Forest Ecosystems
Alfred-Möller-Str. 1, Haus 41/42
16225 Eberswalde
Germany

Email: pcc-icpforests@thuenen.de

Eberswalde, 2016

CONTENTS

1 Introduction

Lichens are an important component of the forest biodiversity and are considered to be among the most sensitive groups of organisms to several types of pollutants (Nimis et al., 2002). They have been used for defining critical levels and loads for different type of ecosystems under the Air Quality Directive and the Convention on Long-range Transboundary Air Pollution, and recently also for revising the critical loads for NH_3 in sensitive ecosystems (e.g. Geiser et al., 2010). Besides their use for evaluating effects in sensitive ecosystems, they can be used in other ecosystems as early-warning indicators since they are most likely the first species group to react. In the present chapter, the methods assessing epiphytic lichen diversity in forest ecosystems are developed.

2 Scope and application

This Part of the Manual aims at providing a consistent methodology to collect sound, harmonized and comparable epiphytic lichen diversity data at selected ICP Forests monitoring plots. Harmonization of procedures is essential to enhance comparability of forest epiphytic lichen data. To have their data used in the international database and evaluations, National Focal Centres and their scientific partners participating in the ICP Forests programme are requested to follow the methods described here.

An overview on assessed variables is given in Table VII.2-1.

Table VII.2-1: Variables assessed within the epiphytic lichen survey

Variable	Level II	Level II core	Level I
Longitude plot centre	o	o	o
Latitude plot centre	o	o	o
Azimuth of sampled tree from plot centre	o	o	o
Distance of sampled tree from plot centre	o	o	o
Tree bark-type group	o	o	o
Aspect of sampling grid	o	o	o
Species codes for lichen species in the sampling area	o	o	o
Frequency of each lichen species in each grid cell	o	o	o
Species codes for lichen species occurring in the complete subplot	o	o	o

o - optional

3 Objectives

The main objective of the epiphytic lichen assessment is to monitor epiphytic lichen diversity and its changes on monitoring plots and to contribute to the understanding and evaluation of forest condition in Europe.

Specific objectives are defined as follows:

- to assess the epiphytic lichen diversity (species richness, abundance and composition) on plots
- to detect temporal changes in lichen diversity (species richness, abundance and composition) on plots
- to relate changing influences of natural and anthropogenic environmental factors (e.g. stand structure, tree composition, depositions) to epiphytic lichen diversity (species richness and frequencies)

4 Location and measurements of sampling

4.1 Sampling design

Assessment of epiphytic lichen diversity is carried out on twelve randomly selected living trees, growing on Level I plots or Level II plots, respectively.

4.1.1 Location, size and shape of subplots

A subplot with a fixed area of 0.25 ha has to be established for the lichen diversity assessment. It must be part of the plot and must be representative for the whole Level I or Level II plot in order to allow comparisons between epiphytic lichen vegetation changes and other parameters recorded on the same plot in future surveys.

Level I plots: The subplot is a circle with a 28.2 m radius (area= 0.25 ha).

Level II plots: The subplot can be a quadrate, a rectangle or circle with an area = 0.25 ha.

4.1.2 Marking the sampling subplot

A permanent marking system has to be installed for the sampling subplot. The precise method to be used is under the responsibility of each country (boundary markers, buried metal stakes, etc.) but it is important that the markers do not affect the lichen vegetation. The markers should be invisible. The presence of markers needs to be checked regularly. It is recommended that maps should be used for recording the spatial arrangement and exact locations of the sampling units. Such maps should use the most permanent system of coordinates possible.

Level I plots: A permanent, but preferably invisible marking of the subplot centre has to be installed.

Level II plots: If the subplot shape is a circle it is recommend to mark the centre. In case of quadrate or rectangular plots it is proposed to mark the four corners.

4.1.3 Pre-stratification of trees

Tree species with different bark types (acidic or neutral, Annex 1) can host different lichen communities. This factor is likely to affect overall species richness of the subplot, and should be taken into account, if possible, for tree selection. Pre-stratification of the trees based on bark-type is possible only on Level II plots, as the species composition of the trees of the subplot is known prior

to the assessment of lichen diversity. This information, however, is not available for the reference area of 0.25 ha chosen for lichen assessment at Level I plots.

Level I plots: Pre-stratification of trees based on bark type is not applied.

Level II plots: Pre-stratification based in bark type is applied as follows: of the trees with dbh ≥13 cm (perimeter > 40 cm) present in the subplot, classify the tree species in two groups, with acidic bark (group A), and with neutral bark (group B). To help with this classification and to avoid inhomogeneous interpretations, a common list of tree species and their corresponding types of bark is provided in Annex 1. In case that there are less than 12 trees with a dbh ≥ 13, pre-stratification is not necessary as all trees will be selected.

4.1.4 Random sampling of trees

4.1.4.1 Level I plots

Random selection of the trees is carried out in the field as follows:

1 Twelve collecting points are located in the plot using a radial scheme (Figure 1 as an example). Each collecting point is defined by an azimuth and a distance from the centre (Table 2). The radial scheme (with 12 radii, with an angle of 30º, starting from azimuth 0, i.e., azimuths 0, 30, 60….360) is common for all Level I plots. But the distances from the centre along each of the 12 radii are randomly selected for each Level I plot. Collecting points are numbered clockwise, starting in the north (azimuth = 0) from 1 to 12.

2 From each collecting point, select the nearest tree with a dbh ≥ 13 cm. Note that the tree must be located in the plot and that a tree can only be investigated once. If there are less than 12 trees suitable for selection on the plot (e.g. plot damaged by a windstorm), note the number of missing trees.

3 Complementary trees: If after selection of these 12 trees they are distributed in the two bark-type groups, and one of the bark-type groups is represented by less than 3 trees, randomly select additional trees of this bark-type group in order to have a minimum of 3 selected trees per bark-type group. Report these trees as “complementary tree” in the forms. Lichen diversity on these trees will be considered as additional information for complementary analyses.

4 For the selected trees, species code and location in the plot (azimuth and distance from centre) have to be recorded.

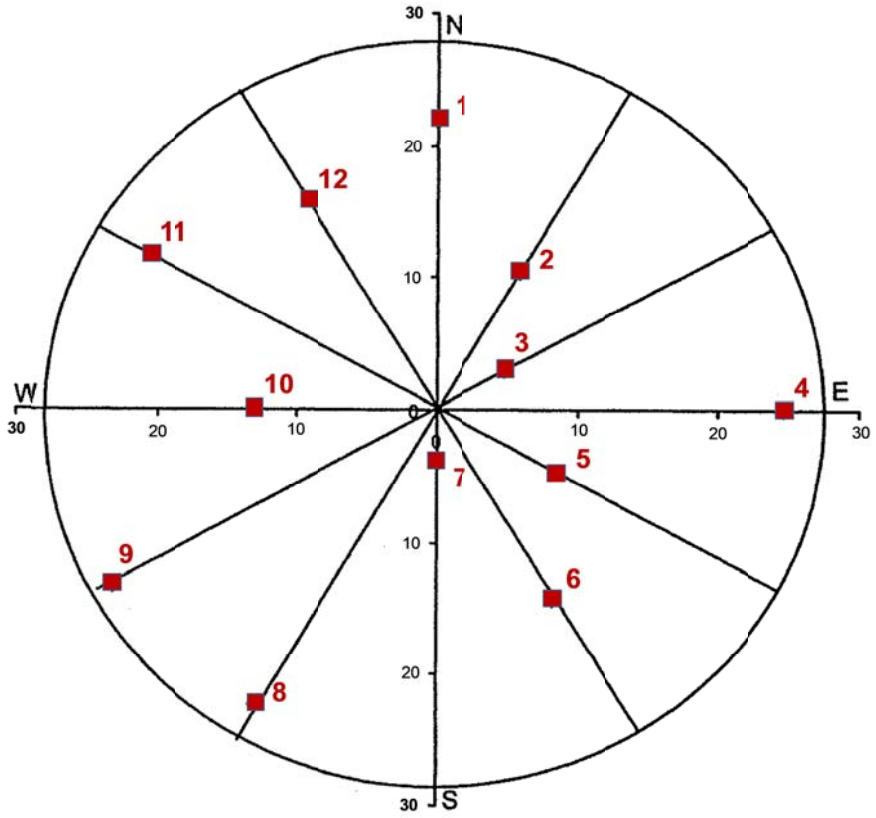

Figure VII.2-1: Example of a scheme for epiphytic lichen assessment (0.25 ha) in a Level I plot. The 12 collecting points are highlighted in red.

Table VII.2-2: Number, azimuth and distance from the centre of the 12 collecting points. The azimuths are fixed but the distance from the centre has to be randomly selected for each Level I plot.

Collecting point no.	Azimuth [360°]	Distance from centre [m]
1	0	22
2	30	12
3	60	6
4	90	25
5	120	10
6	150	17
7	180	4
8	210	26
9	240	27
10	270	13
11	300	24
12	330	18

4.1.4.2 Level II plots

Pre-stratification for each intensive plot is carried out by means of the tree information available from the existing data base at Level II. Disregarding trees with dbh<13 cm, all trees on the sampling subplot are classified into two groups: one with acidic bark (group A), the other with more or less neutral bark (group B) (Annex 1).

Subsequently, 12 sampling trees are selected randomly with the proportion of trees in the two groups calculated as follows:

p1 = (N trees in Group A) / N trees

p2 = (N trees in Group B) / N trees

with: N trees = number of trees on the sampling subplot

p1 + p2 = 1.0

For each of the two pre-stratified groups a proportional number of trees is then randomly selected on the sampling subplot according to the following formula:

Number of sampling trees in Group A = 12 * p1

Number of sampling trees in Group B = 12 * p2

With the total number of sampling trees = 12.

Complementary trees: if in one of the two bark-type groups, there are less than 3 trees on the subplot, randomly select new trees in order to have at least 3 trees per group. Report these trees as "complementary tree" in the forms. Lichen diversity on these trees will be considered as additional information for complementary analyses.

4.1.5 Sample trees

Only standard trees should be selected for the lichen survey. A standard tree is defined as follows:

- it has a dbh ≥ 13 cm (at 130 cm from the ground level. See Figure VII.2-2 for details on how to measure).
- the area of the trunk that is unsuitable for recording (damages, decortication, branches, knots and/or other epiphytes or climbing plants such as ivy, preventing growth of lichens) within each of the 4 grids is < 20%.

Note that if the four grids cannot be adequately positioned on the trunk the tree cannot be selected and it has to be replaced by another one as specified in section 4.1.4.

Stems that have clear separation below 100 cm above the ground should be considered separate trees.

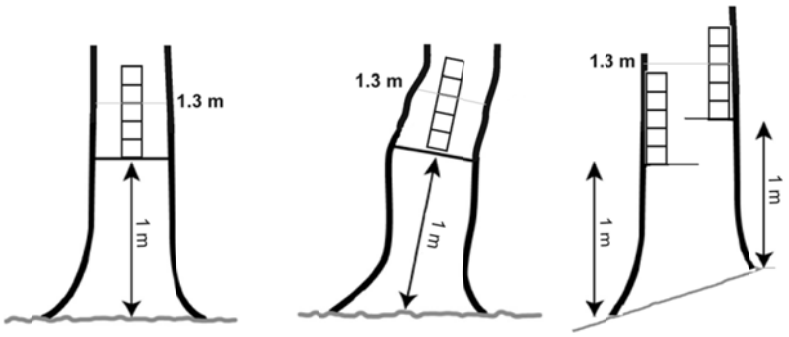

Figure 2: Procedures for measurements of height from the ground level for positioning the lower edge of the grid (1 m) and for measuring the circumference and the inclination of the trunk (1.3 m) (modified from Dobbertin and Neumann, 2010)

4.1.6 Tree relevés

Epiphytic lichen diversity is sampled on selected trees by means of a 10 × 50 cm grid (Fig. 3), subdivided into five 10 × 10 cm quadrates, to be attached to the trunk of sample trees. The grid has to be flexible enough to be easily placed on the bole but also resistant, so as to prevent alteration in the shape and in the dimensions.

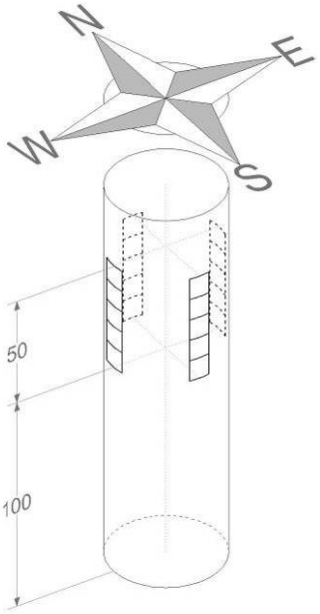

Figure 3: Observation grids attached at four aspects along the trunk

The observation grid is attached along the trunk so that its lower edge is always 1 m above the base of the trunk (see Fig. 3). The grid must be placed at four different aspects of the trunk (NESW). It is possible to relocate each monitoring grid by a maximum shift of 20° (firstly in a clockwise, then in counter clockwise direction), to avoid parts of the trunk which are not suitable for sampling (e.g. wounds, knots, etc). Even if a high lichen cover is present, a grid must be shifted if the overall coverage of disturbances (namely damages, decortications, branches, knots and/or other epiphytes or climbing plants such as ivy, preventing growth of lichens) is higher than 20% (however, muscicolous lichens must be included in the list of species).

At each aspect, all five quadrates of the grid are examined for lichen species. The occurrence of each species within each quadrate is recorded, and the frequency of each species in the grid is calculated (by summing up the quadrates in which the species are found, ranging from 0 to 5) and documented.

The list must include all species. However, a few small crustose lichens are particularly difficult to identify and/or are easily overlooked. Where the identification of certain thalli is difficult both in the field and/or in the laboratory, it is advisable to include them in the calculation of diversity as "Sp. nr. x", having confirmed that they are not damaged or poorly developed forms of species already occurring in the monitoring grid. No lichens should be removed from within the observation grid as future surveys and/or quality control procedures are planned. Where species identification requires laboratory investigation equivalent specimens should be removed from the trunk area outside of the quadrate. If this is not possible a small fragment can be removed from the specimen within the quadrate without causing harm to the thalli.

4.1.7 Additional survey of species outside the sampling units

An additional list of all species of the plot not encountered within the sampling units can be compiled. Such a list will help to interpret future vegetation changes within the sampling units and will be of additional interest regarding the biodiversity. The species list will be submitted separately using the data submission forms. Countries may wish to optionally record additional lichen species occurring on the sample tree but outside of the sampling area. Where recorded, these species should have their location on the tree noted; e.g. on stem below sampling grid on stem above sampling grid, on branches, on twigs.

4.1.8 Compatibility with other surveys

In all cases, the exact location of the lichen assessment plot has to be determined in that way that any conflict with other assessments will be avoided. During the installation of plots, trees already used for other intensive studies should not be taken into account. Adequate information (map, signs) must be provided to all survey teams in order to limit damage on trees within the plot.

4.2 Sampling equipment

Each crew will bring in the field a plan with the position of the trees, appropriate standard forms, markers for the trees (if needed), a standard sampling grid, a tape measure, a compass, a hand lens, bags and tools for collecting samples, and any material important for the identification of the species. For Level I plots, a GPS will also be needed.

4.3 Frequency of sampling

Lichen diversity studies on Level II plots should be undertaken ideally at least every five years. For transnational surveys a new random sampling of the trees has to be applied in order to reflect possible changes in tree composition.

In national assessments, repetitions of lichen diversity in shorter intervals on the same trees (without new random sampling) may be useful to detect small shifts in species composition and frequency.

Heavy disturbances caused by management operations (e.g., thinning or clear cutting operations) or natural events (e.g. storm damage or disease outbreak) preceding or occurring during the sampling year should be recorded.

4.4 Sample collection, transport and storage

Specimens should be collected only when necessary for proper identification, and if not rare within the sampling units. Collection will be carried outside the area of assessment and preferably outside the plot. Specimens will be stored in an official herbarium.

5 Measurements

5.1 Parameters to be assessed and reporting units

5.1.1 Selected variables

The variables considered for epiphytic lichen assessment and their reporting units at ICP Forests monitoring sites of different levels are listed in Table VII.2-3.

Table 3: Variables and reporting units assessed within the epiphytic lichen survey

Variable	Report unit
Longitude plot centre	ddmmss
Latitude plot centre	ddmmss
Azimuth of sampled tree from plot centre	Degree º
Distance of sampled tree from plot centre	m
Tree bark-type group	Classes
Aspect of sampling grid (NESW)	Code
Species codes for lichen species occurring in the sampling area	Code
Frequency of each lichen species in each grid aspect	Nr (1-5)
Species codes for lichen species occurring in the grid	Code
Species codes for lichen species occurring in the complete subplot	Code

5.1.2 Information on assessed trees

For each assessed tree, the bark-type group, azimuth and distance from the plot centre have to be reported. The moss cover inside the grid has also to be reported. Additional information on factors affecting lichen diversity (e.g., isolated tree) or disturbances shall be reported under ”other observations”.

5.1.3 Species studied

Epiphytic lichens present within the sampling area covered by the grid have to be assessed. Optionally, a list of all sampled epiphytic lichen species in the plot can be compiled.

The nomenclature of the lichen species and codes should follow a list of available species prepared and updated by the Expert Panel. Regular updates of the coded list will be necessary, as growing taxonomic knowledge may also require some updates. The countries may prepare lists of species proposed for update and report them to the Programme Coordination Centre for further evaluation. In such a list, apart from species names, also citations of the relevant taxonomic references must be included, e.g. national floras, or scientific papers dealing with these species. An update of the code list will be done by the Programme Co-ordination Centre after consultation and in co-operation with the Expert Panel and occasionally with additional taxonomic experts. To make discussion of problematic cases easier, the countries may indicate into their proposal some national taxonomic experts to be consulted. As the procedure for an update of the code list needs some time, a list of proposals for update should be reported by a country not later than eight weeks before end of deadline for data submission.

Unidentified species should be submitted using the data submission forms with the code "999.999". For different unidentified species in each plot, use the codes 999.001, 999.002, etc. These codes are considered on a plot base (i.e., for example 999.001 reported for two different plots represent different species). Specimens of these species should be sampled and stored in a herbarium for subsequent identification.

5.1.4 Measurement of species abundance

For each lichen species, count the number of unit areas (10×10cm) of the sampling grid in which the lichen species occurs (values ranging from 1 to 5, for four aspects of the grids, max 20 counts per tree).

5.2 Quality Assurance and Quality Control

National Focal Centres (NFCs) are responsible for Quality Assurance and Quality Control procedures. All steps in the procedures should be described in a national quality assurance program.

At the international level, training, intercalibration, and intercomparison courses, as well as the ICP Forests Manual should be organized at the European level in close connection with the Expert Panel on Forest Biodiversity.

Important points of QA/QC in epiphytic lichen assessments include: compilation of a national field manual, repeated national training and calibration of field teams, plausibility checks of data, independent field controls and checks and participation to international cross-calibration courses.

5.2.1 Plausibility limits

For each tree and lichen species, the frequency value at tree level must be between 1 and 20.

5.2.2 Data completeness

Typically 12 trees per plot have to be assessed (up to 14 with complementary trees), but data will be considered complete with less trees in case that there are less than 12 trees available for selection in the subplot. This case has to be reported in the forms.

5.2.3 Data quality objectives

Data quality will be improved by means of intercalibration exercises. The data quality objectives are outlined in Table 4.

Table VII.2-4: Data quality objectives (DQO) for the assessment of lichen diversity.

Type of exercise	Variable	Data Quality Objectives
Intercalibration	Total number of species per plot	Control ± 20%
Intercalibration	Mean number of species per tree	Control ± 20%
Intercalibration	Mean sum of lichen frequency per tree	Control ± 20%
Intercalibration	Total number of macrolichen species per plot	Control ± 20%
Intercalibration	Mean number of macrolichen species per tree	Control ± 20%
Intercalibration	Mean sum of macrolichen frequency per tree	Control ± 20%

5.2.4 Data quality limits

The data are considered of sufficient quality when the following criteria are met (Table VII.2-5):

Table VII.2-5: Data quality limits (DQL) for the assessment of lichen diversity

Type of exercise	Variable	Data Quality Limits
Intercalibration	Total number of species per plot	≥80% of the teams fulfil the DQO
Intercalibration	Mean number of species per tree	≥80% of the teams fulfil the DQO
Intercalibration	Mean sum of lichen frequency per tree	≥80% of the teams fulfil the DQO
Intercalibration	Total number of macrolichen species per plot	≥80% of the teams fulfil the DQO
Intercalibration	Mean number of macrolichen species per tree	≥80% of the teams fulfil the DQO
Intercalibration	Mean sum of macrolichen frequency per tree	≥80% of the teams fulfil the DQO

6 Data handling

6.1 Data submission procedures and forms

All data must be sent to the relevant National Focal Centre after each sampling year. Each Focal Centre is in charge of data validation. The data should be submitted in standard format, following the data submission forms of this manual.

The submission of data to the Programme Co-ordinating Centre of ICP Forests must follow the coded lists for epiphytic lichens available through the Expert Panel on Biodiversity. Species not included in the list should be reported to the Expert Panel for further evaluation, following procedures outlined in Chapter 5.1.2. Data are to be per tree submitted for each grid aspect.

6.2 Data validation

Data checks should be done as soon as results from the analyses are available. Data validation and quality assurance should be applied in accordance with the guidelines for QA/QC procedures as depicted in Chapter 5.2.

6.3 Transmission to co-ordinating centres

All validated data should be sent annually to the European central data storage facility at the ICP Forests Programme Coordinating Centre. Detailed time scheduled is provided by the relevant bodies.

6.4 Data processing guidelines

Results will be presented in form of total number of species per plot, mean number of species per tree, and mean Lichen Diversity Value (LDV) per tree. Analyses will consider both all lichens and only macrolichens. The LDV of a tree is calculated as the sum of the frequencies of all the lichen species present in the tree. The frequency of a species per tree is the sum of the number of squares in which a given lichen species is present.

6.5 Data reporting

All validated data should be sent to the National Focal Centre and submitted annually to the transnational central data storage.

7 References

Nimis P.L., Scheidegger C. and Wolseley P.A. 2002. Monitoring with lichens : monitoring lichens. Kluwer Academic published in association with the NATO Scientific Affairs Division, Dordrecht ; London.

Geiser L.H., Jovan S.E., Glavich D.A. and Porter M.K. 2010 Lichen-based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA. Environmental Pollution, 158, 2412-2421.

Annex I – Minor changes after 2016

Date	Minor change to latest published version in 2016	Affected sections of this document