LIFE MODERN NEC

Air quality, the response of ecosystems

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Action A.2

Report on revised monitoring protocols for existing indicators and on the need of new indicators

Summary

As a preparatory action of the LIFE MODERn (NEC) project, action A.2 aims at reviewing the indicators presently used, to verify: i) if the are able to give a reliable picture of the state of the freshwater and forest ecosystems; ii) if their protocols need to be revised; iii) if new indicators should be activated.

For this purpose, we collected existing protocols, literature and results of relevant projects, suggesting potential revisions of existing protocols.

In total, 46 protocols were reported and examined, 39 of which were totally or partially suitable for monitoring under the NEC directive.

The examination of these protocols gave the opportunity to identify a restricted number of protocols which appears to be more suitable to be applied in the Italian sites selected for the monitoring activities related to the NEC Directive, and they will be used in action B3 for testing their ability to give indication on the ecosystem status and health and on its response to air pollution.

1. INTRODUCTION

The current strategy adopted by Italy to meet the requirement of the NEC Directive mainly follows the protocols developed by the International Cooperative Programs (ICPs), running under the Convention on Long-Range Transboundary Air Pollution (CLRTAP); in particular, ICP-Waters for freshwater ecosystems and ICP-Forests for terrestrial ecosystems.

For the aims of the project, it is necessary to review the indicators presently used, verifying if they are able to give a reliable picture of the state of the freshwater and forest ecosystems, or if new indicators should be activated.

Furthermore, an evaluation is needed of the efficacy and suitability of the protocols used for monitoring, and of the need to revise them or to add new protocols.

For this purpose, Action A.2 reviewed existing protocols, literature and results of relevant projects, suggesting potential revisions of existing protocols, gathering and organizing them for testing within Action B.3.

2. PROTOCOL COLLECTION

In total, 45 protocols were reported and examined, 39 of which were totally or partially suitable for monitoring under the NEC directive.

The number of protocols reported for each monitoring activity is reported in table 1. They are all shortly described in explanatory sheets at the end of this report.

Table 1

Monitoring activity	***# of protocol***	***useful for NEC***
Animal biodiversity	6	4
Air quality	2	2
Atmospheric deposition	3	3
Crown condition and phenology	4	4
Ecosystem chemistry	2	2
Epiphytic lichens	5	5
Freshwater	8	6
Ground vegetation	7	7
Growth	3	3
Meteo	2	1
Ozone injury	2	1
Soil solution	2	2
Visibility	1	1

3. DISCUSSION

3.1 Activity already running

3.1.1. Freshwater ecosystems

Commonly used protocols for freshwater monitoring were developed with the aim of verifying the effect on water bodies of the long range transport of atmospheric pollutants (ICP-Waters network) and of different anthropic pressures (Water Framework Directive). However, they are relatively similar, reflecting a long tradition of biological monitoring of water bodies.

The protocols developed for the Water Framework Directive are however mostly tuned to satisfy the requirement of the methods used for the evaluation of the ecological quality of water bodies on the basis of the response of the so-called Biological Quality Elements, and mainly focused to identify local pollution sources.

For the NEC Directive, the interest is in the effect of diffuse pollution, originated by the deposition of airborne pollutants, and different methods may be used, tested or developed.

For this project, the use of the sampling protocols developed in the ICP-Waters monitoring network is proposed, in order to obtain data with high taxonomic resolution that can be used either with the numerical methods in use in the ICP-Waters programme, or with new numerical methods developed during the project.

3.1.2. Forest ecosystems

- **Air quality**

The evaluation of the ozone effects on forest vegetation requires a good plot-by-plot estimation of ozone levels.

Between 1997 and 2014, ozone levels were measured in the Italian forest plots by means of passive samplers, giving the possibility to estimate bi-weekly and annual ozone average concentration, limited to the growing season.

More precise models of plant response require more detailed information on the seasonal and daily distribution of ozone levels, such as the improved bases of data to developed by the LIFE project MOTTLES to estimate stomatal fluxes of water vapour and ozone uptake, their critical levels and exceedances for the protection of forests, following and revising the indications of the ICP Forests Manual Part VIII Assessment of ozone injury and Part XV Monitoring of air quality, v.05/2016.

In fact, the LIFE+ project MOTTLES (LIFE15 ENV/IT/000183 MOnitoring ozone injury for seTTing new critical LEvelS) proposed to slightly revise the ICP-Forests protocols for monitoring the NEC indicators: stomatal ozone flux exceedance and visible foliar ozone injury (Paoletti et al.

2019). It also proposed a new concept for assessing critical levels for forest protection against ozone, replacing air ozone concentrations with stomatal ozone flux, which measures the ozone effectively taken up by plants.

The application of this protocol to new sites will also give the opportunity to improve its modelling approaches, including new species.

- **Atmospheric deposition**

Beside air quality, atmospheric deposition is the main factor relating airborne pollution and ecosystems and it represents an important pathway for atmospheric pollutants reaching remote areas, such as forest ecosystems and remote water bodies.
Pollutant produced by industry, traffic, agriculture and other human activities are emitted into the atmosphere and they can be transported towards other areas, where they are deposited mainly through wet deposition of compounds dissolved in rain, snow, sleet or similar, and dry deposition of particulate matter, for example through gravity or adsorption on forest canopy.
The amount of pollutant deposited can be modelled, but in-situ measurement is needed because of its relatively high local variability, related to the distribution of pollutant sources and the local topography. In forest and waterbodies, polluted atmospheric deposition may cause water and soil acidification and ecosystem eutrophication.
Even if there are models allowing to estimate the deposition of major pollutants on the basis of their sources, differences between modelled and measured values in a specific site may be large, and in-situ measurement is important.
We compared the protocol used at present, which strictly follow the ICP-Forests manual, with two alternative protocols and we found that the protocol in use is reliable for the purposes of the NEC Directive.

- **Crown condition and phenology**

Evaluation of crown condition and phenology in forest plots aims to monitor the state and change of crown condition and of life events in relation to environmental variables and climatic events, contributing to the evaluation of forest condition.
Crown condition and phenology are presently evaluated following the ICP-Forests Manual, in order to provide assess sound, harmonized and comparable crown condition along time series and geographical gradients,
This method allows to quantify defoliation and damage symptoms on plots and to detect temporal changes that can be related to the changing influences of natural and anthropogenic environmental factors, such as stand structure, tree composition, atmospheric depositions, air quality and climatic events.
The methods are very reliable if used on single plot, but their use for high-resolution survey would requires a large effort and a large number of trained personnel.
An alternative method is represented by Digital Cover Photography (DCP), which is based on collecting images with a camera equipped with a narrow lens, oriented upward, to achieve a restricted field of view, allowing to estimate canopy cover, as the proportion of the ground covered by the vertical projection of tree crowns.

For extending the evaluation from single plot to a large share of forested areas, remote sensing may represent a valid option.
In this project we propose to maintain the visual estimation of crown condition and phenology and to include a specific remote sensing protocol for phenology, in order to compare their results and to evaluate the possibility to extend the evaluation to larger forested areas.

- **Ecosystem chemistry**

Foliar and litter analyses are important monitoring activities allowing the definition of the nutritional state of trees, which is often indicative of processes at the ecosystem level. For example, inadequate nutrient supply or excess of certain elements may cause low tree vitality, or increased sensitivity to air pollution, or can be a signal of high level of pollutant concentration in air or in atmospheric deposition.

At present, both litter and leaves are sampled and analysed following the ICP-Forests protocols, which are relatively standard protocols and fully able to satisfy the requirement for monitoring on the basis of the NEC Directive.

- **Ground vegetation**

The diversity of plant taxonomic groups (i.e., tracheophytes, bryophytes and lichens on mineral soil), measured using classical metrics as species richness and abundance, iscurrently monitored following the indications of the ICP Forests Manual Part VII.1, v.05/2016.

A vegetation probabilistic sampling is adopted to represent the plot-level state (2500m^2 - 50m x 50m ICP Level II plot). Presence and relative species cover by layer are surveyed at a single 100m^2 scale by 12 permanent sampling units on a regular 10 m x 10 m cells grid within the plot, repeated on a distributed comparable system in the buffer zone. Structural data as average layer height, litter, bare soil and total cover are also assessed.

Population level sampling on 100 systematic 50 cm x 50 cm sampling units, within the plot, have been tested optionally.

Surveys are performed in summer, and can be eventually repeated in spring also, by trained teams. A Quality Assessment /Quality Control protocol is described, including observers' calibration. Species nomenclature follows a standardized flora. Results are presented in the form of total number of species per plot, mean number of species per sampling unit, species-abundance per layer per sampling unit, and relative cover by layers.

Other protocols used in national and international networks and scientific programs were also examined in order to identify possible improvements in the revised protocol.

- **Growth**

Tree growth is a key ecological parameter of forests, and is measured as increment, defined as the growth of trees (shoots in coppice forests) and stands within a defined period and can be expressed as increment of diameter, basal area, height and/or volume.

The standard and more reliable measurement of increment uses trunk diameter or circumference, which may be measured manually or electronically. In the ICP-Forests Manual, two protocols are included, one using manual measurement every 5 year, and one using manual or electronic reading of metallic bands installed on the trees. Both have their advantages and disadvantages; manual measurement allows the evaluation of a large number of trees, while metallic bands are usually installed in a smaller number of trees. However, metallic bands can be useful to estimate short term (from hours to months) variability in trunk size, related to physiological and meteorological events.

Terrestrial laser scanning (TLS) can also be used to collect detailed 3-D information of single trees or forest stand, and to assess tree growth based on time series analysis.

- **Meteo**

Forest ecosystems are strongly affected by meteorological variables, that can determine the distribution of natural forests and affect the composition, structure, growth, health and dynamics of both natural and human induced forests. Short-term variability of meteorological variables can strongly affect plant physiology and ecosystem functions. Measurements of meteorological data are highly standardized, but their high spatial variability requires direct measurement in each plot to obtain information that can be related to vitality, growth, phenology, and the crown condition of trees. The ICP-Forest protocol is considered to be able to provide enough information for evaluating the response of the trees and of the forest ecosystem to meteorological variability. More extensive and expensive protocols are available, such for example the ICOS protocol, but the cost and complexity of this measurement structure is excessive for the purpose of relating forest health to air pollution for a large number of forest ecosystems.

- **Ozone injury**

Tropospheric ozone can be an important stress factor affecting forest trees, even in remote areas.
The protocol for the assessment of ozone injury presently used in the Italian forest monitoring network follows the recommendations of the ICP Forests manual. As different tree species may react differently to the same ozone level, an alternative method was developed in the VibEuroNet network and applied in ICP forests, using the wayfarer, a common shrub, as biological indicator of ozone stress. However, the use of this method in the forest monitoring network is limited by the geographic and altitudinal distribution of the shrub, that is found in Northern and central Italy, below 1000 m a.s.l.

- **Soil solution**

Monitoring soil solution composition is the natural follow-up to the monitoring of atmospheric deposition. It allows to investigate the way in which pollutants are transmitted to the soil and then through the soil, to be either absorbed by plants, stored and/or transformed into the soil or transmitted to underground waters. Further, the influence of pollutants on the fate of other substances can be observed.

At the state of the art, modelling these phenomena is out of feasible bounds. Few models exist, which are calibrated on agricultural soils and do not appear to offer satisfactory performance. Concerning the development of methods for soil solution monitoring, a thorough literature survey shows that no basic progress has appeared since this activity was started in Europe on a large scale, within the CLRTAP convention. There are choices to be made about specific details concerning materials, but the Italian program did test alternate materials long ago, and there appears to be no reason to modify present standards.

3.2. New indicators

The present Italian and European network of forest monitoring are focused on the vegetation compartment and on the abiotic parameters. In this project, an attempt will be made to make forest monitoring more complete, including the examination of other biological communities, such as animals and lichens. In this way, a more reliable description of the forest ecosystem will be obtained.

Furthermore, “visibility” will be included as an estimator of the values of cultural and touristic ecosystem services and as an indicator of the presence of anthropic substances like nitrogen oxides and particulate matter.

- **Animal biodiversity**

Animal biodiversity is an important part of forest ecosystem, which is more difficult to monitor than vegetal biodiversity because of animal mobility and low spatial density.

Several protocol have been proposed in small projects or research studies to monitor animal biodiversity, including trap cameras, sound recorders, soil macro-arthropod collection, analysis of soil environmental DNA, Malaise traps for insects. However, no protocol is presently used in large networks, apart from the QBS-ar protocol.

For this reason, different protocols should be refined and tested in action B.3 and their efficacy in describing animal biodiversity evaluated. On the basis of the protocol collection performed in this action, the most promising protocols to be tested in action B.3 are the QBS-ar protocol, passive acoustic monitoring for birds and bats, and analyses of soil environmental DNA.

- **Epiphytic lichens**

Epiphytic lichens, as species number and Lichen Diversity Value (LDV), are already included in the monitoring by the NEC Italian network (2019-2020), following ICP Forests Manual (Part VII.2, v.05/2016), as indicators of the effects of NO_X and SO_2.

At present, a protocol using a sampling grid and species list is used, but quantitative indicators of functional traits (growth form, ecological indicators, reproductive strategy, photobiont) can be added to improve the description of the lichen community.

Other protocols used in monitoring networks, such as the ICP-IM or the USGS network, can be used in complement of the presently used protocol. An interesting addition can be represented by metal analysis in lichens, in particular in areas close to pollution sources. Other more specific protocols, such the Finnish protocol, cannot be simply applied in the Mediterranean ecoregion because of the biogeographic distribution of lichen and their host tree.

- **Visibility**

A unique protocol for air transparency evaluation has been proposed: the I.M.PRO.V.E. protocol has the objective of calculating air transparency in natural areas, associated with the presence of atmospheric pollutants such as particulate matter (PM) and gases of anthropogenic or natural origin. Thus visibility can be used as a physical parameter for assessing the air status, with related information on its quality, in a natural environment with relevant conservation and recreational vocation, such as a natural park, and it also allows to study the impact of atmospheric pollution on ecosystems. The visibility monitoring station for measuring 4 macro-parameters: includes 4 sampling lines: nitrogen oxides, meteorology, fine particles (PM2.5 and PM10) and photographs of the view. The chemical-physical parameters collected are used to calculate the visibility degree through the quantification of a coefficient (bext), describing the extinction of light as a function of the different chemical-physical parameters associated with airborne molecules and particles. The overall analysis involves the study of correlations of this value with weather conditions and with the optical visibility captured by the photographs.

There is no monitoring of visibility currently ongoing within the NEC Italy Network. As an AFTER LIFE task following LIFE Smart4Action (LIFE13ENV/IT/000813 Smart4Action), a one-year test of visibility monitoring has been performed at a single site, inside Circeo National Park (Sabaudia, Latina, Central Italy), adopting the U.S. I.M.PRO.V.E. protocol, which has shown to be a unique scientific-sound method to measure air quality and air transparency in natural environments, adding useful data to fulfil the NEC Directive requirements.

4. CONCLUSIONS

A relevant number of protocols potentially useful to improve the Italian forest and freshwater monitoring activity were examined and discussed. More than 40 protocols proved potentially useful for the evaluation of ecosystem status following the NEC Directive.

The examination of these protocols gave the opportunity to identify a restricted number of protocols/groups of indicators which appears to be more suitable to be applied in the Italian sites selected for the monitoring activities related to the NEC Directive. The above protocols/groups, together with eventual updates or new punctual indicators from already adopted groups, will be proposed and defined in action B.2, and further used in action B.3 for testing their ability to give indication on the ecosystem status and health and on its response to air pollution.

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MONITORING ACTIVITY:

MONITORING OF AIR QUALITY - OZONE

PROTOCOL IN USE AT PRESENT

MOTTLES - “MOnitoring ozone injury for seTTing new critical LEvelS”

Source: E. Paoletti, A. Alivernini, A. Anav, O. Badea, E. Carrari, S. Chivulescu, A. Conte, M.L. Ciriani, L. Dalstein-Richier, A. De Marco, S. Fares, G. Fasano, A. Giovannelli, M. Lazzara, S. Leca, A. Materassi, V. Moretti, D. Pitar, I. Popa, F. Sabatini, L. Salvati, P. Sicard, T. Sorgi, Y. Hoshika. **Toward stomatal–flux based forest protection against ozone: The MOTTLES approach**. *Science of The Total Environment*, Volume 691, 2019, Pages 516-527, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2019.06.525.

Short description: Ozone concentration is measured by an active monitor (2B Technologies, Inc., Boulder, Colorado, USA). The sensors are installed at 2 m above ground level, following recommendations of the ICP Forests and recorded in open areas. Data acquisition is based on data loggers equipped with a custom–made program. The power supply is assured by solar panels or mains, when present, and backup batteries.

Sampling/mensuration frequency: Data are acquired with about 2 to 10 s (depending on the sensor), and the average is stored every hour

Sampling/mensuration scale: Site-specific and should be carried out on plots where meteorology and deposition data are available.

Used by: MOTTLES - “MOnitoring ozone injury for seTTing new critical LEvelS”

Advantages: Ozone monitoring with a high temporal resolution (1 h interval) permits the proper calculation of Phytotoxic Ozone Dose above a threshold Y of uptake (PODY) which is a promising a biologically significant metric for the quantitative O3 risk assessment (Level II approach). This MOTTLES concept contributes to expanding monitoring at existing long-term forest sites to realize a stomatal O3 flux-based assessment which is considered a promising approach for the protection of forests against O3 following the revised EU National Emission Ceilings Directive (2016/2284/EU, hereafter NEC Directive).

Problems: The monitoring stations can be considered expensive compared to passive samples and require constant maintenance.

File name of the description: https://www.sciencedirect.com/science/article/pii/S0048969719331110

Reported parameters: Hourly ozone concentrations, AOT40, PODY

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

MONITORING OF AIR QUALITY - OZONE

PROTOCOL IN USE AT PRESENT

ICP-FORESTS

Source: ICP-Forests Manual, part XV, http://icp-forests.net/page/icp-forests-manual

Short description: Ozone concentration is monitored using passive sampling measured near, but outside the forest. Passive samplers should be installed at 2 m above soil level in the open-field plot.

Sampling/mensuration frequency: The sampling is carried out on a 2-week basis and covers the period of 1 April - 30 September. For Mediterranean conditions, it is recommended to cover all 12 months of the year.

Sampling/mensuration scale: Site-specific and should be carried out on plots where meteorology and deposition data are available

Used by: ICP Forests network

Advantages: simple, inexpensive

Problems: Low temporal resolution (from one week to one month, mainly dependent on the magnitude of air concentrations). It does not provide a variation of O_3 concentrations during a daytime when plants actively absorb O_3 and can not be used for the proper calculation of Phototoxic Ozone Dose (PODY).

File name of the description: ICP_Manual_2020_part15_Air_Quality_version_2020-1.pdf

Reported parameters: Ozone concentrations in µg m-3, AOT40

Suitable for monitoring under the NECD: yes but with limitation

MONITORING ACTIVITY:

ANIMAL BIODIVERSITY

OTHER PROTOCOLS

Passive acoustic monitoring (PAM) for birds species richness and diversity

Source: experimental protocol to be refined

Short overview

Forest bird species are appropriate indicators to test the effectiveness of ecological forestry because they occupy a broad range of forest habitat types and food sources, are responsive to the types of changes in forest conditions caused by forest management, vocally defend breeding territories, can be cost-effectively and unobtrusively monitored, and are a high conservation priority and responsibility for resource managers (Rempel et al. 2016). Thus, estimation of avian biodiversity is a cornerstone measure of ecosystem condition.

Surveys conducted using Passive Acoustic Monitoring (PAM) by using autonomous recorder units (ARU), are often more efficient at estimating diversity than traditional point-count surveys. However, there is limited research into the optimal temporal resolution for sampling the trade-off between the number of samples and sample duration when sampling a survey window with a fixed survey effort despite autonomous recorders allowing easy repeat sampling compared to traditional survey methods.

There is evidence that high temporal resolution (HTR) sampling outperforms low temporal resolution (LTR) sampling in every metric, with HTR sampling predicted to detect approximately 50% higher alpha diversity, and 10% higher gamma diversity. This effect is primarily driven by increased coverage of variation in detectability across the morning, with the earliest period containing a distinct community that is often under sampled using LTR sampling. When considering multiple independent detections of species, HTR sampling detected three times more uncommon species than LTR sampling.

There are good theoretical reasons to believe sampling at very high temporal resolution across a broader overall time window may produce better estimates of species richness and more accurate species inventories. Thus, when the focus of monitoring is the estimation of alpha and beta diversity, HTR sampling of passive-acoustic monitoring-based surveys should be considered the primary method for estimating the species richness of bird communities in forests.

On the contrary, if the focus is the study of the soundscape, which is defined as the product of the relationships between the sounds of the environment and the listener (Schafer, 1977), it is important to standardize the time window for recording and recording should be done possibly simultaneously at different sites. Recording can then be used to calculate different caustic indexes

Sampling frequency: continuous

Sampling/mensuration scale: site-specific

Used by: NONE

Advantages: PAM does not require active presence of users for monitoring making possible to standardize the collection of acoustic data

Problems: Define right timing for sampling of ARU to maximize data collection and reduce memory and battery drainage to perform longer sampling periods

Reported parameters

- Species diversity
- Acoustic Complexity Index (ACI) from Pieretti et al. 2011
- Normalized Difference Soundscape Index (NDSI) from REAL (http://www.real.msu.edu) and Kasten et al. 2012
- Bioacoustic Index from Boelman et al. 2007.
- Acoustic Diversity Index (ADI) from Villanueva-Rivera et al. 2011
- Acoustic Evenness Index (AEI) from Villanueva-Rivera et al. 2011

Suitable for monitoring under the NEC: YES

OTHER PROTOCOLS

Passive acoustic monitoring (PAM) for bat species richness and diversity

Source: experimental protocol to be refined

Short overview

Bats are mostly forest mammals. Many species roost and/or forage in the forest, or use forest patches and corridors for commuting and migration stopovers. The presence and activity of bats in the forest are highly influenced by the forest age and structure, and, in turn, forest management.
Bats show responses to anthropogenic stressors linked to changes in other ecosystem components such as insects, and as K-selected mammals, exhibit fast population declines. This speciose, widespread mammal group shows an impressive trophic diversity and provides key ecosystem services. For these and other reasons, bats might act as suitable bioindicators in many environmental contexts. However, few studies have explicitly tested this potential, and in some cases, stating that bats are useful bioindicators more closely resembles a slogan to support conservation than a well-grounded piece of scientific evidence. Based on the limited number of studies available, the use of bats as bioindicators is highly promising and warrants further investigation in specific contexts such as river quality, urbanisation, farming practices, forestry, bioaccumulation, and climate change. Whether bats may also serve as surrogate taxa remains a controversial yet highly interesting matter. Some limitations to using bats as bioindicators include taxonomical issues, sampling problems, difficulties in associating responses with specific stressors, and geographically biased or delayed responses.

Advantages: Passive Acoustic Monitoring (PAM) does not require active presence of users for monitoring making possible to standardize the collection of acoustic data. The use of bats as bioindicators has pros and these comprise: (a) relative taxonomic stability; (b) wide geographic ranges; (c) rich trophic diversity; (d) provision of key ecosystem services, (e) graded responses to environmental alteration correlated with those of other biodiversity components, such as insects; (f) rapid population declines due to slow population growth; (g) possibility of measuring several variables (population size, feeding activity, etc.); and (h) the role of bats as reservoirs of emerging infectious diseases whose epidemiology could reflect environmental stress.

Problems PAM of bat assemblages is still an emerging field in bat research and conservation. Probably due to a general lack of methodological standards and the lack of common ecological indices, few long-term bat acoustic monitoring programs are currently active and data is rarely shared and compared between regions.

Sampling frequency: continuous

Sampling/mensuration scale: site-specific

Reported parameters: species list and activity indices

Used by: NONE

Suitable for monitoring under the NEC: YES

OTHER PROTOCOLS

QUALITÀ BIOLOGICA DEL SUOLO, QBS-ar

Source: http://www.expeeronline.eu/images/ExpeER-Documents/Handbook_of_standardized_ecosystem_protocols.pdf

Short description

The QBS-ar index (Qualità Biologica del Suolo) is based on the following concept: the higher is the soil quality, the higher is the number of microarthropod groups morphologically well adapted to this soil habitat. The fluctuation of the soil quality can be related to direct human inputs (including land management practices) or to long-term processes such as climate change. QBS-ar has been developed by an Italian team (Parisi et al. 2005) and it has been applied to several ecosystems, including agricultural lands, grasslands, urban soils, woods at different levels of wilderness, and degraded soils. It does not require identifying the fauna to the species level. It is applied to the soil microarthropod community, separated according to the biological form approach with the intention of evaluating the microarthropods' level of adaptation to the soil environment life, and overcoming the well-known difficulties of taxonomic analysis to species level for edaphic mesofauna. In addition, this index is rather inexpensive, both in terms of equipment required and time/energy needed in the sampling activity and the analysis of the samples.

The high number of applications in Italy, European and non-European countries signal the potential of QBS-ar. It is a good candidate index for continuous biomonitoring of soil communities to describe patterns and processes in the microarthropod biodiversity across the landscape. A deeper knowledge of soil biodiversity in response to landscape use will provide guidance in effective management planning for sustainable renewable resource use and nature conservation.

This protocol is intended for the assessment of the soil macrofauna present in a studied site through a simple index, which doesn't involve species identification skills. A high index represents a high diversity of fauna adapted to life in the soil and corresponds to a good biological quality of the soil. Soil samples are collected in situ, and the soil fauna are extracted by a dynamic extraction method (Berlese Tüllgren funnel). The fauna is identified at the level of Recognizable Taxonomic Unit (RTU), which means order or class depending on the specialization of the order. Each RTU found in the sample receives a score from 1 to 20 according to its adaptation to soil environment, following a score grid. The final index sums up these scores. Some orders are consistent in their performance and all the orders get the same score and so identification or RTU is to the level of order. Some orders are heterogeneous in their performance and there is a need to identify to family, which is the RTU. The data include:

- Location, area, soil cover. These are the site metadata, plus data for every spatial unit inside the site if necessary.

- Soil fauna diversity. For each type of soil fauna, information about presence/absence and score of adaptation to edaphic life, according to the scoring grid supplied.

- Soil quality index. Aggregation of the score of each fauna type in a single index. Soil organisms are separated into biological forms according to their morphological adaptation to soil environments. Each of these forms is associated with a score named the EMI (eco-morphological index), which ranges from 1 to 20 in proportion to the de- gree of adaptation. The QBS-ar index value is obtained from the sum of the EMI of all collected groups. If in a group, biological forms with different EMI scores are present, the higher value (more adapted to the soil form) is selected to represent the group in the QBS-ar calculation. This choice is based on the consideration that the examined soil is able to support well adapted and consequently more vulnerable biological form. Parisi et al. (2005) provides tables to easily calculate the index.

sampling/mensurational frequency: In stable conditions it is adequate to collect the soil sample once a year (e.g. in the woods, grasslands), in spring or in autumn, since the composition of the soil fauna partially varies with the seasons. The winter should be avoided, cold temperatures reducing the activity/presence of the soil fauna.

sampling/mensurational scale: Data should be entered on for each identified spatial unit. In each site for each unit, three soil cores, 100 cm2 and 10 cm deep are collected with a standard soil corer.

Used by: Regional environmental protection agency of Piemonte, Marche, Emilia Romagna, Veneto (Italy)

Advantages: It does not require identifying the fauna to the species level overcoming the well-known difficulties of taxonomic analysis to species level for edaphic mesofauna. In addition, this index is rather inexpensive, both interms of equipment required and time/energy needed in the sampling activity and the analysis of the samples. It is well adapted to be applied to several types of soil and landscape.

Problems: The respect of the protocol for the extraction stage is particularly important for the quality assurance of the indicator.

File name of the description: "Handbook for Standardised Ecosystem Protocols" is the deliverable No D.2.3 of the FP 7 Project: Distributed Infrastructure for Experimentation in Ecosystem Research (ExpeER)

Reported parameters

- Order or class level of soil fauna collected (Recognizable Taxonomic Unit).
- Abundance per RTU.
- Scoring of each fauna group depending on its adaptation level to life in the soil.
- Soil biological quality index – calculated by adding the scores.

Suitable for monitoring under the NEC: YES

OTHER PROTOCOLS

Environmental DNA (eDNA) of soil

Source: experimental protocol to be refined

Short overview:

Environmental DNA (eDNA) metabarcoding is a novel method of assessing biodiversity wherein samples are taken from the environment via water, sediment or air from which DNA is extracted, and then amplified using general or universal primers in polymerase chain reaction and sequenced using next-generation sequencing to generate thousands to millions of reads. From this data, species presence can be determined, and overall biodiversity assessed. It is an interdisciplinary method that brings together traditional field-based ecology with in-depth molecular methods and advanced computational tools. As an emerging monitoring method, there are many pitfalls and roadblocks to be considered and avoided, but the method may still have the ability to revolutionize modern biodiversity surveys for the molecular era.

Sampling/mensuration frequency: una tantum

Sampling/mensuration scale: site-specific

Used by: none

Advantages:

Despite being a relatively new method of surveying, eDNA has already proven to have enormous potential in biological monitoring. Conventional methods for surveying richness and abundance are limited by taxonomic identification, may cause disturbance or destruction of habitat, and may rely on methods in which it is difficult to detect small or elusive species, thus making estimates for entire communities impossible. eDNA can complement these methods by targeting different species, sampling greater diversity, and increasing taxonomic resolution. Additionally, eDNA is capable of detecting rare species, but not of determining population quality information such as sex ratios and body conditions, so it is ideal for supplementing traditional studies. Regardless, it has useful applications in detecting the first occurrences of invasive species, the continued presence of native species thought to be extinct or otherwise threatened, and other elusive species occurring in low densities that would be difficult to detect by traditional means.

Problems:

Degradation of eDNA in the environment limits the scope of eDNA studies, as often only small segments of genetic material remain, particularly in warm regions. Additionally, the varying lengths of time to degradation based on environmental conditions and the potential of DNA to travel throughout media such as water can affect inference of fine-scale spatiotemporal trends of species and communities

Reported parameters: OTU list

Suitable for monitoring under the NEC: YES

OTHER PROTOCOLS

LIFEPLAN

Source: https://www2.helsinki.fi/en/projects/lifeplan/instructions

Short description:

At each sampling location, a one-hectare (100 m x 100 m square) sampling plot is established. The plot should be placed in a habitat type that represents vegetation typical to the area, e.g. a common forest type.

Five types of sampling are conducted within this area: audio recording, camera trapping, cyclone sampling, Malaise trapping, and soil sampling (Fig. 2). The sampling plot has a single cyclone sampler and a single Malaise trap, placed near the center of the plot. The sampling plot also has five camera traps and five audio recorders, placed as five pairs, one near the center of the plot and one at each corner. Each pair of a camera trap and an audio recorder is attached to the same tree.

Sampling/mensuration frequency: Continuous, weekly visit required

Sampling/mensuration scale: Site-specific

Used by: Life-plan A Planetary Inventory of Life

Advantages: provides a large amount of data about different animal groups

Problems: treatment procedure of such a large amount of data still not finalized

File name of the description: n/a

Reported parameters: not yet defined

Suitable for monitoring under the NECD: NO

OTHER PROTOCOLS

Insect metabarcoding using Malaise trap

Source: Sire, L., Yáñez, P.S., Wang, C. et al. Climate-induced forest dieback drives compositional changes in insect communities that are more pronounced for rare species. Commun Biol 5, 57 (2022)

Short description:

Insect sampling is conducted from late spring to early autumn using Townes-style Malaise traps with black walls and white roof. Malaise trap sample-bottles are filled with a mixture of 20% monopropylene glycol and 80% pure ethanol. Samples are retrieved once per month, resulting in a total of four samples per plot over the sampling period.

Insects are first filtered from the trapping solution and rinsed with ultrapure Milli-Q water to remove monopropylene glycol residue, then placed within sterile and disposable Petri dishes on clean absorbing paper to dry overnight at ambient temperature. Once dried, insects are size-sorted using decontaminated forceps. Insects larger than a European honey bee are removed and only the head or a part of the abdomen was retained in order to reduce the biomass and improve the detection of rare or small species. Insect bulk samples are then ground and homogenized into fine powder for DNA extraction and analysis.

Sampling/mensuration frequency: monthly in late summer and autumn

Sampling/mensuration scale: Site-specific

Used by: CLIMTREE project

Advantages: produce very comprehensive list of insect

Problems: large amount of manual activity not compatible with long-term data series on a number of sites

File name of the description: s42003-021-02968-4.pdf

Reported parameters: Insect species list

Suitable for monitoring under the NECD: NO

MONITORING ACTIVITY:

ATMOSPHERIC DEPOSITION OF POLLUTANTS

PROTOCOL IN USE AT PRESENT

ICP-FORESTS

Source: Clarke N, Žlindra D, Ulrich E, Mosello R, Derome J, Derome K, König N, Lövblad G, Draaijers GPJ, Hansen K, Thimonier A, Waldner P, Arne Verstraeten, 2020: Part XIV: Sampling and Analysis of Deposition. Version 2020-1. 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, 34 p. + Annex

Short description:

Bulk precipitation is collected with three funnel-type polyethylene collectors (cylinders during snow season). Throughfall is sampled with 16 funnel-type collectors of the same design as the collectors used in the open field, systematically located in the plot in a 10x10-m grid. Samples are collected once every week, mixing together (separately) all open field samples and all throughfall samples. Collectors are washed or replaced at each sampling.

For stemflow analysis, a gutter is coiled in a spiral around the stem of selected trees. The stemflow runs down in a container. Every week, the collected stemflow is accurately mixed and a subsample is taken for analysis.

All samples are sent by post to the lab for analysis. A strict QA/QC is applied to chemical analyses, including control charts, internal tests and ring tests.

Optional wet-only electric sampler opening automatically during rain and snow events are also foreseen, but are not presently used in the Italian network.

sampling/mensurational frequency: weekly sample, weekly to monthly analysis

sampling/mensurational scale: plot-level

Used by: ICP Forests network, with differences in sampler shape and position between country.

Advantages: simple, robust, tested, no need of power supply (apart for wet-only samplers)

Problems: requires weekly manual sampling, does not allow quantification of nitrogen total deposition

File name of the description: ICP_Manual_2020_part14_Depo_20211115.pdf

Reported parameters (separately for open field, throughfall, stemflow and optionally wet deposition):

Precipitation amount (mm), pH, alkalinity and concentration of Ca^{++}, Mg^{++}, Na^{+}, K^{+}, NH_4^{+}, NO_3^{-}, SO_4^{--}, Cl^{-}, Dissolved organic C, Total N, Total (or reactive) P

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS:

TEFLON STRINGS

Source: Ferm and Hultberg (1995) Method to estimate atmospheric deposition of base cations in coniferous throughfall. Water, Air, Soil Pollut., 85: 2229-2234 as used by P.E. Karlsson, G. Pihl Karlsson, S. Hellsten, C. Akselsson, M. Ferm, H. Hultberg (2019) Total deposition of inorganic nitrogen to Norway spruce forests – Applying a surrogate surface method across a deposition gradient in Sweden. Atmospheric Environment, 217: 116964.

Short description:

A string sampler is placed under a transparent roof made of polycarbonate. It consisted of 13 m of Teflon strings with a diameter of 0.4 mm. It is sprayed with 150 ml deionized water once a month, year round. Sprayed water is collected by a bottle under the strings and the samples are sent to the laboratory for chemical analysis. During wintertime the string sampler sometimes must be brought indoors before spraying with de-ionized water. The roof above the Teflon string sampler is needed to avoid that precipitation would enter the bottle under the strings. The ratio of the deposited amount of a compound to the ratio of the deposited amount of sodium is used in calculations.

sampling/mensurational frequency: monthly sampling and analysis

sampling/mensurational scale: plot-level

Used by: not officially used

Advantages: allows quantification of nitrogen total deposition

Problems: experimental protocol to be further tested

Filename of the description: FermandHultberg1995.pdf

1-s2.0-S135223101930603X-main.pdf

Reported parameters:

Calculated total nitrogen deposition (NO_3^-, NH_4^+ and total N)

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS

AUTOMATIC FRACTIONING OF STEFLOW DEPOSITION

Source: https://www.wsl.ch/en/about-wsl/instrumented-field-sites-and-laboratories/lwf-demonstration-site/10-atmosphere/10-stemflow-collector.html

Short description:

A silicone gutter is coiled in a spiral around the stem of selected trees. The stemflow runs down in this gutter and is collected in an automated tipping bucket connected to a counter. Each tipping of the lever increases the counter value and redirects a small fraction of stemflow into a collecting vessel.

The stemflow collectors remain in place all year round. The quantity of stemflow is recorded every other week and samples are brought back to the laboratory for chemical analyses.

sampling/mensurational frequency: weekly sample, weekly to monthly analysis

sampling/mensurational scale: plot-level

Used by: SwissICP-Forest network

Advantages: simpler collection of stemflow samples

Problems: more complex mechanically

Filename of the description: (10) Stemflow collector - WSL.html

Reported parameters (stemflow deposition, only)

Precipitation amount (mm), pH, alkalinity and concentration of Ca^{++}, Mg^{++}, Na^{+}, K^{+}, NH_4^{+}, NO_3^{-}, SO_4^{--}, Cl^{-}, Dissolved organic C, Total N, Total (or reactive) P

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

CROWN CONDITIONS AND PHENOLOGY

PROTOCOL IN USE AT PRESENT

ICP-Forests (crown condition)

Source: Eichhorn J, Roskams P, Potočić N, Timmermann V, Ferretti M, Mues V, Szepesi A, Durrant D, Seletković I, Schröck H-W, Nevalainen S, Bussotti F, Garcia P, Wulff S, 2020: Part IV: Visual Assessment of Crown Condition and Damaging Agents. Version 2020-3. 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, 49 p. + Annex

Short description:

This Manual aims at providing a consistent methodology to assess sound, harmonized and comparable crown condition data at the ICP Forests monitoring plots, both Level I and Level II. Its main objective is to monitor the state and change of crown condition on permanent plots in relation to environmental variables and climatic events, so contributing to the evaluation of forest condition in Europe. Specific objectives are: i) to assess defoliation and damage symptoms on plots; ii) to detect its temporal changes; iii) to relate changing influences of natural and anthropogenic environmental factors (e.g., stand structure, tree composition, depositions, climatic events) to defoliation and damage symptoms.

The assessment of crown condition is carried out on trees growing in a fixed surface (circular area with 17 m radius on Level I plots; 50x50 m wide plot on Level II plots). Only trees with dominant crown are assessed. Evaluation of defoliation and damage symptoms is carried out by trained field crews.

Results are presented in form of mean defoliation, percentage of trees with defoliation over the standard thresholds (>25%: moderate; >60%: severe; 100%: dead).

sampling/mensurational frequency: annual surveys

sampling/mensurational scale: tree-level

Used by: ICP Forests network

Advantages: standardised, robust, reproducible, tested, including QA/QC procedures. The basic and fundamental parameter, defoliation, is reliable and reproducible from trained field crews and allows comparison between different countries and projects.

Problems: there are still problems and uncertainties in the assessment of biotic damage and damage causes and agents. That requires further training and the redaction of “ad hoc” iconographic manuals.

File name of the description: ICP_Manual_2020_part04_Crown_20210512.pdf

Reported parameters Defoliation descriptors at tree an plot level; tree mortality; general statistics on symptoms and damage agents.

Suitable for monitoring under the NECD: YES

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Eichhorn J, Szepesi A, Ferretti M, Durrant D, Roskams P, 2006: Part II: Visual Assessment of Crown Condition. UNECE ICP Forests Programme Co-ordinating Centre, Hamburg. [http://www.icp-forests.org/Manual.htm]

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Bussotti, F., Bettini, D., Cenni, E., Ferretti, M., Sarti, C., Nibbi, R., Capretti, P., Stergulc, F., Tiberi, R., 2016 + updates. PARTE 2 – Valutazione della condizione delle chiome. Procedure di rilievo nelle aree di saggio e valutazione della condizione delle chiome. Manuale di campagna. Roma, Ministero delle Politiche Agricole, Alimentari e Forestali.

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PROTOCOL IN USE

ICP-FORESTS (phenology and damages)

Source: Raspe S, Fleck S, Beuker E, Bastrup-Birk A, Preuhsler T, 2020: Part VI: Phenological Observations. Version 2020-3. 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, 14 p. + Annex [http://www.icp-forests.org/Manual.htm]

Short description:

Weekly visit to the plot and manual recording of phenological events:

- Occurrence of flushing, flowering, fruiting, Lammas shoots or secondary flushing, colour change and leaf/needle fall
- Biotic damage (pests and/or diseases)
- Abiotic damage (e.g. frost, wind, hail)

The observations can be made manually by a field observer or with the use of automatic cameras that take pictures of the selected trees with a certain time interval.

sampling/mensurational frequency: weekly

sampling/mensurational scale: plot-level

Used by: ICP-Forests

Advantages: simple procedure, weather independent

Problems: low observation frequency due to weekly visit

Filename of the description: ICP_Manual_2020_part06_Phenology_version_2020-3.pdf

Reported parameters date of start and end of phenological events

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS

Digital cover photography (DCP) for assessment of crown structure and phenology

Soruce:

Chianucci, F., 2016. A note on estimating canopy cover from digital cover and hemispherical photography. *Silva Fennica*, *50*(1), pp.1-10.

Chianucci, F., Bajocco, S. and Ferrara, C., 2021. Continuous observations of forest canopy structure using low-cost digital camera traps. *Agricultural and Forest Meteorology*, *307*, p.108516.

Chianucci, F., Ferrara, C. and Puletti, N., 2022. coveR: An R package for processing Digital Cover Photography images to retrieve forest canopy attributes. *bioRxiv*.

Short descripition:

Digital canopy photography has been a widely used tool in forestry. Digital cover photography (DCP) is amongst an increasingly popular method (Macfarlane et al. 2007), which is based on collecting images with the camera equipped with a narrow lens, oriented upward, to achieve a restricted field of view (30° FOV). The method allows to estimate canopy cover, defined as the proportion of the ground covered by the vertical projection of tree crowns (Jennings et al. 1999). The method maximizes the full-frame, which bring several advantages: firstly, all the image pixels are used to sample a restricted portion of the crown, which allows to separate gap fraction into large, between-crowns gaps and small, within-crown gaps. Second, the sky luminance is even, and therefore the method is less sensitive to sky condition, camera exposure, and thresholding (Chianucci 2016; Macfarlane 2011). More importantly, the method can provide a reliable, replicable and more precise estimation of canopy cover, which can be used as a proxy of crown conditions, than traditional visual methods, which rely on the ability of observers and observation periods (Kennedy and Addison 1987), as well as the inability of most observers to distinguish between cover intervals smaller than 10% (Hahn and Scheuring 2003), and the quality of produced estimates cannot be inspected by users.

DCP can be used manually to estimate canopy cover. Images can be acquired with either a camera equipped with a short lens (e.g. a 50 mm lens) or by a smartphone. Images can be processed in R using the open library 'coveR' which is specifically tailored to process this kind of images (Chianucci et al. 2022)

Crown condition:

Sampling/mensurational frequency: twice in the vegetation peak (summer)

sampling/mensurational scale: plot-level

Used by: none

Advantages: simple procedure, transparent, robust, replicable

Problems: The method estimate canopy (crown and foliage) cover, not directly defoliation

Tree phenology:

DCP can be implemented in continuous-camera systems to achieve a daily estimate of canopy cover, from which it is possible to infer key transitional stage such as start of season (SOS) and end of season (EOS). An example is given in Chianucci et al (2021) which used the time-lapse feature of camera traps to achieve daily estimates of canopy cover, which has been also compared with satellite phenology metrics. Images can be processed in R using the open library 'coveR' which is specifically tailored to process this kind of images, and also has an EXIF feature for repeated photographs (Chianucci et al. 2022)

Phenology

Sampling/mensurational frequency: automated daily estimates using time-lapse cameras

sampling/mensurational scale: plot level

Used by: none

Advantages: simple procedure, transparent, robust, replicable , cheap

Problems: it allows to estimate sos, eos, los, peak, max, not suited for detecting flowering, fruiting, color changing (unless the camera is oriented inclined to the crown)

Reported parameters start of season (sos) , end of season (eos), length of season (los), peak of season (peak), max value (maximum value at the peak of season)

Suitable for monitoring under the NECD: YES

Chianucci, F., 2016. A note on estimating canopy cover from digital cover and hemispherical photography. *Silva Fennica*, *50*(1), pp.1-10.

Chianucci, F., Bajocco, S. and Ferrara, C., 2021. Continuous observations of forest canopy structure using low-cost digital camera traps. *Agricultural and Forest Meteorology*, *307*, p.108516.

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SENTINEL-2 TIME SERIES (TS) ANALYSIS TOOL (sen2rts)

Source: https://sen2rts.ranghetti.info/

Short description:

“sen2rts” is an R library which helps to extract and manage time series from Sentinel-2 archives (L. Ranghetti (2021). “sen2rts: Build and Analyse Sentinel-2 Time Series”. R package version 0.4.0. doi: 10.5281/zenodo.4682829, URL: https://sen2rts.ranghetti.info). S2 time series are processed to extract information about seasonality. The following steps are implemented:

1. produce raster archive with sen2r;
2. extract time series over spatial features;
3. smooth raw time series;
4. fill temporal gaps;
5. cut cycles;
6. assign cycles to specific seasons;
7. interpolate cycles;
8. extract phenological metrics;
9. aggregate time series over seasons.

Phenological metrics extracted are, for example: start of season (sos) , end of season (eos), length of season (los), peak of season (peak).

sampling/mensurational frequency: Not applicable

sampling/mensurational scale: plot-level/spatial feature

Used by:

Advantages: automatic processing (interpolating, gap filling, fitting) to extract phenological metrics from Sentinel-2 time series

Problems: low observation frequency due to high cloud cover, need of entire time series (at least year) to estimate phenological metrics

Filename of the description: 1-s2.0-S0168192321002008-main.pdf

Reported parameters start of season (sos) , end of season (eos), length of season (los), peak of season (peak), max value (maximum value at the peak of season)

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

Ecosystem chemistry

PROTOCOL IN USE AT PRESENT

ICP-FORESTS (Foliar Nutrients)

Source: Rautio P, Fürst A, Stefan K, Raitio H, Bartels U, 2020: Part XII: Sampling and Analysis of Needles and Leaves. Version 2020-3. 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, 16 p. + Annex

Short description:

The nutritional state of trees is often indicative of processes at the ecosystem level. Inadequate nutrient supply may be a direct cause of low tree vitality or a factor which increases adverse air pollution effects. High concentrations of certain elements in the leaf or needle tissues may be the effect of intoxication or of high air-pollution levels. Unfavorable chemical conditions in the rooting zone of the soil may also lead to imbalances in the nutrient supply and subsequently to unbalanced nutrition of the trees. Thus, sampling and analysis of needles and leaves is essential. The objective of foliar analysis is to estimate the nutritional status of trees and the impact of air pollutants at the monitoring sites, the detection of time trends and spatial patterns and to contribute to the understanding and quantification of forest condition in Europe. Specific objectives are the quantification of the mean element concentrations of N, S, P, Ca, Mg, K and C and other nutrients and heavy metals.

Following the ICP-Forest program sampling and analysis must be performed at least every two years, but many environmental changes might require annual sampling to be detected. In case sampling is performed biannually, the sampling should be performed in uneven years (2021, 2023, 2025 etc.) to obtain simultaneous data from different countries.

Used by: ICP Forests network and national monitoring networks

Advantages: robust, reproducible, tested, including QA/QC procedures

Problems: As trees must not be felled the operator has to reach the upper third of the crown climbing the tree,- this can be facilitated by installing a permanent pulley and rope. Hydraulic lift are not usable in the Italian sites. Sample transport and storage does not lead to contamination of the sample. For sample analysis quality control is particularly important.

File name of the description: ICP_Manual_2020_part12_Foliage_version_2020-3.pdf

Reported parameters:

foliar N, S, P, Ca, Mg, K and C concentration and the most significant ratios of such concentrations.

Suitable for monitoring under the NECD: YES

PROTOCOL IN USE AT PRESENT

ICP-FORESTS (Litterfall)

Source: Ukonmaanaho, L., Pitman R, Bastrup-Birk A, Breda N, Rautio P, 2020: Part XIII: Sampling and Analysis of Litterfall. Version 2020-1. 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 for Forests Ecosystems, Eberswalde, Germany, 18 p. + Annex

Short description:

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.

Litterfall analysis have to be performed at regular time intervals in order to establish potential relationships between changes in the stand condition and changes of the nutritional status

It is recommended to sample litterfall from at least 10 collectors per plot under uniform forest canopy, distributed all over the plot area, and litterfall is collected at least monthly and even bi-weekly in periods of heavy fall.

Litterfall must be sorted at least in foliar and non-foliar fractions before analysis, or in more detailed fractions.

Used by: ICP Forests network and national monitoring networks

Advantages: robust, reproducible, tested, including QA/QC procedures

Problems: need frequent visits to the plot, and sorting foliar and non-foliar fragments is time consuming

File name of the description: ICP_Manual_2020_part13_Litterfall_version_2020-1.pdf

Reported parameters:

C N S P and heavy metal concentration

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

EPIPHYTIC LICHENS

PROTOCOL IN USE AT PRESENT

ICP-Forests Manual, Part VII.2

Source: 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://icp-forests.net/page/icp-forests-manual]

Short description:

This Manual aims at providing a consistent methodology to collect sound, harmonized and comparable epiphytic lichen diversity data at selected ICP Forests monitoring plots. Its main objective is to monitor lichen diversity and its changes on monitoring plots and to contribute to the evaluation of forest condition in Europe. Specific objectives are: i) to assess the epiphytic lichen diversity (species richness, abundance and composition) on plots; ii) to detect its temporal changes; iii) to relate changing influences of natural and anthropogenic environmental factors (e.g., stand structure, tree composition, depositions) to epiphytic lichen diversity.

Assessment of epiphytic lichen diversity is carried out on 12 randomly selected living trees, growing on Level I or Level II plots. Epiphytic lichen diversity is sampled with a 10×50 cm grid, subdivided into five 10×10 cm quadrates, placed at each of the four cardinal points of the trunk (N, S, E, W) at 1 m above the ground on the trunk of the sample trees.

Results are presented in form of total number of species per plot, mean number of species per tree, and mean Lichen Diversity Value (LDV) per tree (sensu Asta et al. 2001; EN 16413 2014). Analyses consider both all lichens and only macrolichens (foliose and fruticose species). The LDV of a tree is calculated as the sum of the frequencies of all the lichen species present in the tree.

sampling/mensurational frequency: annual surveys

sampling/mensurational scale: tree-level

Used by: ICP Forests network

Advantages: standardised, robust, reproducible, tested, including QA/QC procedures

Problems: method based on a medium to high level of expert assessment. It requires specialists with a good taxonomical knowledge of the most frequent epiphytic lichen species

File name of the description: ICP_Manual_2016_part07_2_Lichen_version_2016-2.pdf

Reported parameters (separately for all lichens and only macrolichens): i) Tree level: mean number of species; mean Lichen Diversity Value (LDV); ii) Plot level: total number of species

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS:

ICP-Integrated Monitoring

Source: Manual for Integrated Monitoring (1998) Finnish Environment Institute, ICP IM Programme Centre, Helsinki, Finland. www.syke.fi/nature/icpim > Manual for Integrated Monitoring. (Accessed 12.01.2021).

Subprogramme EP: Trunk epiphytes (Chapter 7.20) (1998). In: Manual for Integrated Monitoring. 1998. www.syke.fi/nature/icpim > Manual for Integrated Monitoring. (Accessed 12.01.2021).

Short description:

The aim is to obtain sensitive bioindication in epiphytic lichens of changes in, primarily, acidifying deposition. The method is based on lichens on the bark of living trees. Observe either all lichen species or, if this is not possible, at least most fruticose and foliose plus a few crustose species which can be easily identified and have indicator values. The observations are made on tree trunks between 50 and 200 cm above ground, either by the line cover (A), the point frequency (3) or the species list (C) methods, each of them including observations on lichen health (D).

Method A: line cover. Note lichens along a measuring tape fastened around the trunk of each sample tree at one or more levels above the ground (120 cm is mandatory; 60, 90, 120, 150 cm are optional).

Method B: point frequency. The method is based on a quadrat with a number of points, where species hits are counted.

Method C: list of lichen species occurring between 50 and 200 cm above ground on each tree.

Method D: lichen health. Additional parameters to methods A-C. Thallus length (measure the tallest thallus for each pendulous lichen species on each tree) and vitality (overall vitality of common species such as *Hypogymnia physodes* or *Parmelia sulcata*, ordinal 5-classes scale of damage).

sampling/mensurational frequency: from one to five years

sampling/mensurational scale: tree-level

Used by: ICP IM Programme

Advantages: easy to apply, the combination of several methods provides complementary information

Problems: it requires expert assessment; the experimental protocol is not sufficiently rigorous or standardised, and it leaves too open and subjective choices in the various phases of the method (for example which of the methods to use, whether to consider all species or only the foliose or fruticose ones)

Filename of the description: ICP IM Manual - August 1998.pdf

Reported parameters: list of epiphytic lichen species, lichen health of single common species

Suitable for monitoring under the NECD: YES. The method of Stofer et al. (2016) can be considered as one of the possible methods listed in your ICP IM Manual (1998), and in particular method C (§ 7.20.2.2), that suggests to 'establish a species list of lichens occurring between 50 and 200 cm above ground on each tree on the plot'.

OTHER PROTOCOLS:

Forest Inventory and Analysis (FIA) Lichen Communities Indicator field protocol

Source: USFS, 2011. Phase 3 Field Guide – Lichen Communities. Version 5.1 October, 2011. United States Forest Service. Web site accessed 12.01.2022. https://www.fia.fs.fed.us/library/field-guides-methods-proc/index.php

Short description:

Phase 3 of the monitoring programme consists of a subset of Phase 2 sample plots which are measured for a broader suite of forest health attributes including tree crown conditions, lichen community composition, understory vegetation, down woody debris, and soil attributes.

The purpose of the lichen community indicator is to use lichen species and communities as biomonitors of change in air quality, climate change, and/or change in the structure of the forest community.

The objectives are to determine the presence and abundance (from rare to abundant, 4-classes ordinal scale) of epiphytic macrolichen species (fruticose and foliose) on living and dead trees (including also recently fallen branches and logs) in each plot and to collect samples to be mailed to lichen specialists.

Note that the crew member responsible for this task is not required to accurately assign species names to the lichens (that is done later by a specialist) but must be able to make distinctions among species.

As a reference of papers reporting the results of this method refer to: Jovan and McCune (2005), Geiser and Neitlich (2007), Geiser et al. (2014, 2021), McDonough et al. (2015).

sampling/mensurational frequency: annual surveys

sampling/mensurational scale: plot-level

Used by: United States Forest Service - Forest Inventory and Analysis (FIA)

Advantages: it does not require the expert assessment of specialists during field surveys, easy to apply since it considers only macrolichens (foliose and fruticose species)

Problems: with respect to the European method (Stofer et al. 2016) it includes only macrolichens, and lichen diversity (species list and abundance) is assessed at plot level; the lack of specialists in the field makes difficult the identification of very similar foliose or fruticose species (e.g., species belonging to *Usnea*, *Cladonia, Ramalina* genera)

Filename of the description: field_guide_p3_5-1_sec21_10_2011.pdf

Reported parameters: list and abundance of epiphytic macrolichen species (plot level)

Suitable for monitoring under the NECD: YES. Simplified method that does not require the involvement of lichen specialists in the field surveys

OTHER PROTOCOLS

Finnish air quality epiphytic macrolichen survey method

Source: Mayer A. L., Vihermaa L., Nieminen N., Luomi A., Posch M., 2009. Epiphytic macrolichen community correlates with modeled air pollutants and forest conditions. Ecological Indicators 9: 992–1000. https://doi.org/10.1016/j.ecolind.2008.11.010; SFS standard 5670, 1990. Air Quality. Bioindication. Mapping of Epiphytic Lichens. Finnish Standards Association, Helsinki, [In Finnish]. See also Kinnunen et al. (2003)

Short description:

The aim is to monitor air quality through the presence of a set of epiphytic macrolichen species, and the abundance and degree of damage observed in two species with known pollution sensitivity, *Hypogymnia physodes* and *Bryoria* spp. Epiphytic macrolichen species presence and abundance are quantified on the trunk bark of 5 standard trees per each forest site. A 30×40 cm transparent sampling grid (divided into 100 squares of equal size, with each center marked with a dot) is placed on the two opposite sides of each tree (e.g., according to the prevailing wind direction), from 120 to 160 cm above the ground. The abundance of *H. physodes* and *Bryoria* spp. is measured by counting the number of dots in each grid hosting these species. A five-class damage score is assigned to the most damaged *H. physodes* individual observed between 50 and 200 cm above the ground. The presence of the following epiphytic macrolichen species at 100-200cm height is assessed: *H. physodes*, *Parmeliopsis ambigua*, *Imshaugia aleurites*, *Parmeliopsis hyperopta*, *Hypocenomyce scalaris*, *Bryoria* spp., *Usnea* spp., *Platismatia glauca*, *Cetraria pinastri*, *Pseudevernia furfuracea*, *Cetraria chlorophylla*, *Parmelia sulcata* and *Scoliciosporum chlorococcum*. This list includes common epiphytic species found on pine trees with a range of air pollution sensitivity. Starting from the presence/absence data for the epiphytic macrolichen species, the Index of Atmospheric Purity (IAP) is calculated for each forest site.

sampling/mensurational frequency: annual surveys

sampling/mensurational scale: plot-level

Used by: Finnish Forest network

Advantages: standard method; it provides information on pollution sensitive macrolichens; it gives quantitative measures

Problems: the list of species is suitable only for pine trees; some parts are subjective (e.g., the identification of the most damaged *H. physodes* individual)

Filename of the description: https://doi.org/10.1016/j.ecolind.2008.11.010

Reported parameters: i) Tree level: presence, abundance and vitality of *Hypogymnia physodes* and *Bryoria* spp.; presence and abundance of a ‘closed list’ of macrolichen species; ii) Plot level: Index of Atmospheric Purity (IAP)

Suitable for monitoring under the NECD: YES. But a new ‘closed’ list of species should be specifically selected for Italian or Mediterranean forest types

OTHER PROTOCOLS

Guidelines for the use of lichens as bioaccumulators (Italian method, ISPRA)

Source: Giordani P., Benesperi R., Bianchi E., Brunialti G., Cecconi E., Contardo T., Di Nuzzo L., Fortuna L., Frati L., Loppi S., Monaci F., Munzi S., Nascimbene J., Paoli L., Ravera S., Tretiach M., Vannini A., 2020. Guidelines fo the use of lichens as bioaccumulators. ISPRA Manuali e Linee Guida 189/2019. ISBN 978-88-448-0966-9. https://www.isprambiente.gov.it/files2020/pubblicazioni/manuali-e-linee-guida/guidelines-for-use-of-lichens-as-bioindicators_def.pdf

Short description:

The use of lichens for assessing atmospheric levels and deposition patterns of trace elements is well-established. These guidelines (developed by the Italian Lichen Society) address the use of epiphytic lichens (bioaccumulation by native lichens and lichen transplants) to monitor spatial and temporal trends of element concentrations (macro-elements and trace elements) related to air pollutant depositions.

Native lichens: samples of the selected species (foliose or fruticose lichens) are collected on the trunk or on the branches of standard trees above 100 cm from the ground to avoid terrigenous contamination. The outermost portions of lichen thalli (the dimension is species-specific) is selected and cleaned from pieces of bark or other extraneous materials in order to properly obtain a sample.

Lichen transplants: samples of foliose or fruticose epiphytic lichens are collected in a background area (responding to a strictly defined standard protocol). The lichen material is prepared for field exposure (e.g., preparation of exposure devices). The lichen samples (or exposure devices containing them) are exposed in the survey plots for a period ranging between 4 and 12 weeks (to be *a priori* defined).

Preparation and chemical analysis: each lichen sample (both native and transplanted) is pulverized or finely chopped, mineralized by means on an acid digestion and subject to the analytical determination of macro-elements and trace elements (to be selected according to the aim of the project; mostly Al, As, Cd, Cr; Cu, Hg, Ni, Pb, Ti, V, Zn)

sampling/mensurational frequency: annual surveys, together with lichen diversity assessment

sampling/mensurational scale: plot-level

Used by: ISPRA

Advantages: standard method from sampling to data processing and interpretative scales (see also Cecconi et al. 2019); easy to survey in the field

Problems: need to identify more than one biomonitor species suitable for national use

Filename of the description: guidelines-for-use-of-lichens-as-bioindicators_def.pdf

Reported parameters: content in lichen thalli of macro-elements and trace elements (to be selected according to the aim of the project; mostly Al, As, Cd, Cr; Cu, Hg, Ni, Pb, Ti, V, Zn)

Suitable for monitoring under the NECD: YES. It can represent a good complementary method to lichen diversity assessment, in order to obtain information on trace element pollution in forest sites

MONITORING ACTIVITY:

WATER CHEMISTRY

PROTOCOL IN USE AT PRESENT

ICP-WATERS

Source: ICP Waters Programme Manual 2010. ICP Waters Report 105/2010. Report No. 6074-2010: 91 pp.

Short description:

Specific criteria for site selection are provided. Sites should be selected avoiding situations of impacts from local pollution sources (e.g., domestic sewage, industrial wastewater, agriculture etc.). Sites to be selected are likely to change in response to air pollution. Drainage lakes (i.e., with an outlet) and with a moderate water renewal period are best suited for monitoring. A small river or brook is preferable, but large enough to sustain a permanent flow throughout the year. The presence of upstream lakes should be minimal.

Catchments and site information should be provided using specific forms to the Programme Centre, once for each monitoring site. Registration forms for all sites and forms for data reporting are available at: http://www.icp-waters.no/.

Specific indications for both river and lake samplings are provided in the Manual: as a rule, the sample should be representative of the whole water body and avoid local gradients. Cautions should be taken to avoid contamination of the samples.

The use of adequate methods is the responsibility of each national focal centre providing the data. However, the use of international standard methods such as those prescribed by ISO/CEN is recommended. A list of ISO and CEN methods to be used for analysis and QA/QC procedures in the laboratory is provided in the Manual. In addition to the QA/QC adopted by each laboratory, a quality check of the data is performed using dedicated forms for data reporting, including the ionic balance and the comparison between measured and calculated conductivity.

The ICP WATERS Programme Centre organizes an intercomparison exercise on annual basis, usually considering the following variables: pH, Conductivity, Alkalinity, NO_3-N, Cl, SO_4, Ca, Mg, Na, K, TOC, Tot-P, Al, Fe, Mn, Cd, Pb, Cu, Ni, and Z. The participation is recommended for the laboratories providing data to the Programme. Further information are available at: https://www.icp-waters.no/data/quality-control/

sampling/mensurational frequency: monthly sampling when possible. Alternatively, the samples must be taken at the same time of the year each year, year, preferably shortly after fall overturn for lakes. Monthly sampling is recommended for stream site, due to the higher interannual variability of water chemistry. At Italian sites, monthly sampling is used for stream sites, annual or biannual sampling for lakes.

sampling/mensurational scale: site level

Used by: ICP WATERS network

Advantages: criteria for site selection and procedures for sampling, analyses and quality check of the data are clearly provided and are specific for sites which must give information on the impacts of atmospheric pollution, so that the protocol is particularly suitable for the monitoring under the NECD. Parameters fit with those required by Annex V of the NECD. Data are already collected using standard forms/templates which fit well with the present reporting scheme of the NECD.

Problems: requires quite frequent sampling and an extended set of parameters to be considered; a high level of analytical quality is required. Monitoring should be maintained in the long-term, so that dedicated resources are needed

File name of the description: 6074_BMW_ICP_Waters_Manual _April_2011_Revised_Sept_2015

Reported parameters (separately for lakes and rivers): Alkalinity, SO_4, NO_3-N, Cl, DOC/TOC, pH, Ca, Mg, Na, K, NH_4-N, LAL, Specific conductivity, TOTP (mandatory); water temperature, runoff, TOTN, ORTP, Dissolved oxygen, SiO_2, F, colour, turbidity and Total Al (optional).

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS:

Water Framework Directive (WFD; 2000/60/CE)

Source:

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02000L0060-20141120

DIRETTIVA 2000/60/CE DEL PARLAMENTO EUROPEO E DEL CONSIGLIO del 23 ottobre 2000 che istituisce un quadro per l'azione comunitaria in materia di acque

https://www.mite.gov.it/sites/default/files/direttive-acque/direttiva_2000-60-ce.pdf

Short description:

The purpose of the Directive is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater. Member States shall establish programmes for the monitoring of water status within each identified river basin district. For surface water these programmes shall cover both the ecological and chemical status. Member states identify the location and boundaries of bodies of surface water, establish reference conditions, identify pressures and assess the susceptibility of the surface water status to these pressures. Annex V specifies the quality elements for the classification of ecological status (biological elements, and hydromorphological and chemical and physico-chemical elements supporting the biological elements) and defines the monitoring of ecological status and chemical status for surface waters (design, frequency etc.). Monitoring results led to a classification of ecological status and ecological potential into 5 classes: high, good, moderate, poor and bad.

sampling/mensurational frequency: depending on the type of monitoring and on the quality elements (e.g., usually 3 months for physico-chemical elements)

sampling/mensurational scale: surface water bodies

Used by: all member States

Advantages: clearly defined and standardised protocols for monitoring of surface water bodies, shared by all Member States. Monitoring data are already available.

Problems: the monitoring under the WFD is not specifically targeted to assess the effects of atmospheric pollutants, if this is not the main pressures. Sites are in most cases not suitable to this type of assessment, especially in countries where other pressures than acidification/deposition of atmospheric pollution are more relevant (e.g., eutrophication). Even tough data are routinely collected, it would be necessary to identify proper sites (e.g., "reference" sites within the WFD monitoring networks of each region), gather the data and adapt them to the present NECD reporting scheme.

Filename of the description: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02000L0060-20141120

Reported parameters:

See Annex V of Directive 2000/60/EC

Suitable for monitoring under the NECD: Partially. Some of the monitored parameters can be useful for the assessment under the NECD. However, not all the required parameters by Annex V of the NCD are considered by the WFD; further, frequency under the WFD is often too low.

MONITORING ACTIVITY:

Macroinvertebrates - High altitude lakes

PROTOCOL IN USE AT PRESENT

ICP-WATERS and ISPRA protocol "Biological methods for inland surface waters"

Source: ICP Waters Programme Manual 2010. ICP Waters Report 105/2010. Report No. 6074-2010:

https://drive.google.com/file/d/0B_DU7Rk3lFWYQmwzZEZCSnh2VEk/view?resourcekey=0-qV3yQS4dzDj3O4YqQPBpJQ

ISPRA Manuals and Guidelines 2014:

https://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/MLG__111_2014_Metodi_Biologici_acque.pdf

Short description:

Semi-quantitative macroinvertebrate samples are taken, during the ice-free season and before insect diapause, through a disturbance-removal sampling after 2-5 min handle-netting the substrate (250 µm mesh size), possibly complementary with sampling for water chemistry. Different habitats are considered (silt, gravel, pebbles, boulders, rock faces) on ≥ 0.5-meter-wide littoral- or river-reaches. The samples are then fixed with 80% alcohol and transported to the laboratory. Sorting is performed at genus/species level; identification involves the use of specific taxonomic keys in use at national and at European levels: several guides related to macroinvertebrates in general (AA.VV. 1977-1985); Timm (2009) and Schmelz and Collado (2010) for oligochaetes; Andersen et al. (2013) for Chironomids.

sampling/mensurational frequency: once or twice per year, depending on site

sampling/mensurational scale: site level (replicated samples at each site to consider different habitats)

Used by: ICP WATERS network

Advantages: the identification at species level allows the application of several metrics, including those in use for the evaluation of the acidification status within ICP WATERS, or other metrics that could be useful to assess the overall ecological status of the freshwater site.

Problems: sampling and laboratory protocol are time-consuming; the identification at specie level requires a high level of expertise. The ICP WATERS protocol and the suggested indicators are targeted to acidification, but other pressures could be relevant (e.g. N enrichment, climate change). Further, indicators are often based on species from Nordic countries (e.g. Norway) and river habitats while it would be useful to develop suitable indicators for other ecoregions and specific for lakes.

File name of the description: 6074_BMW_ICP_Waters_Manual _April_2011_Revised_Sept_2015

MLG__111_2014_Metodi_Biologici_acque.

Reported parameters: list of invertebrate taxa (possibly at species level) and relative abundance

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

Macroinvertebrates - Lowland lakes

PROTOCOL IN USE AT PRESENT

Source: Benthic Quality Index for Italian Lakes (BQI)

Boggero A., S. Zaupa, B. Rossaro, V. Lencioni, L. Marziali, F. Buzzi, A. Fiorenza, M. Cason, F. Giacomazzi, & S. Pozzi. 2013. Protocollo di campionamento e analisi dei macroinvertebrati negli ambienti lacustri. MATTM-APAT, Roma, 17 pp.

Boggero A., Zaupa S., Cancellario T., Lencioni V., Marziali L., Rossaro B. 2016. Italian Classification method for macroinvertebrates in lakes. Method summary. Report CNR ISE, 03.16: 16 pp.

Short description:

In lakes were obvious depth gradient exists the sampling may be laid out in the form of a stratified sampling, working on transects with sampling sites arranged to give equal coverage of the different zones. Each site is located along transects connecting the shore-line to the maximum depth, in 3 different sampled areas (littoral, sublittoral and profundal). A sample consists of 3 grab replicates per site (minimum effort to obtain good data set) to a maximum of 9 replicates in a transect. Sampling is performed through the use of an Ekman grab with an area of 0.0225 m^2 of lake bottom. The number of transects depends on lake area and their position is based on expert knowledge. After taking samples, each of them has to be washed through a sieving net with a mesh size of 250 µm to efficiently catch even the smallest organisms, placed in a plastic bottle and preserved with formalin (5%). The sample treatment in the lab includes: sorting of organisms under a stereo-microscope; identification to species level, including Chironomids and Oligochaetes. The genus level is considered when the occurrence of immatures hindered species identification.

sampling/mensurational frequency: The sampling period is February to April (Spring) and September to October (Autumn) due to Insects life-cycles, with a biannual sampling frequency (i.e. during turnover and after stratification periods).

sampling/mensurational scale: site level

Used by: Regional Agencies for Environmental Protection (ARPA)

Advantages: the protocol has been developed in compliance with the WFD, and allows a high level of comparability with other national networks. The identification at species level, when available, allows the application of several metrics, including those in use for the evaluation of the acidification status within ICP WATERS, or other metrics that could be useful to assess the ecological status of the site.

Problems: sampling and laboratory protocol are time-consuming; the identification at specie level requires a high level of expertise. The protocol is targeted to assess eutrophication as specific pressure, not for atmospheric pollution impact: proper metrics/indicators should be developed.

File name of the description: REPORT CNR-ISE_Italian Classification method for macroinvertebrates in lakes (see http://www.vb.irsa.cnr.it/wfd-en)

Reported parameters: List of invertebrate taxa. Numbers are expressed as individual counts abundance and related to area as number of individuals per 1 m^2 (ind m^{-2}).

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

Macroinvertebrates – Wadable streams and rivers

PROTOCOL IN USE AT PRESENT

Source: Protocollo di campionamento dei macroinvertebrati bentonici dei corsi d'acqua guadabili (national method to collect samples of macroinvertebrates in wadable rivers in compliance with the requirements of the WFD 2000/60/EC).

https://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/metodi-biologici-acque/fiumi-macroinvertebrati.pdf

Short description:

The protocol focus on a multi-habitat scheme, where habitats are sampled in proportion to their presence within a reach of about 25-50 m. Sampling is usually performed with a Surber net (area 0.05 to 0.01 m^2; mesh size 0.5 mm). A full sample consists of 10 sampling units from different microhabitat types occurring in at least 10% of the sampling sites. Abundance of most taxa may be estimated in the field; however, when taxonomic details are needed, laboratory analysis is required. In general, the level of identification will be different according to the monitoring scheme and to the metric required to evaluate the ecological status.

sampling/mensurational frequency: seasonal

sampling/mensurational scale: site level

Used by: Regional Agencies for Environmental Protection (ARPA)

Advantages: the protocol is highly detailed and standardised and allow a high level of comparability among sites and networks.

Problems: sampling and laboratory protocol are time-consuming. The identification at family level is enough for the operational monitoring, but affects metrics and indicators that can be applied. The application of the available metrics is affected also by the altitudinal extreme of temperatures limiting the occurrence of eurithermal species. Further, the protocol is not targeted to assess specific pressure such as atmospheric pollution impact: proper metrics/indicators should be developed.

File name of the description: MACROINVERTEBRATI ACQUATICI notiziarioirsa_mar2007 (see http://www.irsa.cnr.it/index.php/ita/prodotti-della-ricerca/notiziario)

Reported parameters: List of invertebrate taxa. Numbers are expressed as individual counts abundance and related to area as number of individuals per 1 m^2 (ind m^{-2}).

Suitable for monitoring under the NECD: Partly

MONITORING ACTIVITY:

Epilithic diatoms

PROTOCOL IN USE AT PRESENT

ICP-WATERS/ ISPRA protocol "Biological methods for inland surface waters"/CEN EN 13946:2014

Source:

ICP Waters Programme Manual 2010. ICP Waters Report 105/2010. Report No. 6074-2010: https://drive.google.com/file/d/0B_DU7Rk3lFWYQmwzZEZCSnh2VEk/view?resourcekey=0-qV3yQS4dzDj3O4YqQPBpJQ

ISPRA Manuals and Guidelines 2014: https://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/MLG__111_2014_Metodi_Biologici_acque.pdf

CEN EN 13946:2014 - Water quality - Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes: https://standards.iteh.ai/catalog/standards/cen/7a0de152-2689-4d1b-b012-46e980cb045a/en-13946-2014

Short description: At stream sites at least 5 cobble size stones are selected from pools in three discrete locations over a 50 m reach. Stones should be collected from a depth below that of minimum flow. At lake sites at least 5 cobble size stones are selected from a depth of 20 - 30 cm from 2 - 4 surveyed locations around the shore, avoiding stream inflows. Total surface considered should be at least 1 m^2. Stones with attached macrophytes or those covered in sediment should be avoided. Diatoms are removed by brushing into a tray or funnel, and decant into plastic vials. Sample may be prepared using standard techniques. In the laboratory, samples were treated with H_2O_2 and HCl according to standard techniques and mounted with Naphrax on glass slide. Three hundred to five hundred valves should be counted from each sample and identified to species level where possible.

sampling/mensurational frequency: multiple or once per year depending on site. Samples should be collected during the same season each year. The same location and substrate has to be sampled at each site on each sampling occasion.

sampling/mensurational scale: site level (multiple sampling points at each site)

Used by: ICP WATERS network; Regional Agencies for Environmental Protection (ARPA)

Advantages: stone surfaces provide a stable and easily comparable habitat; further, epilithon is usually preferred for biomonitoring. The identification at species level allows calculating several ecological indexes, as well as the classification by pH preference, used in evaluation of the acidification status.

Problems: the identification at species level requires a good level of expertise. It would be advisable to develop specific indicators according to the typical species of a given site/region. With this method, relative abundance can be calculated, but there is not a quantitative assessment (e.g. in relation to a surface unit).

File name of the description: 6074_BMW_ICP_Waters_Manual _April_2011_Revised_Sept_2015

MLG__111_2014_Metodi_Biologici_acque

Reported parameters (separately for lakes and rivers): list of diatom taxa (possibly at species level) and relative abundance; classification according to pH preference

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS

The UK Environmental Change Network Protocols for Standard Measurements at Freshwater Sites – FTD Protocol for epilithic diatoms

Source:

UK Environmental Change Network - http://www.ecn.ac.uk/measurements/freshwater/fdt

Battarbee, R.W. 1986. Diatom analysis. In: Handbook of Holocene palaeoecology and palaeohydrology, edited by B.E.Berglund, 527–570. Chichester: Wiley.

Patrick, S.T., Waters, D., Juggins, S. & Jenkins, A. eds. 1995. The United Kingdom Acid Waters Monitoring Network. Site descriptions and methodology report. London: ENSIS Ltd.

Short description: Standing waters: three spatially discrete littoral locations, which are not unduly influenced by inflow streams or localised catchment disturbance, heavy shade etc, are selected around the shore. The locations are recorded on a sketch map. Running waters: 50 m length of stream is selected; three sampling locations, which are not unduly influenced by inflow streams, localised catchment disturbance, heavy shade, etc, are selected along this length. Grid references of each sampling location are recorded. At each location five permanently submerged cobble-sized stones, ideally from a depth >30 cm in lakes, are selected and epilithic diatoms removed and preserved. The stones should be rinsed 2–3 times with a few drops of distilled or filtered water and re-brushed. The bulk sample for each sampling location is homogenised in the tray, and a subsample is then decanted to fill a plastic vial and preserved with 2–3 drops of Lugol's iodine. At sites where a suitable habitat for epilithon is unavailable, an epiphytic sample can be collected. Each sample is identified uniquely. Samples should be stored in the dark in a cool environment.

sampling/mensurational frequency: Sampling is carried out preferably three times each year, in March–April, June–July and September–October. If only annual sampling is possible, this should be carried out during September.

sampling/mensurational scale: site level (multiple sampling points at each site)

Used by: ECN (follows that used for the United Kingdom Acid Waters Monitoring Network)

Advantages: same as ICP WATERS Protocol.

Problems: the identification at species level requires a good level of expertise. As for the ICP WATERS protocol, this method provides a qualitative (relative abundance) but not quantitative assessment.

File name of the description: ECN_UK_FDT.pdf

Reported parameters: annual mean percentage frequency (from replicate samples) of taxa.

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS

Freshwater ecology: quantitative periphyton biomass sampling methods

Source:

New Zealand Department of Conservation – Te Papa Atawhai

https://www.doc.govt.nz/globalassets/documents/science-and-technical/inventory-monitoring/im-toolbox-freshwater-ecology/im-toolbox-freshwater-ecology-quantitative-periphyton-biomass-sampling-methods.pdf

https://www.doc.govt.nz/globalassets/documents/science-and-technical/inventory-monitoring/im-toolbox-freshwater-ecology/im-toolbox-freshwater-ecology-periphyton-taxonomy-methodology.pdfBiggs, B.J.F.; Kilroy, C. 2000: Stream periphyton monitoring manual. Prepared for the New Zealand Ministry for the Environment. National Institute of Water and Atmospheric Research, Christchurch.

https://niwa.co.nz/sites/niwa.co.nz/files/import/attachments/peri_complete.pdf

Short description: Quantitative sampling methods designed to estimate biomass for a known area. It provides data suitable for statistical testing of differences amongst sites to detect impact effects. Sampling points are located along a single transect and 10 points sampled. The methods described in Biggs & Kilroy (2000) can be used on different substrates such as gravel, cobble, boulder, sand and silt.

sampling/mensurational frequency: depending on monitoring purpose

sampling/mensurational scale: site level (10 or more sampling points at each site)

Used by: New Zealand Department of Conservation – Te Papa Atawhai

Advantages: Provides reliable, precise and detailed information about periphyton biomass in a stream reach.

Problems: the identification at species level requires a good level of expertise. Material may be lost or degraded during sample collection and transport, negatively biasing estimates of biomass. Samples must be processed rapidly. Stream must be wadable.

File name of the description:

im-toolbox-freshwater-ecology-quantitative-periphyton-biomass-sampling-methods.pdf

Reported parameters: average algal biomass and composition

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:

GROUND VEGETATION

PROTOCOL IN USE AT PRESENT

Source: Canullo R, Starlinger F, Granke O, Fischer R, Aamlid D, Dupouey JL, 2020: Part VII.1: Assessment of Ground Vegetation. Version 2020-1. 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, 14 p. + Annex

ISBN: 978-3-86576-162-0 [http://icp-forests.net/page/icp-forests-manual]

Short description:

This Manual aims at providing a consistent methodology to collect high quality, harmonized and comparable forest ground vegetation data at selected UN/ECE ICP Forests monitoring plots (within monitoring sites). Its main objective is to to estimate the status and changes in the diversity of plant communities at the monitoring plots and to contribute to the understanding and evaluation of forest condition in Europe. Specific objectives are: i) to census plant species and their relative abundance per vegetation layer; ii) to distinct the structural diversity in terms of species composition, percentage cover and vertical stratification; iii) to estimate the species richness (density) in terms of mean species number / area; IV) to detect temporal changes in vegetation and mean species density on a plot.

The assessment of ground vegetation diversity should take into account the trade-off between the accuracy of species cover estimation and the surveyed area, the expected ranges in the spatial pattern (homogeneity) and the structure of forest vegetation within Europe. In the view of this clarification two different sampling designs are proposed to achieve a common sampling area (CSA) to survey Level I plot (2000 m^2) and level II sites (2500 m^2):

- for Level I plot (2000 m^2) and level II sites (2500 m^2) vegetation surveys are performed over a large area, utilizing sampling units of a surface ≥ 100 m^2, with a low to medium accuracy in specific cover estimates
- for level II sites vegetation surveys are concentrated on detecting small-scale dynamics in plant populations. Small sampling units (in general ≤ 10 m^2) are used for a more accurate estimation of specific cover

  CSA of 400 m^2 representative for the ground vegetation of the plot, is mandatory for Level II plots. This area will be reached as the sum of smaller sampling units within the plot. Countries are free to select the shape of the sampling units; number (thus size) of sampling units must fulfill the objective of representativeness.

Selection and location of the sampling units

In the case of Level I, the use of four not contiguous 10 m x 10 m sampling units randomly placed within the circular plot generated from Level I points (radius of 25.24 m = 2000 m^2 and excluding the inner circle having a radius of 11.28 m (400 m^2). For level II the sampling units should be not contiguous, placed as far apart as possible to minimize spatial autocorrelation and should exclude major heterogeneities at any scale of sampling (boulders and cliffs, tracks and paths, fire places, streams and ponds, ditches and channels,peaty pools).

Layers to be recorded

- moss layer (i.e. terricolous bryophytes and terricolous lichens),
- herb layer (all non-ligneous irrespective of height, and the ligneous only if ≤ 0.5m height), including eventual seedlings and browsed trees,
- shrub layer (only ligneous and all climbers) > 0.5 m and ≤ 5 m height,
- tree layer (only ligneous and all climbers) > 5 m height.

With available lists of species (to be derived from the specific cover by layers) it is possible to estimate the mean species density (with associate error and interval of confidence when national data per sampling units at national level are available). When possible, the estimated mean number of species can be related to the total number of species surveyed on the overall plot area, in order to test the indicator value of the estimate. Species-specific cover values per layer and global layer coverage may be used to define the compositional structure according to recognized vegetation types.

sampling/mensurational frequency: Vegetation studies on Level II ICP Forests Monitoring sites must be undertaken at least every five years. In order to help separating short-term fluctuations from long-term vegetation dynamics, it is recommended that vegetation assessment is undertaken every year. A clear indication for sampling frequency for Level I plots is not reported.

sampling/mensurational scale: ground vegetation

Used by: ICP Forests network

Advantages: systematic sampling for Level I plots, standardized, robust, reproducible, tested, including QA/QC procedures

Problems: method based on a medium to high level of expert assessment, preferential sampling for level II sites (but sites representative of the forest type to be surveyed)

File name of the description:

ICP Forests_Manual_2020_part07-1_Ground_Vegetation_v.2020-1

Reported parameters:

- Tree layer cover sampling area;
- Shrub layer height sampling area;
- Shrub layer cover sampling area;
- Herb layer height sampling area;
- Herb layer cover sampling area;
- Moss layer cover sampling area;
- Baresoil cover sampling area;
- Litter cover sampling area

Suitable for monitoring under the NECD: YES, partially

PROTOCOL IN USE AT PRESENT

Manuale di campo per lo studio della biodiversità vegetale – rete NEC Italia

Source: application of ICP Forests principles, *http://www.scienzadellavegetazione.it/sisv/libreria/braun-blanquetia/BRBL48.pdf*

Short description:

This Manual aims at providing a consistent methodology to collect sound, harmonized and comparable data on plant diversity at taxonomic level, as response factor. Target organisms are terricolous vascular plants, lichens and bryophytes. A vegetation probabilistic sampling is adopted to represent the plot-level state (2500m^2). Species abundance by layers are surveyed at a single 100m^2 scale by 12 permanent sampling units on a regular 10 m x 10 m grid within the plot, repeated on a distributed comparable system in the buffer zone. Structural data as layers, litter, bare soil and total cover are also assessed.

Surveys are performed in summer, and can be optional repeated in spring also, by trained teams. A QA/QC protocol is described, including observers' calibration.

Population level sampling on 100 systematic 50 cm x50 cm sampling units, within the plot, have been tested optionally

Species nomenclature follows a standardized flora. Results are presented in form of total number of species per plot, mean number of species per sampling unit, species-abundance per layer per sampling unit, and relative cover by layers.

sampling/mensurational frequency: annual to (at least) five years

sampling/mensurational scale: Sampling Unit level

Used by: CONECOFOR - Italy.

Advantages: standardized bases, robust assessment of species density, widely tested, including QA/QC procedures.

Problems: overlooked functional scale-dependent data; observers' effect to be always tested; plot fencing implies inside and outside fence surveys.

File name of the description: 2012 GV CONECOFOR BRBL48manuale.pdf

Reported parameters: Total coverage, Average height and cover visual assessment of layers, presence and relative species cover by layer; derived species density per plot.

Suitable for monitoring under the NECD: YES, partially

OTHER PROTOCOLS

Terrestrial Ecosystem Research Network (TERN) - Australian territory

Wood S., Stephens H., Foulkes J., Bowman D. (2014) AusPlots Forests Survey Protocols Manual. Version 1.6. University of Tasmania. http://www.ausplots.org/science-outputs#MANUALS

Short description:

The overall objective of the (TERN) AusPlots Forests Survey Protocols Manual is to "provide a national institutional infrastructure network for terrestrial ecosystem research" under which AusPlots Forest aims to: Establish a continental-scale plot based monitoring network that improves our understanding of tree growth, forest productivity and carbon dynamics in eucalypt forests in relation to macro-environmental gradients across Australia. In this context AusPlots Forests has closely followed survey protocols developed by established national and international plot networks. This methodological consistency ensures that the data can be seamlessly integrated into existing forest inventory databases (e.g. Forestplots.net) and contribute to global meta-analysis of forest dynamics.

The one hectare plot is 100m x 100m location is determined before going into the field and is dependent on the hypotheses and research questions that motivate the establishment of the AusPlot. The plot center or the plot corners area accurately located and stored in a GPS or recorded on a high quality map or imagery. This initially involves speaking to local forest ecologists and a desktop exercise using GIS layers of vegetation type (and structure) and satellite imagery, followed by a reconnaissance field trip to assess a range of identified forest stands. The plot is then divided up into twenty-five 20m x 20m subplots.

Despite the AusPlots Forest is focused on tree level a description of understorey survey approach is provided as follow: brief qualitative description of the floristic and structural nature of the understorey. Include information on the dominant guild (e.g. sclerophyll, fern, grass, rainforest), dominant species, approximate cover and height and stratum. This information provides useful context when interpreting the dynamics of the overstorey trees. Use subplots to detail spatial distribution of notable vegetation communities.

Used by: Australian public bodies/partners

sampling/mensurational frequency: not reported

sampling/mensurational scale: Sampling Unit level

Advantages: Most recent, choice of plot location dependent on the research question or hypothesis

Problems: Focused on tree level description with a brief qualitative description of the floristic and structural nature of the understorey.

Reported parameters: species, diameter, height, spatial location and general characteristics of each tree ≥10cm in the 100 x 100m plot

File name of the description: AusPlots Forests Manual Species List and Understorey v1.6.pdf

Suitable for monitoring under the NECD: YES, partially

OTHER PROTOCOLS

FunDivEUROPE - Functional significance of forest biodiversity in Europe

"Description of the understorey vegetation composition and quantification of the understorey biomass"

http://project.fundiveurope.eu/wp-content/uploads/Sampling_Protocol_Understorey-Vegetation_Dec_2012.pdf

Short description:

This sampling protocol aims at providing a consistent methodology to collect high quality, harmonized and comparable forest understorey vegetation data at the exploratory and experimental platforms of FunDivEUROPE. Aiming to further enhance comparability between FunDivEUROPE and other research initiatives, ICP Forests (Canullo et al., 2010) and Futmom (http://www.futmon.org/index.htm) protocols were used to write it. Under the main hypothesis that diversity and abundance of the understorey vegetation increases with increasing overstorey diversity in the canopy, the general objective of this understorey vegetation assessment is to estimate the status and differences in diversity, biomass and nutrient stocks and status of plant communities between the plots at the exploratory and experimental platforms.

For each of the plots per focal region, the core plot will be divided in nine quadrants. In three quadrants, a subplot of 5 m x 5 m will be marked for identification and estimation of cover of understorey vascular plant species. Within this subplot, understorey vegetation will be clipped in a zone of 0.5 m x 0.5 m, where understorey vegetation is relatively abundant and where vegetation composition is representative for the whole subplot. This results in a total of 215 (plots) x 3 (subplots) = 645 subplots for identification of understorey vegetation, biomass sampling and chemical analyses.

sampling/mensurational frequency: The frequency and time of sampling should take in consideration: 1) the occurrence of vernal species; 2) maximum biomass values. This may imply more than one sampling, but for logistical reasons only one sampling period per exploratory region is scheduled. However the protocol is aimed to try optimizing as much as possible criteria 1) and 2).

sampling/mensurational scale: Sampling Unit level

Used by: project partners

Advantages: sampling strategy based on a clear research hypothesis

Problems: limited to project activities, need to understand if plot are spatially representative of "focal region" (no definition of "focal region")

File name of the description: FunDivEUROPE Sampling_Protocol_Understorey-Vegetation_Dec_2012 (v1.0).pdf

Reported parameters:

Total cover of shrub (> 1.3 m and stem < 7.5 cm diameter) and tree layer (stems

> 7.5 cm diameter (% of sampling area); Total cover of the understorey vegetation (% of sampling area)

Total cover of mosses (% of sampling area); Total cover of bare soil (% of sampling area); Total cover of litter (% of sampling area).

Suitable for monitoring under the NECD: YES, partially

OTHER PROTOCOLS

UNECE Convention on Long-range Transboundary Air Pollution

International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems

Manual for Integrated Monitoring. 1998. Finnish Environment Institute, ICP IM Programme Centre, Helsinki, Finland. www.syke.fi/nature/icpim > Manual for Integrated Monitoring.

Short description:

The main aim of the “Vegetation - intensive plot” subprogramme (VG) is to obtain sensitive bioindication of changes in pollutant deposition or other atmospheric factors, e. g. warming, on a representative plant community and its species. The understorey vegetation includes soil-growing vascular plants, bryophytes and lichens, not fungi or algae. Another aim is to obtain data on the dynamics of tree biomass and canopy structure representative at least for the intensive area where a number of subprogrammes (e.g., Vegetation structure and species cover - VS - optional) are also being conducted.

For the VG subprogramme (mandatory) is indicated to to establish one or two permanent intensive plots about 40 m x 40 m (preferably between 20 m x 20 m and 50 m x 50 m) in a homogeneous part of one or two plant communities representative of the monitoring site and preferably also widespread in the region. Establish the plot when most plants are fully developed. It is practical to orientate the plots in north-south/east-west directions. Mark permanently the corners of the plot. Distribute randomly or by stratified random sampling a sufficient number of sample plots, e. g. 50 cm x 50 cm, on the intensive plot and mark them permanently. In order to counteract unwanted activity from animals on the sample plot the marking should be insignificant. 20-40 plots should be sufficient, depending on the variability of the understorey vegetation and the size chosen of the sample plots. Exclude plots where unwanted substrates, e.g. stone or log occupy more than a certain part. Make sure that disturbing influence, e.g. trampling, especially on the small sample plots, from various monitoring activities are excluded.

For the VS subprogramme (optional) the observations are carried out on permanent plots, preferably circular. An area of 100 m^2 (radius 5.64 m) makes cover estimates easy (1 m^2=1%). The plots could preferably be distributed along transect mapping lines on or near the plots of the BI (Tree bioelements and tree indication) subprogramme (otional), i.e. in a quadratic network, using the same station codes. If a plot is "impossible" with regard to topography, heterogeneity etc. or if the dominant plant community does not cover 50% or more, it is rejected. Whatever design that is used, the observed parameters should be representative for the entire catchment.

sampling/mensurational frequency:

For VG subprogramme (mandatory) Tree and shrub layers should be observed every five years. Depending on the stability and vulnerability of the vegetation, the intervals between observations of the field and bottom layers could be 1 - 5 years. In order for a time-series to be established as soon as possible annual observations are recommended. Make observations when the majority of species are fully developed. In deciduous forest there may be two peaks of development, one before leafing and one later.

For VS subprogramme the survey is repeated after 10-20 years or after major changes, such as heavy management measures, increased grazing, fire, extensive storm felling, avalanche and landslide. The season for the inventory should coincide with maximum development of vegetative and reproductive organs of plants.

sampling/mensurational scale: Sampling Unit level

Used by: partners participating in the UNECE Convention

Advantages: two subprogrammes (Vg mandatory and Vs optional) using the same layers and coverage calculation to obtain sensitive bioindication of changes in pollutant deposition or other atmospheric factors

Problems:

File name of the description: "ICP-IM veg Manual & annex" (folder)

Reported parameters: not reported the sampling scheme to establish the number of the plot representative of the region of interest

VG subprogramme

> Tree layer and dead wood - Observe living and dead trees that belong to the tree layer according to definition and, if feasible, also logs and stumps over the whole intensive plot. Map of living trees.
>
> Shrub layer - Map living and dead trees and shrubs in the shrub layer
>
> Field and bottom layers - the cover of the field and bottom layers and their species. Cover percentage (%) of the sample plot is the area that above-ground living parts of a plant occupy when projected vertically onto the ground (shade when sun in zenith)
>
> Vigour (optional) - Estimate presence of flowering organs (bud, flower, fruit, fruit rest of the current year) of species in the field layer using the fertility codes:
>
> 0 = species sterile
>
> 1 = <10 % of all shoots/individuals with flowering organs
>
> 2 = >10 % of all shoots/individuals with flowering organs

VS subprogramme

> Estimate the total cover of each vegetation layer (same of VG subprogramme) and the cover of each species in each layer for each sample plot. Normally percentage cover-classes are used.

Suitable for monitoring under the NECD: YES, partially

OTHER PROTOCOLS

LIFE14 ENV/IT/000514, FutureForCoppiceS

Biodiversity

Source: Manuale di rilevamento: Diversità di piante vascolari, licheni epifiti, funghi lignicoli e uccelli come indicatori di gestione forestale sostenibile a scala locale - Guida per studi in campo

Short Description:

Within each treated area, a rectangular macroplot (20 m x 40 m) is randomly selected with a buffer zone indicated as buffer zone of 2.5 m and an internal zone indicated as core area. The detection of vascular plants must be carried out within a 10 m x 10 m plot identified with a random procedure. The plots will be identified within the core areas of each macroplot. To position the starting vertex of the plot on the field, two coordinates expressed in meters were generated and, starting from a vertex of the macroplot (selected in turn randomly), the y coordinate on the long side of the macroplot and the x coordinate on the short side were identified. Once the vertex of the plot was identified, pegs and measuring wheels will be used to delimit it. The coordinates of the starting vertex will be reported in the survey sheet. In the event that part of the plot falls on discontinuity of the substrate (ditches, charcoal pits, etc.) it is necessary to recalculate the random coordinates and proceed with the identification of a new plot.

sampling/mensurational frequency: not reported

sampling/mensurational scale: Sampling Unit level

Used by: project partners

Advantages: protocol to be used on treated areas

Problems: limitated to project purpose

File name of the description: folder “B4_Manual_F4C SFM biodiv.pdf”, file B4_Manual_F4C SFM biodiv.pdf

Reported parameters: Land cover for each level (trees, shrubs and herbs) using Braun-Blanquet’s scores

Suitable for monitoring under the NECD: YES, partially

OTHER PROTOCOLS

LIFE14 ENV/IT/000514, FutureForCoppiceS

Protection service

Source: Manuale Campionamento per la stima della copertura del piano di vegetazione arboreo superiore, inferiore, arbustivo e delle briofite, dello spessore della lettiera e della regimazione idrica - Guida per studi in campo

Short description:

Within each treated area, a rectangular macroplot (20 m x 40 m) is randomly selected with a buffer zone indicated as buffer zone of 2.5 m and an internal zone indicated as core area. 1). The measurements carried out to calculate the indicators relating to the coverage of the upper and lower vegetation plan should be carried out at regular intervals, using photographic instruments, following a systematic grid for the localization of the sampling points. The measurement of the indicators, relating respectively to the depth of the litter layer and the coverage of the bryophytes, will be carried out by creating a linear transept of 40 m, along one of the two diagonals of the macroplot, randomly drawn. The measurements will be made starting from 2 m from the vertices of the diagonal, to avoid making measurements inside the buffer zone. The measurements of the litter depth will be carried out at a distance of 2 m from each other (for a total of 20 measurements per macroplot). To estimate the coverage of the bryophytes, a distance of 20 cm will be used between one sampling point and the next (for a total of 200 observations in each macro-plot). The measurements will be carried out in summer (between July and August), i.e. at maximum leaf expansion. As regards the determination of the water regulation capacity, the information collected for the estimate of the coverage of the lower floor will be integrated with data relating to the geomorphology of the macroplots.

sampling/mensurational frequency: not reported

sampling/mensurational scale: Sampling Unit level

Used by: project

Advantages: protocol for treated area

Problems: limited to project purpose

File name of the description: folder "B4_Manual_F4C SFM biodiv.pdf", file B5_Manual_F4C SFM protettiva coperture ecc.pdf

Reported parameters: Litter depth, bryophyte cover, soil water regulation capacity

Suitable for monitoring under the NECD: YES, partially

MONITORING ACTIVITY:

TREE GROWTH

PROTOCOL IN USE AT PRESENT

ICP-FORESTS (periodic increment of all trees on the plot)

Source: Dobbertin M, Neumann M, Levanič T, Sanders TGM, Skudnik M, Krüger I, 2020: Part V: Tree Growth Level II. Version 2020-1. 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, 19 p. + Annex

Short description:

Tree growth is a key ecological parameter of forests. The manual focuses on growth assessment on Level II plots and follows the BioSoil manual on growth assessment at Level I plots. Tree species is a basic and fundamental information and all effort should be taken at Level II plots to provide it.

Increment is defined as the growth of trees (shoots in coppice forests) and stands within a defined period and can be expressed as increment of diameter, basal area, height and/or volume.

A full inventory including in-growth and regeneration is mandatory every ten years on each plot except for young stands; all trees with at least 5 cm diameter over bark (at least 3 cm for stools and shoots in coppice forests) must be individually identifiable by numbering. The location of dbh measurements must also be indicated at the stem. Dbh can be measured by clubbing (two diameters and taking the mean) or by diameter bands.

Dbh, tree height, height to crown base, removals and mortality must be undertaken **every fifth year** and subsequently reported and detailed with proper codes.

Optional: interannual growth measurements by girth bands on a subsample of trees.

Used by: ICP Forests network and national monitoring networks

Advantages: simple, standardised, robust, reproducible, tested, including QA/QC procedures

Problems: For forest growth measurements, quality control is particularly important, because many of the measured variables will be used in combination to compute additional values. A careful training of field teams is foreseen with an adequate plausibility tests for data collection.

Need long data period to identify variability and trends.

File name of the description: ICP_Manual_2020_part05_Growth_version_2020-1.pdf

Reported parameters Tree Species, Dbh of all trees above the threshold, tree height and height to crown base.

Suitable for monitoring under the NECD: YES

OTHER PROTOCOL IN USE AT PRESENT

ICP-FORESTS (permanent monitoring of selected trees using electronic dendrometers and/or girth bands)

Source: Dobbertin M, Neumann M, Levanič T, Sanders TGM, Skudnik M, Krüger I, 2020: Part V: Tree Growth Level II. Version 2020-1. 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, 19 p. + Annex

Short description:

Tree growth is a key ecological parameter of forests. The manual focuses on growth assessment on Level II plots and follows the BioSoil manual on growth assessment at Level I plots. Tree species is a basic and fundamental information and all effort should be taken at Level II plots to provide it.

Increment is defined as the growth of trees (shoots in coppice forests) and stands within a defined period and can be expressed as increment of diameter, basal area, height and/or volume.

Interannual growth measurements by girth bands on a subsample of trees is estimated during weekly visits

Used by: ICP Forests network and national monitoring networks

Advantages: simple, standardised, robust, reproducible, tested, including QA/QC procedures

Problems: For forest growth measurements, quality control is particularly important, because many of the measured variables will be used in combination to compute additional values. A careful training of field teams is foreseen with an adequate plausibility tests for data collection.

Requires installation of girdle bands or electronic dendrometers.

File name of the description: ICP_Manual_2020_part05_Growth_version_2020-1.pdf

Reported parameters: Dbh of selected trees.

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS:

Source:

E. Bogdanovich, O. Perez-Priego, T. S.El-Madany, M. Guderle, J. Pacheco-Labrador, S. R. Levick, G. Moreno, A. Carrara, M. P. Martín, M. Migliavacca (2021). Using terrestrial laser scanning for characterizing tree structural parameters and their changes under different management in a Mediterranean open woodland. Forest Ecology and Management Volume 486. https://doi.org/10.1016/j.foreco.2021.118945

Short description:

Terrestrial laser scanning (TLS) is a tool able to collect detailed three dimensional information of a single tree as well as of entire forest stand. Among other applications, TLS can be powerfully adopted for assessing tree growth based on time series analysis, as it allows a level of scrutiny not achievable using established destructive techniques. Using specific modelling approaches (e.g. Quantitative Structure Models, QSMs), we were able to assess the annual growth of the individual trees in terms of diameters (of both stem and branches) and tree height. The development of multitemporal QSMs, based on TLS-derived data, allowed the accurate determination of crown length and width, as well as the volume reduction as the result of the tree pruning, if present.

Sampling/mensuration frequency:

One acquisition/year during leaf off conditions (winter).

Used by: internal protocol (Crea Forestry Research Centre) for research activities

Advantages:

Detailed information on tree dimension for both stem and crown.

Problems:

Well-trained staff for (i) field data collection and (ii) post processing.

Filename of the description: TLS.pdf

Reported parameters

All the "tree architectural traits" such as:

- DBH and other stem diameters at different heights;
- Branches dimension;
- Crown attributes.

Suitable for monitoring under the NECD: YES

MONITORING ACTIVITY:
METEOROLOGICAL MEASUREMENTS

PROTOCOL IN USE AT PRESENT
ICP-FORESTS

Source:
Raspe S, Fleck S, Beuker E, Preuhsler T, Bastrup-Birk A, 2020: Meteorological Measurements. Version 2020-1. 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, 18 p. + Annex

Short description:
Meteorological variables affect composition, structure, growth, health and dynamics of forest ecosystems. The measurement of meteorological data at forest monitoring plots is essential for the interpretation of climate change effects. The magnitude and changes in time of the meteorological variables should be assessed as accurately as possible in order to be able to use the meteorological data as explanatory factors of the many other observations made at the Level II plots. Meteorological data from national weather stations are in most cases not sufficient for representing forested areas. Geographic features affect airflow and limit the representativity of the meteorological data by spatial heterogeneity (e.g. location, altitude, exposition, slope). Meteorological monitoring at the Level II plots provides local, inside forest area information on the basic driving and influencing factors for forest ecosystems. Meteorological data are used when deriving fluxes and deposition of air pollutants to forest stands, as well as the water- and element cycles, the vitality, growth and phenology, and the crown condition of trees.

Sampling/mensuration frequency:
Data measured every 10 seconds and collected in 2 files, hourly and daily.

Used by:
ICP Forests network and national monitoring networks.

Advantages:
simple, standardized, robust, reproducible, tested.

Problems:
Meteorological data is collected by control units with multiple sensors, it is very important that the system is well powered and that the solar panels are able to charge the battery pack. In addition, some sensors, such as the rain gauge, need to be cleaned periodically especially if under the canopy. At least one maintenance must be scheduled annually.

File name of the description:
ICP_Manual_2020_part09_Meteorology_version_2020-1.
https://storage.ning.com/topology/rest/1.0/file/get/9995552074?profile=original

Reported parameters
In the open field meteorological station: Air Temperature (AT), Relative Humidity (RH), Global Radiation (SR), Precipitation (PR), Wind Speed (WS), Wind Direction (WD) and Soil Temperature

(ST). In the plot meteorological station: Air Temperature (AT), Relative Humidity (RH), Precipitation (PR), Soil Temperature (ST) and Soil Moisture (WC) (only core plot).

Suitable for monitoring under the NECD: YES

OTHER PROTOCOLS:
ICOS

Source:
Instruction on air meteorological measurements, http://www.icos-etc.eu/icos/documents/instructions/airmet

Short description:
Several meteorological variables need to be measured at ICOS monitoring stations in order to characterize them, analyse the drivers explaining GHG fluxes, and properly calculate, partition and aggregate these fluxes and their seasonal budgets from eddy covariance (EC) measurements, the storage profile, automatic chambers, etc. This protocol deals with all the relevant air characteristics to be measured at ICOS stations, i.e. air temperature (TA), air relative humidity (RH), air pressure (PA), wind speed (WS), wind direction (WD). Radiations (SW in, SW out, LW in, LW out, PAR in, PAR out, RN, RG and RDiff) and Precipitations (PR) are kept separated in dedicated documents.
Furthermore, in order to reduce the occurrence of gaps in meteorological data and to have a reference, ICOS stations have to be equipped with a backup meteorological station in the vicinity of the main one. The steps needed to install, maintain, calibrate the sensors, up to the data collection, processing and submission are described in this protocol. The info are contained in the following sections: - Measurements: in this section the sensors are described, and the steps to perform from the installation to the data collection are illustrated. - Maintenance and calibration: in this section all the maintenance and calibration guidelines are described, including the general timeline and how to deal with repairs. - Submission: it is the section related to the data preparation and submission to the ETC. It includes a summary of the submission workflow described in a dedicated document and lists all metadata and ancillary parameters to be sent to the ETC, and how to submit them. - Processing: this section is for internal use of the ETC and describes the processing applied to the single variable/info/sample submitted.
It is important to remind that the exact application of the protocol at station level must be also discussed with the ETC in order to reach agreed solutions for specific cases. If a specific part is relevant only for a specific ecosystem or site Class, this is also reported in the document.

Sampling/mensuration frequency:
Data measured every 20 seconds, collected in a daily file and submitted every day on ETC Servers.

Used by:
ICOS Network.

Advantages:
Standardized, robust, reproducible, tested.

Problems:
The system is very expensive because the components must have very strict specifications. In addition, a backup station is required for the most important measurements.

Reported parameters
Air Temperature (AT), Relative Humidity (RH), Wind Speed (WS), Wind Direction (WD), Pressure (PA), Precipitation (PR) and Radiations (SW, LW, PAR, RN, RG and RDiff)

Suitable for monitoring under the NECD: NO

MONITORING ACTIVITY:

OZONE INJURY

PROTOCOL IN USE AT PRESENT

ICP-FORESTS

Source: ICP-Forests Manual, part VIII, http://icp-forests.net/page/icp-forests-manual

Short description:

The methodology applied is based on the ICP Forest Manual (Part VIII).

Assessment on main tree species - The percentage of symptomatic, current year's leaves and needles on main tree species conducted within the Intensive Monitoring Plot. A minimum of 3 branches (minimum of 30 leaves each) per tree and 5 trees per plot are assessed.

Broadleaf trees. All leaves per branch are examined under best light conditions. The percentage of symptomatic leaves per branch is estimated and scored.

- 0 = No injury;
- 1 = 1 – 5% of the leaves per branch show ozone symptoms;
- 2 = 6 – 50% of the leaves per branch show ozone symptoms;
- 3 = 51 – 100% of the leaves per branch show ozone.

Conifer trees. The different needle age classes are identified. Only current year needles (C) and current + 1 year needles (C+1) are assessed. Needles have to be placed close to each other and examined in full sunlight. The chlorotic mottling will be scored for each needle age class in percentage of total surface affected. The resulting percentages per branch and needle age are then transformed to the corresponding score.

- 0 = No injury present;
- 1 = 1 – 5% of the surface is affected;
- 2 = 6 - 50% of the surface is affected;
- 3 = 51 – 100% of the surface is affected.

Less Exposed Sampling Site (LESS) - A LESS is established within the vicinity of the open-field meteorological monitoring station where ozone concentrations are actively recorded. 25 quadrates (2 x 1 m) are randomly selected along the forest edge (50 m long) in order to assess the presence and absence of ozone visible injury on the respective species. The symptomatic and asymptomatic species within LESS is assessed.

Sampling/mensuration frequency: The mandatory is every 2 years, but an annual assessment is preferred.

Sampling/mensuration scale: plot-level

Used by: ICP Forests network, with differences in altitude, climate and species composition among countries.

Advantages: simple, robust, tested

Problems: requires well-trained observers (they should participate in Intercalibration Courses on the Assessment of Ozone Visible Injury organized by ICP-Forest Expert Panel on Ambient Air Quality).

File name of the description: ICP_Manual_2020_part08_Ozone_version_2020-1.pdf

Reported parameters:

Assessment on main tree species – Percentage of symptomatic branches, reported as frequency classes (% of branches Score 0, 1, 2 and 3).

Less Exposed Sampling Site (LESS) - The following variables reported:

- Total number of species recorded per LESS;
- Number of symptomatic species per LESS;
- Percentage of symptomatic species per LESS;
- Lists of symptomatic and asymptomatic species per LESS.

Suitable for monitoring under the NECD: YES

OTHER PROTOCOL

VibEuroNet

Source:

Gottardini E, Ferretti M, Schaub M (2017) VibEuroNet - Viburnum lantana observation Network in Europe. Manual to implement a pilot study. Developed within the ICP Forests Expert Panel on Ambient Air Quality, 14 p. DOI: 10.13140/RG.2.2.17680.61449

(available at: https://www.researchgate.net/publication/318013788)

Short description:

The shrub species *Viburnum lantana* L. meets several requirements to be considered as a valuable in-situ bioindicator to assess ozone impact on vegetation in forested areas, such as wide distribution, high sensitivity to ozone and specific symptom development, consistency between ozone levels and response indicator (i.e. visible foliar symptoms).

30 *V. lantana (or V. opulus*) plants are randomly selected in the Light Exposed Sampling Sites (LESS) within a 1x1 km quadrate. If less than 30 plants are found, other plants are selected in 3 further open areas, tracks, or pathways randomly selected. For each plant presence/absence of ozone foliar injury, proportion of leaves with ozone injury, plant height class, aspect and shaded portion are assessed and for each open area and/or track soil moisture class is evaluated.

Sampling/mensuration frequency: annual

Sampling/mensuration scale: plot-level

Used by: This method was applied in a pilot study testing its ability to assess ozone injury within the ICP Forests programme.

Advantages: simple, robust

Problems: The geographic distribution of Viburnum species does not entirely cover the Italian peninsula.

File name of the description: VibEuroNet_manual.pdf

Reported parameters: % symptomatic plants

Suitable for monitoring under the NECD: NO

MONITORING ACTIVITY:

SOIL SOLUTION COLLECTION AND ANALYSIS

PROTOCOL IN USE AT PRESENT

ICP-FORESTS

Source: Nieminen TM, De Vos B, Cools N, König N, Fischer R, Iost S, Meesenburg H, Nicolas M, O'Dea P, Cecchini G, Ferretti M, De La Cruz A, Derome K, Lindroos AJ, Graf Pannatier E, 2016: Part XI: Soil Solution Collection and Analysis. Version 2016-2. 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, 20 p. + Annex

Short description:

Soil solution is sampled by gravity samplers at the base of the forest floor and by tension, ceramic cup, samplers placed at three depths: 15-25 cm, 30-50 cm, 50-70 cm. Tension samplers are evacuated at -60 kPa at every sample collection. Three to five replicas for each site are used for gravity samplers, five replicas for each depth and site for tension samplers.

Used by: ICP-Forests network, with differences in sampler types and positions between countries

Advantages: simple, robust, amply tested, no need of power supply

Problems: requires bi-weekly manual sampling; samplers are subject to damage by soil creeping in sloping sites and may require frequent replacement

File name of the description: ICP_Manual_2016_part11_Soil_Solution_version_2016-2.pdf

Reported parameters:

Sample volume, pH, electrical conductivity (EC) and concentration of Ca^{++}, Mg^{++}, Na^{+}, K^{+}, NH_4^{+}, NO_3^{-}, SO_4^{--}, Cl^{-} Total N

Suitable for monitoring under the NECD: YES

OTHER PROTOCOL:

variations included in the section above, with respect to the alternatives actually used in Italy.

a) Use of different types of tension samplers; a comparison is reported in ICP Manual, 2016, part11 Soil Solution, Annex II. From it can be seen that there is no 'ideal' type of tension sampler, and that the presently used type is in no way inferior to any other. Actually, PTFE tension samplers were tested at the beginning of the soil solution sampling program in Italy. In our tests, they showed a higher release of relevant analytes with respect to ceramic cup samplers. This may have been a 'new sampler' effect, subsiding with time.
b) Use of gravity samplers for subsoil sampling; this is done in several foreign sites within the ICP-Forests network. The main reported advantage is that it allows to definitely sample only non-retained water, the water fraction that actually moves through the soil. Disadvantages, are however, marked.
    a. Use of gravity samplers in subsoil implies a high degree of disturbance of tree roots; in a previous experience, the sampling pit had to be cleaned of new-growth roots every year, with the attendant disturbance to trees
    b. The amount of space occupied, due to the need of a sampling pit, is considerable and does not appear compatible with the size and degree of occupancy of Italian Level II sites
    c. A specific research has demonstrated that a standard gravity sampler does not exist; commercial production samplers are not suitable for sampling at the base of the forest floor, for which purpose samplers are usually hand-built, using widely different shapes and materials; this raises serious doubts on the comparability of results

The amount of retained water actually sampled by tension samplers is very small; -60 kPa is a very high potential, and the amount of soil water to be found between 0 and -60 kPa is always quite small; further, such tension is only held for a short period as, when there is adequate soil moisture, the filling of samplers by water causes a rapid decrease of tension; then, the applied vacuum is mostly only to ensure sample transfer to gathering vessels.

OTHER PROTOCOL

ESTIMATION OF LEACHING FLUXES

Source: Raspe S, Fleck S, Beuker E, Preuhsler T, Bastrup-Birk A, 2020: Meteorological Measurements. Version 2020-1. 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, 18 p. + Annex

Short overview

The possibility of estimating leaching fluxes is briefly mentioned in the section of ICP-Forests Manual on soil solution collection and analysis. It is clearly desirable, as it would allow quantification of pollutant fluxes through the soil and towards underground waters.

The content of this chapter was written to the standards of the early 2000s, and does not take account of progress in instrument technology. Further, it is clearly written for conditions in which semi-continuous plot maintenance by highly qualified personnel is performed. This is clear in the recommendation of measuring soil water potential by using conventional tensiometers, instruments which, in average Italian conditions, would require multiple visits each year by research team personnel. In the last 10-20 years, different types of alternate water potential instruments have appeared, which can measure soil water potential across the whole range of significant interest, while requiring substantially less care.

As a result, it is current practice to install only water content measuring instruments, using TDR technology. This is also the final recommendation of the ICP-Forests manual chapter. These instruments are fully suitable to long-term monitoring conditions and can function continuously without any human intervention.

Modelling of water fluxes by exclusive use of mathematical model, without actual measurements, is a commonly accepted solution. The necessary set of data (precipitation, temperature, soil properties) is available. A considerable variety of soil-water models is available.

MONITORING ACTIVITY:

VISIBILITY

PROTOCOL IN USE AT PRESENT

I.M.PRO.V.E., Interagency Monitoring of Protected Visual Environment

Source: http://vista.cira.colostate.edu/Improve/

Short description:

The term "visibility" means an estimation of air transparency which allows an observer to clearly distinguish an object at a certain distance from an observation point, against the background of the horizon,. The I.M.PRO.V.E. protocol has the objective of calculating "visibility" in natural areas from a given observation point, associating reductions of this parameter with atmospheric pollution from particulate matter (PM) and selected gas molecules, of both anthropogenic and natural origin. Thus visibility can be used as physical parameter for assessing the air status, with related information on its quality, in a natural environment with relevant conservation and recreational vocation, such as a natural park, and it also allows to study the impact of atmospheric pollution on ecosystems. The visibility monitoring station for measuring 4 macro-parameters: includes 4 sampling lines: nitrogen oxides, meteorology, fine particles ($PM_{2.5}$ PM_{10}) and photographs of the view. The chemical-physical parameters collected are used to calculate the visibility degree through the quantification of a coefficient (b_{ext}), describing the extinction of light as a function of the different chemical-physical parameters associated with airborne molecules and particles. The overall analysis involves the study of correlations of this value with weather conditions and with the optical visibility captured by the photographs.

Visibility assessment scale: the calculation of b_{ext} is at landscape scale

Used by: U.S. National Park Service; University of Colorado (U.S.A.)

Advantages: visibility is an innovative parameter in the environmental quality monitoring framework; it can support the study of other environmental monitoring, including the study of the impacts of atmospheric pollution on ecosystems

Problems: It relies on high technology monitoring equipment, with high automation and the need of remote control and maintenance

File name of the description: USA Visibility protocol.pdf

Reported parameters:

- meteo parameters: air temperature, relative humidity, wind direction, wind speed, precipitation;
- gravimetric $PM_{2,5}$ and PM_{10};
- chemical gas phase parameters: nitrogen oxides (NO, NO_2, NO_x);
- chemical PM parameters: sulfates, nitrates, chloride, elemental carbon, organic carbon, total organic matter, metals and trace elements (Ti, Si, Ca)
- pictures of the view from the monitoring station.

Sampling time resolution/ frequency of the measured parameters:

- meteo parameters: hourly maximum, minimum and average, daily;
- gravimetric $PM_{2,5}$ and PM_{10}: 24 h, every three days;
- chemical gas phase parameters: continuous sampling, daily;
- chemical PM parameters: 24 h, every three days;
- photos: 1 picture every 5 minutes, every three days.

Suitable for monitoring under the NECD: YES