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MANUAL

on

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

Part XIII

Sampling and Analysis of Litterfall

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1. Introduction

Litterfall is a key parameter in the biogeochemical cycle linking the tree part to the water and soil part. Both the biomass of the litter and its chemical content (including heavy metals) are needed to quantify the annual return of elements and organic matter to the soil. Litter decomposition is a major pathway of nutrient fluxes and determines the organic matter input to forest soils and has a strong influence on forest productivity and soil nutrient status.

Effects of anthropogenic and natural factors, such as climate change, could influence both litterfall production and its seasonal progression. Processes like carbon cycling and carbon sequestration are closely related to stand leaf area index (LAI) and litterfall.

Changes in litterfall are responses to disturbances caused by biotic factors such as insect pests and/or environmental factors like spring frost, drought, wind, or pollution. Litterfall production is a quantitative parameter of stand vitality and gives additional information to the visual assessment of canopy condition already observed in each plot. Direct observation of abnormalities of the leaves can be performed on the collected litter (leaf size, fungi, and necrosis) for symptomatology.

Litterfall can also provide temporal and quantitative information about phenological development of the stand. The quantification of the foliage amount, flowering and fruiting patterns allows direct measurements of year-to-year variation in phenology as a reaction to short term weather patterns, long term climate, and tree vitality.

Litterfall area of leaves is also one of the components of direct estimate of LAI, the stand leaf area per ground area expressed in $m^2 m^{-2}$. LAI describes a fundamental property of the plant canopy in its interaction with the atmosphere, especially radiation, energy, momentum and gas exchange (Monteith and Unsworth, 1990). LAI plays a key role in the interception of radiation, canopy interception (rainfall and deposition), in the carbon assimilation and water evapotranspiration during the diurnal and seasonal cycles, and in the pathways and rates of biogeochemical cycling within the canopy-soil system (Bonan, 1995; Van Cleve et al., 1983, Vesterdal et al.,2008). Finally, various soil-vegetation-atmosphere models use LAI (Sellers et al., 1986; and Bonan, 1993). For evergreen species the annual litter represents the turn-over of needle/leaf area. For deciduous species, litterfall collection throughout one year and sorting among species is probably the most accurate way of measuring total LAI, and of calculating the contribution of each species to the total (e.g. Breda, 2003). This measure can be used as an alternative to mid season instrument based methods of LAI estimation within the plot (e. Thimonier et al., 2009), or as an annual addition to a base line obtained by selected destructive harvesting of trees around the plot. LAI for one species is not simply related to its density or basal area contribution to the stand and cannot be derived from dendrometrical stand information alone.

2. Scope and application

This part of the Manual aims to provide sufficient methodological advice to allow participating National Focal Centres to sample and prepare an accurate measurement of the quantity and quality of litterfall, from selected plots of the ICP Forests intensive monitoring system. Harmonization of procedures of collection and chemical analysis is essential to ensure comparability of the chemical composition of litterfall, and accurate assessment of LAI. Only data obtained by the methodologies described in this chapter will be accepted for submission into the international database of the ICP Forests programme.

An overview on the variables assessed in the litterfall survey is given in Table 1. Litterfall chemistry is optional on standard Level II plots but mandatory on Level II core plots.

Table 1: Status of variables for measurements at various levels

Form	Variable	Level I	Level II	Level II core
Biomass measures				
LFM	Dry weight per m^2 [kg/m^2] for total litter biomass	n	o	m
LFM	Dry weight per m^2 [kg/m^2] for foliar litter biomass	n	o	m
LFM	Dry weight per m^2 [kg/m^2] for other litter biomass	n	o	m
LFM	Dry mass of 100 leaves or of 1000 needles [g]	n	o	o
LFM	Area of 100 leaves or of 1000 needles [m^2]	n	o	o
Chemical analyses				
LFM	C [g/100g]	n	o	m
LFM	N [mg/g]	n	o	m
LFM	S [mg/g]	n	o	m
LFM	P [mg/g]	n	o	m
LFM	Ca [mg/g]	n	o	m
LFM	Mg [mg/g]	n	o	m
LFM	K [mg/g]	n	o	m
LFO	Zn [µg/g]	n	o	o
LFO	Mn [µg/g]	n	o	o
LFO	Fe [µg/g]	n	o	o
LFO	Cu [µg/g]	n	o	o
LFO	Pb [µg/g]	n	o	o
LFO	B [µg/g]	n	o	o
LFO	Cd [ng/g]	n	o	o

o - optional m - mandatory n – not assessed

Litterfall sampling is strongly recommended on Level II sites where meteorology data is available.

3. Objectives

The main objectives of litterfall sampling and analysis are to quantify litterfall production and its chemical composition over time. This will enable:

Quantification of litterfall amounts at any one plot, to be expressed in g m^{-2} (or kg ha^{-1}).

The option to assess the local seasonal variation of litterfall components at any one forest plot, and between plots of different species. (N.B. Annual totals only need to be reported)

Accurate measurement of litterfall chemical quality, to be prepared from oven dried and **bulked annual** samples, or the **means** of periodic analysis, and expressed as concentrations of specific elements.

Measurement of specific leaf area of deciduous species on each 'core' plot of the intensive monitoring network in each year, allowing a direct assessment of LAI in m^2 m^{-2} as an alternative to field based methods.

Evaluation of the data will then allow for

Comparisons of litterfall quantity variation across latitudinal and longitudinal gradients by species

Investigation of relationships with insect vectors, weather phenomena, soil changes and climate variation by inter-plot comparisons

Greater understanding of the role of litterfall in nutrient cycling, across gradients of temperature, soil moisture and soil type, and in particular to improve knowledge of the N, P and C cycles.

Accurate estimates of the effects of year on year variation of leaf area for use with assessments of water budgets on forest plots with differing soils across a variation of climate types.

4. Sampling requirements and field systems

Litterfall sorting is time-consuming and hence an expensive analysis. Within the ICP Forests monitoring system, fine sorting of the fractions is mandatory only on Level II core plots where meteorology, soil water, soil solution, and phenology are also performed (see Table 2). On standard Level II plots litterfall collection is optional. When it is carried out on Level II plots, at least a less detailed level of sorting to determine foliar and non-foliar litterfall mass is recommended. Plot data should be recorded and submitted on Form *.LFP (see ICP Forests Manual Part XVII Data handling and data submission forms)

4.1 Field sampling design

4.1.1 Number of replicates

It is recommended to sample litterfall from at least 10 collectors per plot under uniform forest canopy, but up to 20 or 30 collectors under mixed species or in larger plots with uneven topography. Leaves from deciduous trees are more susceptible to turbulent air movement than

conifer needles. This effect may be mitigated either by increasing the number of litterfall traps (e.g. 10 traps for coniferous species and 20 traps for deciduous species) or by increasing the collecting area of each trap (especially for species with large leaves e.g. *Populus*).

4.1.2 Sampling scheme

As litterfall is a canopy parameter, and not a tree one, litterfall traps should be distributed all over the plot area. It is recommended that the traps are set up in a design enabling comparisons with deposition and soil water results. The traps are fixed and may be placed randomly or systematically e.g. at regular intervals and in sufficient number to represent the whole plot and not only the dominant tree species.

4.2 Sampling equipment

The countries are free to select the type of traps for the monitoring of litterfall. Figure 1 gives examples of two litterfall trap designs.

It is recommended that the litterfall traps are not fixed too close to the ground, to ensure adequate water drainage. The opening area of the collectors must be horizontal, and if necessary, special trap fixation should be prepared for mountainous plots. A top height between 1.0 and 1.3 m should ensure that there is clearance from the ground on the up-slope side, whilst still allowing capture of leaves from shrub vegetation. Canopy leaves and other litterfall inputs can be collected in nets or litter bags which are attached to a frame of durable material, with a known catching area (minimum 0.18 m^2 but preferably over 0.25 m^2).The total sampling area must be sufficiently large to be able to determine litter amount and quality. There may be a need to trim tall ground vegetation from just beneath the trap itself, to avoid interference with the nets/bags, which is acceptable as long as the trap position is not within the ecological survey area. For tree species with very large individual leaves e.g. *Populus,* the collecting area of individual traps must be increased (i.e. up to 0.5 m^2).

Mesh trap

Solid funnel with bag

Figure 1: Potential collector design

It is recommended that the litter bags or collecting funnels are at least 0.5 m deep to prevent litter from blowing out of the traps. Deposition of litter into these traps due to lateral movements by wind is assumed to be minimal. The material of the mesh must not interact with the litterfall sample. Litter nets/bags of inert materials like cotton, polyethylene or nylon are suitable materials, not interfering with the major ions present in litter. However, natural materials like fine cotton stitching will decay quickly on site under sustained high temperature and moisture levels. The mesh size of the bags must be large enough to allow for easy drainage of water. It is recommended to adapt mesh size to the dimension of smallest elements, i.e. for needles from coniferous species up to 0.5 mm, but if there is interest in the finest 'frass' material (caterpillar droppings), then the texture needs to be much smaller. During the winter season in areas of heavy snowfall, traps may lowered on to the ground to avoid breakage of the collector structures, preferably on to a plastic mesh sheet to avoid direct contact with the soil.

4.3 Frequency of sampling

It is recommended that litterfall be collected at least monthly and even bi-weekly in periods of heavy fall, which may be co-incident with heavy rainfall. This is to avoid pre-collection decomposition in the traps and chemical leaching of the material during rain episodes. It is particularly vital to obtaining true weights of the fine flower and bud components in spring, which very quickly become compressed and unidentifiable. The samples may be pooled to periodic or annual totals – the litterfall year for reporting purposes should run from spring to spring i.e. beginning of April (yr 1) to the end of March (yr 2). In regions with snow in the winter or which are remote, it may be impossible to collect samples at regular intervals. Litterfall may then be collected once before the winter period and once after snowmelt, as frost will limit both drainage and litter decomposition. Total values for this period should then be subdivided proportionally to the months passed since the last collection.

4.4 Sample collection, transport and storage – quality control in the field

The collection bags must be carefully labelled with site number, trap number and date before removing them from the site. It is recommended that a record sheet is taken to the field at each bag change to record any unusual conditions or missing samples, and that this should be sent in each time with the bags and be stored in suitable files in the analysing laboratory. If collection is made from fixed nets by hand then powder-free vinyl gloves should be worn to lessen sample contamination ahead of chemical analysis. Alternatively, suspended bags may be replaced at each visit, and possibly cleaned and re-used.

Ideally all samples should be transferred immediately to the laboratory, preferably in cool boxes, or if necessary temporarily stored at 4°C, but not frozen.

5. Laboratory measurements

5.1 Variables to be assessed

The variables of interest concern quantity (mass measurements) and chemical quality of litter, and the possibility to measure specific leaf area (SLA) values from the foliar fraction. In standard Level II plots the litterfall survey is optional, but at least litterfall quantity is recommended, along with

measures of dry mass (Table 1), but in the Level II designated 'core' plots chemical analysis is requested of finer fractions (see section 5.3, Table 2).

A procedural flow diagram to facilitate assessment of all these variables is given in Figure 2.

Reception

Litter samples should be checked and counted into the lab on arrival, using non-contaminating gloves, and the paper work filed. This is a vital part of the quality control of samples from the field to the laboratory.

If the samples are damp, this may be an opportunity to measure leaf area for pines, which are particularly difficult when dry, as the longer needles tend to warp and twist. Incoming samples should then be kept damp, but cooled, and processed as soon as possible so that decay does not start. In all cases samples are easier to sort when dried, and could be left covered for several days in a warm, dry place to air dry – alternatively they may be oven dried at temperatures below 70^0C for at least 24 hours. Any insect life in the bags should be noted, and identified if in large numbers.

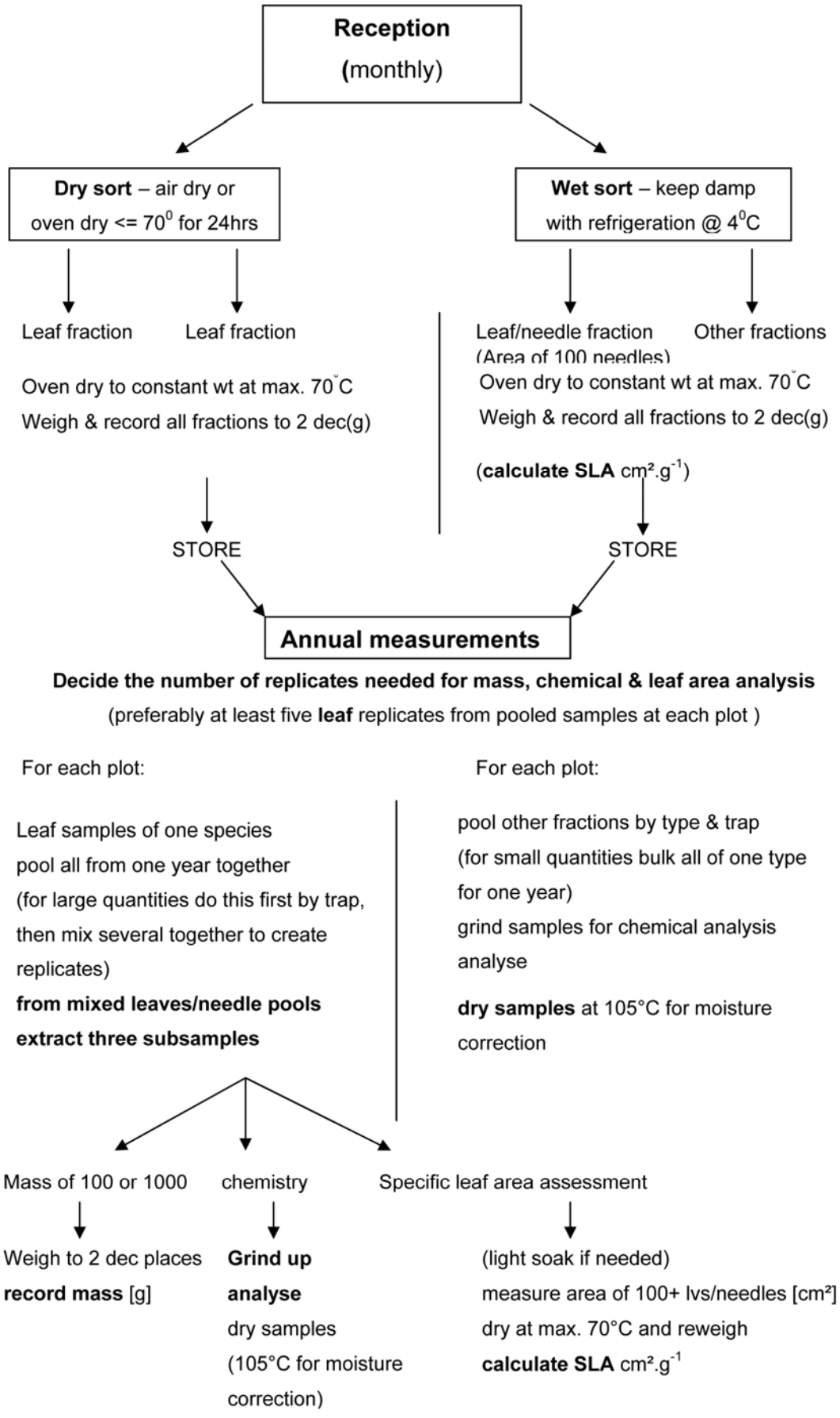

Figure 2: Procedural flow diagram for variable assessment

5.2 Litter sorting and quantity measurement

All litter sorting into fractions should be made wearing non-contaminatory gloves, both for personal safety and to allow chemical analysis afterwards. If the samples are dusty, a light weight face mask should also be worn during lab work. Paper bags can be used to contain the various fractions during oven drying at temperatures at maximum 70^0C.

Any litter collected from Level II plots should be sorted into at least foliar and non-foliar fractions for reporting purposes. If the plot has been designated as Level II core plot, then litter should be further separated into the fractions shown in Table 2.

Table 2: Fractionation of litterfall

Fraction of Litterfall	Level II	Level II core plot
Total litter biomass kg/m^2 (all species)	o	m
Foliar litter total (all species)	o	m
Foliar litter (main species)	o	m
Foliar litter (other tree species)	o	m
Non foliar litter total (all species)	o	m
Flowers total (including catkins)	o	o
Flowers (main species)	o	o
Flowers (other species)	o	o
Fruits/seeds total (all species)	o	m
Fruits/seeds total incl. green cones (main species)	o	m
Fruit capsules + empty cones (main species)	o	o (m*)
Rest of fruiting	o	o
Fruits /seeds total incl. green cones (other species)	o	m
Fruit capsules + empty cones (other tree species)	o	o
Bud scales	o	o
Wood fraction (Twigs <2 cm D/branches/bark)	o	o
Fines and frass (<1mm)	o	o
Other biomass (lichen, moss etc)	o	o

o = optional, m = mandatory

m* mandatory only for the main tree species = *Fagus sylvatica*

It is assumed that large branches >2 cm diameter, not often captured within the litter traps, will be recorded as part of the deadwood estimates of the plot, as taken during ecological surveys. The various fractions should be dried separately at maximum 70°C until constant weight is achieved (at least 24 hrs for fine fractions and leaves, but longer for substantial woody debris), and weighed to 2 decimal places (g). Annual totals will be reported on Form *.LFM, but there is also the facility to report mass/m^2 with other time periods as both start and end date are to be recorded in form .LFM. Storage may then be made until the annual total of material is accumulated (see flow chart Fig 2.).The monthly mass of the various fractions can then be totalised from April to March to achieve annual litterfall mass at the plot in kg m^{-2}, and submitted to the data centre on form*.LFM (Part XVII). Stored material may then be pooled at the end of the year, *well mixed* and subsamples taken for assessment of the weight of 100 leaves or 1000 needles (minimum requirement). Two further subsamples of the annual total can then be taken for chemical analysis (5.3) and more detailed specific leaf area (SLA) assessment if desired (see Chapter 5.4). In the case of foliar material from the main canopy species, it is recommended that a series of replicates should be prepared from the pooled total to allow some assessment of both the chemical and SLA variability of the material, although only the mean is required for reporting

purposes. However, *litter material present in only small quantities* at the end of the year, such as flowers (or bud scales), may be pooled across all the traps and chemically analysed as one total sample.

5.3 Quality of litterfall – chemical analysis

The chemical analysis of litter is similar to that of the foliar component. For techniques and analytical methods in more detail see Part XII of the ICP Forests Manual on Sampling and Analysis of Needles and Leaves. Analysis will be made on an **annual** sample of the various fractions, determined by pooling the monthly collection through the year (April – March).

For chemical analysis the litterfall samples are dried to constant weight in an oven at maximum 70°C, and samples are ground to a homogeneous powder in a suitable mill. For large twig fractions and tough seed cases and cones, this may mean a two-stage pre-treatment to achieve chipped material of a suitable size for laboratory grinding. All chemical element concentrations should be reported moisture corrected from dry ground material mass by drying subsamples to 105°C. For Quality control recommendations see section 6.

Reporting on annual chemistry of element concentrations should be made on Form *.LFM and *.LFO.

5.4 Specific Leaf area measurements for Leaf Area Index estimation

5.4.1 Introduction

The litterfall based method is an *optional* approach for leaf area index (LAI) estimation which has been frequently used in the past for broadleaf stands (Breda, 2003: Thimonier et al.,2009). The most suitable definition of LAI is half the total green leaf area (one-sided area for broad leaves) in the plant canopy per unit ground area (Chen and Black, 1992). The leaf area subtended by deciduous trees for each year can be computed from total leaf litter dry biomass of that species in that year (March-February) per m^2, multiplied by a ratio to convert dry weight to leaf area. This ratio of leaf area (A): dry mass (m) is named *Specific Leaf Area* and its alternative expression is as LMA (leaf mass per area):

$$SLA = A/m \text{ (cm}^2 \text{ per g.)} \qquad LMA = m/A \text{ (g per cm}^2\text{)}$$

Canopy leaf area (LAI) is the composite measure from all tree and tall shrub species in the plot and can only be obtained from litterfall if foliar SLA is determined for each of the component species. SLA can be measured leaf by leaf, as may be needed in photosynthesis or porometry research, or in bulk as an annual value smoothing out the variations of the individuals. However, this requires suitable laboratory equipment for accurate leaf area measurement, such as the Delta-T scanner or the Li-cor CI-203 laser area machine.

5.4.2 Methodology

SLA can be made on both fresh weight and dry weight bases, but the latter gives better standardisation between sites. It has to be determined *for each main canopy species* from a random subsample of litter leaves (at least100 leaves from different traps). Preferentially, several replicates form one years leaf litter total should be analysed to obtain a measure of the variability of the material from the site accruing through the year.

If litterfall leaves are dry, either naturally following abscission, or through storage or oven treatment, they will be more fragile than green leaves. If they are taken wet, or have been some time in the litter trap, they are likely to be dirtier than freshly fallen leaves, and may need to be cleaned and flattened before leaf area measurement. The option to retain or cut off petioles may

not be present in litter leaves, but if canopy representative measures of SLA are needed, leaves as complete as possible need to be measured, or at least have a balance of mid rib and petiole. The exception to this would be in cases where measurements of area eaten are required.

Where a bulk only measurement of SLA is required, a known number of leaves can be selected and dry weighted before other treatment is undertaken. These must all then be measured to obtain cumulative area, and other mean measures (such as leaf size) can then be calculated.

For dried litter leaves either folded or curled, a soaking technique may be required to ensure sufficient flexibility for measurement. This is possible for most broadleaves, though excessive soaking leaches out humic acids and weight loss may ensue. Occasionally for very thin leaves (e.g. *Fraxinus excelsior*), area losses may also occur. Test on each species collected should be conducted to establish a standard treatment with a known effect. In the case of dehisced *Fagus sylvatica* leaves, which dry folded into a concertina, a brief soaking in hot water (60-70°C) has been found to flatten leaves sufficiently for measurement, but weight losses of 5% have been recorded after longer overnight soaking. However, for *Quercus robur* and *Q.petraea* leaves weight loss is minimal over the same time period. For thinner leaves such as *Corylus avellana*, or *Fraxinus*, a soak of an hour or so will be sufficient, as weight losses of up to 15% have been recorded after long soaking. Any weight loss due to a soaking procedure should be incorporated into the SLA calculation as a correction factor before LAI is calculated from the litterfall weights. The use of flattening devices, such as a plant press, has been found helpful to ensuring accurate expansion of soaked broadleaves.

For short conifer needles which have dried (e.g. *Picea* sp), area measurement is often obtainable after only prelimonary cleaning, as they remain woody in nature and do not change area. However, finer needles (e.g. *Larix* sp) are difficult to prepare, and twist on drying. These would need a short soak and would be best measured on a leaf area machine where they can be laid on a flat bed under slight pressure. Longer needles (e.g. some *Pinus* sp) also twist on drying, and are difficult to soak out, as they then break up. Area measurements are best made from these if they can be kept damp from abscission.

All samples should then be dried at maximum 70°C for 24 hours before weighing for calculation of SLA. Previously soaked leaves must not be used for chemical analysis.

6. Quality Assurance and Quality Control

The quality of the litterfall analytical data is controlled by regular Interlaboratory comparison ring tests of plant material by the Forest Foliar Co-ordinating Centre. It is anticipated that there will be increasing need for these tests on non-foliar litter material, in order to establish the limits of expected and acceptable variation, as and when such material is available in sufficient quantity and homogeneity. All countries wishing to report litterfall chemistry should regularly take part in laboratory inter-comparisons.

Guidelines for QA/QC procedures in the laboratory are given in the Manual part XVI on laboratory QA/QC. Documentary proof of the QA/QC adopted in each laboratory should be submitted, together with the annual results, to the European-level data centre.

6.1 Plausibility limits

Table 3 summarises the current suggested plausibility limits on the reported chemical composition of litterfall samples. It is anticipated that these limits will be frequently revised as increasing numbers of litterfall results become available in the central database, and the full range in chemical composition of the different fractions of litterfall is established.

Table 3: **Plausible range of element concentrations in the foliar-litter of different species** *(indicative values in grey)* *.Source: Forest Foliar Co-ordinating Centre. ICP Forests*

Tree Species (Foliar litter)	Limit	C mg/g	S mg/g	N mg/g	P mg/g	K mg/g	Ca mg/g	Mg mg/g	Zn µg/g	Mn µg/g	Fe µg/g	Cu µg/g	B µg/g
Betula pendula	low	*290*		*7.30*	*0.20*	*0.30*	*5.,00*	*1.00*	*105.00*	*600*	*45.0*	*6*	
	high	*330*		*21.00*	*1.20*	*1.40*	*12.50*	*2.00*	*170.00*	*3000*	*300.0*	*19*	*38*
Castanea sativa	low	*390*		*9.00*	*0.20*	*0.20*	*4.50*	*1.40*	*35.00*	*700*		*5*	
	high	*420*		*13.00*	*0.70*	*0.55*	*10.50*	*2.00*	*45.00*	*2500*	*90.0*	*13*	*100*
Fagus sylvatica	low	460	1	9.00	0.50	2.00	4.00	0.80	25.00	650	70.0	4	2
	high	510	2.2	19.00	1.90	8.00	17.00	2.00	35.00	1600	140.0	7	40
Fraxinus excelsior	low	*470*		*12.00*	*0.75*	*0.40*	*20.00*	*2.00*	*15.00*	*110*	*120.0*	*7*	
	high	*470*		*18.00*	*1.50*	*1.40*	*25.00*	*3.50*	*20.00*	*200*	*200.0*	*9*	*50*
Quercus frainetto (Q. conferta)	low		1.1	8.00	1.10	4.50	14.00	1.20					
	high		1.1	11.70	1.30	5.20	18.30	1.40					
Quercus petraea	low	460		8.00	0.30	2.00	7.00	1.30	14.00	700	50.0	5	
	high	510		12.00	0.60	4.00	10.00	2.00	25.00	1700	200.0	8	35
Quercus robur (Q. pedunculata)	low	460	0.85	10.00	0.82	4.00	5.00	1.00	15.00	1000	90.0	6	7
	high	510	1.7	19.00	2.00	8,00	13.00	2.00	25.00	1200	150.0	7	35
Abies cephalonica	low			8.00		2.70	11.00	1.00					
	high			13.00		8.30	24.00	1.50					
Picea abies (P. excelsa)	low		1	6.50	0.60	1.00	2.50	0.70					
	high	520	1.5	12.60	1.20	4.20	16.00	2.20					
Picea sitchensis	low	440	1	6.00	0.60	1.50	4.00	0.60	15.00	250	40.0	2	
	high	530	1.1	13.00	1.10	3.00	11.00	1.00	35.00	1400	120.0	4	35
Pinus sylvestris	low	490	0.62	5.00	0.40	1.00	2.00	0.50	20.00	180	35.0	2	
	high	530	0.62	10.00	0.80	3.00	11.00	0.80	45.00	800	150.0	5	45

6.2 Data completeness

Table1 outlines for all the chemical variables the conditions under which they are mandatory or optional to report. When a country/federal state decides to report optional variables, they should be fulfilling the data quality requirements outlined in the methodology.

6.3 Data handling, submission procedures and forms

Forms for data submission lab quality information and explanatory items are found in Manual part XVII - Data handling and data submission forms in this Manual (and electronically on the ICP Forests web page, at http://www.icp-forests.org/ Manual.htm). The quality information from the labs has to be sent together with the relevant data submission forms to the data centre using form LF.LQA.

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