

Methodological approach for long-term ecological research in forest sites

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Abstract. The dynamic of the processes in an ecosystem requires long-term observations. This paper presents a specific methodology for estimating forest ecosystem characteristics as a part of the Long-Term Ecological Research (LTER) network. Permanent sample plots in Western Stara Planina in Bulgaria are determined to monitor the processes in forest ecosystems. Methods based on remotely sensed vegetation index are commented on. The main phenology's life cycle events, such as the start, peak, end, and length of the growing season, are described. Methods for phytocoenotic structure are presented: complete floristic composition, total projective coverage of horizons, and species abundance. Methods for stand growth and yield estimation, diameter calculation, basal area, volume increment, and description of tree diameter distribution are summarized. Chemical methods for deposition determination are commented on.

Key words: LTER, forest sites, phenology, stand growth, phytocoenotic structure, deposition.

Introduction

The information on long-term changes is the key to understanding the present and predicting the future of ecosystem structure and functions. Thus, the history of ecological changes is essential in ecosystem monitoring. But some changes exceed human life resolution. Forests grow for hundreds of years, and their species composition orderly changes.

According to Mirtl et al. (2018), the overall purpose of the International Long-Term Ecological Research Network is to provide a globally distributed network and infrastructure of long-term research sites for multiple uses in the fields of ecosystem, biodiversity, critical zone and socio-ecological research, and to secure the highest

quality interoperable services in close interaction with related regional and global research infrastructures and networks.

The complex approach to assessing forest ecosystems allows us to understand the impact of abiotic factors on their processes and functions (Leuschner & Ellenberg, 2017). One of the main parameters of the environment, such as solar radiation, air temperature, relative air humidity, and the amount and chemical composition of precipitation, determine the ecosystems' productivity, species diversity, and their sustainability. The influence of environmental factors has a different time scale – from the daily dynamics of main physiological processes, through annual dynamics of phenological manifestations and

biomass accumulation, to the longer-term successional changes, biodiversity, and soil characteristics.

Gas-exchange parameters of trees, such as photosynthesis, dark respiration, and transpiration rate, as well as water-, carbon-, and light-use efficiency, determine potential of net primary productivity of forests. Increased temperature increases evaporation, increasing water stress, even if precipitation remains unchanged from past periods (Williams et al., 2013). A change in temperature from a species' optimal temperature for photosynthesis can positively or negatively affect water-, carbon-, and light-use efficiency (Gustafson et al., 2015). Respiration rates increase exponentially with temperature, reducing net photosynthesis and increasing the metabolic cost of tissue maintenance (Atkin et al., 2007).

Phenology is a significant determinant of the plant species range and could be used to assess the consequences of climate changes on forests' health, physiological condition, and productivity (Chuine & Beaubien, 2001). Remotely sensed vegetation phenology is typically accomplished using time series of vegetation indices. However, most vegetation indices do not have a biophysical character and, especially for phytocoenoses with a robust canopy, are unsuitable for tracking phenological phases. Abedi & Bonyad (2015) showed that the correlation between spectral band information and some forest variables is weak due to heterogeneous conditions of forest stands. According to Kross et al. (2015) the most used vegetation index NDVI (normalized difference vegetation index) saturates rapidly with increasing foliage, reaching its maximum levels at a Leaf Area Index (LAI) of about 3-4 and then not changing significantly. Another frequently used vegetation index – the enhanced vegetation index (EVI), reacts more sensitively to phenological changes but is much more influenced by the time of the capture, as well as by the gas composition of the atmosphere, such as fine dust particles, water vapor, etc. aerosols (Tariq et al., 2021). The Plant Phenology Index (PPI) is a new vegetation index optimized for efficient monitoring of vegetation phenology. PPI is derived from the solution to a radiative transfer equation, is computed from red and near-infrared (NIR) reflectance and has a nearly linear relationship with canopy green leaf area index (LAI), enabling it to

depict canopy foliage density well. Comparison of satellite-derived PPI to ground observations of plant phenology and gross primary productivity (GPP) shows strong similarity of temporal patterns (Jin & Eklundh, 2014).

Different approaches to classifying vegetation are applied in some geographical regions (Russia and Western countries) and by other scientific schools. Before 2000, the Dominant approach for vegetation classification was predominantly used in Bulgaria (Lyubenova, 2004). In recent years, work has been carried out on the floristic approach (Pavlov, 2006) adopted in Western countries, which has recently become increasingly popular. The analysis of the vegetation according to the floristic method includes investigation as the complete floristic composition, structure, and physiognomy of the plant communities, quantitative participation of species, presence, and absence of characteristic species connecting the studied phytocoenoses with a certain syntaxon, etc. An essential part is describing the full species composition of plant communities. In Bulgaria, authors such as Dimitrov & Dimitrova (2012), Tzonev et al. (2023), Vassilev et al. (2012), Sopotlieva & Apostolova (2007), Alexandrova et al. (2020) are working following this approach, etc.

To understand and interpret the influence of abiotic factors on vegetation, information is needed not only on atmospheric gases, aerosols, and deposition processes but also on stand height and age, structure, leaf area index, canopy density, vitality, and biotic stress. The throughfall deposition is a result of the interaction between the canopy and the rainfall. Processes such as foliage exchange, when some ions are leached and other are uptake, interception of particles can change the chemical composition of precipitation (Berger et al., 2008; Tonello et al., 2021). Dry deposition contributes to the enrichment of throughfall with potassium, calcium, magnesium, manganese. During the rainfall, the dry particles are washed out from the canopy and deposit on the forest soils (Damyanova & Tonchev, 2019). The growth and increment of stands are considered as the result of the growth and increment of individual trees. To make adequate decisions regarding the monitoring and management of forest ecosystems, it is necessary to have accurate data on the dynamics

of the growth process, both of individual trees and forest stands (Pretzsch et al., 2022).

The main purpose of this publication was to present the complex of ecological indicators, investigated at the Petrohan forest site, which is the part of the LTER network.

Methodological approaches

Site Petrohan's description

The site Petrohan is located in the territory of Training and Experimental Forestry Enterprise "Petrohan", situated in the West Balkan range with a total area of 7192 ha. The region's relief is mountain, steep, with deeply cut river valleys and secondary watersheds with the lowest point at an altitude of 350 m a.s.l and highest at 1900 m a.s.l. (Fig. 1).

Due to the long range of systematic biometrical, chemical, and physiological data storage, Petrohan is a representative forest's site in the national network for long-term research and the European ecological network LTER. The part of the protected forest on the territory of Training and Experimental Forestry Enterprise "Petrohan" is 74 %, differentiated as an area for water resources, including drinking water. The first forest management plan of forests in the site dates from 1893. The total growing stock is 1 989 695 m³, volume per ha is 298 m³, whole annual increment per year is 24 271 m³, and mean annual volume increment per ha is 3.64 m³ year⁻¹. Cambisols are the main soil type in this region (Hristov et al., 2021).



Fig. 1. Map of site "Petrohan" and sample plots position.

Table 1. Sample plots description.

	SP-1	SP-2	SP-3
Altitude, m	1447	1413	717
Latitude, °	43.121	43.120	43.158
Longitude, °	23.121	23.128	23.150
Slope, °	21.24	10.65	5.78
Aspect, °	143	272	268
Main tree species	<i>Fagus sylvatica</i> L.	<i>Picea abies</i> Karst.	<i>Fagus sylvatica</i> L.
Tree height, m	28	30	31
DBH, cm	34	34	52
Age, years	140	90	170
Canopy closure, %	80-85	60-75	50-80

The site Petrohan was established in 1986. Since 2020, the sample plots are three, including beech and spruce stands (Table 1).

Ecological indicators include soil chemistry, air temperature, humidity, phenology, annual growth, biomass and precipitation chemistry. Measured parameters in bulk and throughfall depositions are the following: pH, electroconductivity, and chemistry of all compartments (NO_3^- , SO_4^{2-} , Cl^- , NH_4^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Pb^{2+} , Cd^{2+}) (Damyanova & Tonchev, 2019).

Phenological observations

The High-resolution vegetation phenology and productivity (HR-VPP) database from the Copernicus Land Monitoring Service is used to determine the phenological occurrences in the experimental sites. The process starts with Sentinel-2 raw data pre-processing, which include two main steps - retrieval of the angles from the metadata, and Atmospheric and Topographic correction (SEN2COR). Sun zenith angle and top of canopy reflectance in red (B04) and near infrared (B08) bands are used for calculation of the Plant Phenological Index (PPI) - a physically

based vegetation index (Eq. 1), which improved monitoring of plant phenology and strongly related with Leaf Area Index (Jin & Eklundh, 2014):

$$PPI = -K \times \ln \left(\frac{DVI_{max} - DVI}{DVI_{max} - DVI_{soil}} \right) \quad (1)$$

where DVI is the difference of near-infrared and red reflectance; DVI_{max} is the temporal maximum of DVI; DVI_{soil} is the soil DVI; K is a gain factor given by the canopy light extinction efficiency, which depends on leaf inclination angle, solar angle and the diffuse fraction of solar radiation. According to Jin & Eklundh (2014), $K \approx 0.5$.

Seasonal trajectories of the PPI (ST-PPI) are constructed according Jönsson et al. (2018), which the model function is a sum over double logistic functions (Fischer, 1994), adapted by Beck et al. (2006) for NDVI:

$$STPPI_{DOY} = MINV + (MAXV - MINV) \times \left(\frac{1}{1 + e^{-L_{slope} \times (DOY - SOSD)}} + \frac{1}{1 + e^{R_{slope} \times (DOY - EOSD)}} - 1 \right) \quad (2)$$

$STPPI_{DOY}$ is used to derive thirteen VPP-indicators (Table 2) calculated after the end of the growing season.

Table 2. List of indicators calculated by the Seasonal trajectories of the PPI dataset for each growing season (Smets et al., 2022).

Phenological indicators	Description	Unit	Range
SOSD	Start of season date		
EOSD	End of season date	Day of year	0 - 365 (366)
MAXD	Day of seasonal maximum		
SOSV	DVI at SOSD		
EOSV	DVI at EOSD		
MAXV	DVI at MAXD	Dimensionless	0 - 3
MINV	Average DVI_{min} before SOSD and after EOSD		
AMPL	MAXV - MINV		
LENGHT	SOSD - EOSD	Days	1 - 365 (366)
L _{SLOPE}	Slope of greening up period	PPI day ⁻¹	0.01 - 0.5
R _{SLOPE}	Slope of senescent period		
SPROD	Seasonal productivity	PPI day	0 - 1095 (1096)
TPROD	Total productivity		

Phytocoenotic characteristics

The phytocoenotic characteristic is made using the floristic method (Braun-Blanquet, 1964; Westhoff & Van Der Maarel, 1978). Phytocoenotic relevés are made in typical areas in 100 m² sample plots in beech and spruce forest. The relevés include: 1) complete floristic composition - all vascular plants like ferns, gymnosperms - plants without

flowers, angiosperms - flowering plants. The mosses are not included; 2) total projective coverage of horizons determined with an accuracy of 5% - a horizon of trees, a horizon of shrubs, and a horizon of herbaceous plants; and 3) species abundance, assessed with the Braun-Blanquet 9-point scale (Braun-Blanquet, 1964) (Table 3).

Table 3. Brown-Blanquet abundance and coverage scale.

Coverage	Number of individuals	Abundance
< 1%	1-2	r
< 1%	2-5	+
< 5%	< 50	1
< 5%	many	2m
6-15%	no matter	2a
16-25%	no matter	2b
26-50%	no matter	3
51-75%	no matter	4
> 75%	no matter	5

Stand growth and yield

The annual increase in diameter, height, basal area, volume, and biomass is defined as growth, and the magnitude with which this increase occurs is increment (Mihov, 2005). Growth can be calculated for each parameter already mentioned and determined for different periods. The current increment represents the growth of an individual tree or stand over a given period of time. When this period is one year, the term current annual increment is used. For a longer period of time - for example, 3, 5, or 10 years - the increment is current periodic. Due to the influence of various environmental factors on the growth of an individual tree, the current increment can also be calculated as an average over a given period of time.

The tree's growth or the stand according to the various parameters (diameter, height, volume, etc.) can be described with different mathematical models, also known as growth functions (Husch et al., 2003; Mihov, 2005). The first derivative of the growth function represents the current increment, and the second derivative represents the growth rate. The mean increment is defined as the average annual growth of the tree or the stand-up to the time of the survey, i.e., the ratio of the parameter (height, diameter, etc.) and age at that moment. Growth can be calculated in addition to absolute units. As a percentage of the value of tree (stand) parameters, this is a very suitable way to conduct comparative studies.

Periodic measurements of the diameter of the trees in the sample plots can be carried out using dendrometers, calipers, or girth bands (Husch et al., 2003). It is recommended that all trees in the sample plots with a diameter of 1.3 m equal to or greater than 10 cm be measured at a diameter of

1.3 m. The diameter is measured directly with the caliper in two perpendicular directions. The diameter is measured using girth bands through the stem circumference to the nearest 1 mm on trees that are thicker than the caliper length. The diameter of all trees is determined with an accuracy of 0.1 cm. When the stems have a common root, they are measured separately if the measurement point (1.30 m from the base) is above the point of branching.

On the part of the trees of different diameter classes, the age is determined by means of a core, taken at the height of the root neck with the help of an increment borer (Husch et al., 2003). The increment cores are processed in a laboratory, their surface being refreshed using a specialized microtome. The drying of the crown and the presence of damage of a biotic or abiotic nature are determined on each tree. On some trees of different diameter classes, the height is measured with a Hagl6f IV hypsometer with an accuracy of 0.1 m. The same hypsometer determines the distance from the measurement point to the tree. To characterize the spatial distribution of trees on the sample plot, the horizontal distance and azimuth from each tree's center to the plot's center are indicated, or rectangular coordinates are determined in a local Cartesian coordinate system (Moeur, 1993).

Chemical deposition

The most used way to monitor precipitation and throughfall is a bulk method. According to the methodology (WMO, 2008), the commonly used precipitation gauge consists of a collector placed above a funnel leading into a container where the accumulated water and melted snow are stored

between observation times. At least three collectors are recommended, located under the various canopy parts – closed to the stem, the edge, and the middle. The size of the orifice of the collector of 200 to 500 cm² will probably be found most convenient (WMO, 2008). The sampling area must be horizontal, as the collecting area will otherwise be reduced. The material of which the collector and its containers, tubes, glue, bird wires, etc., is made must not interact with the sample solution (Clarke et al., 2022). Permanently opened polyethylene plastic collectors, which stand approximately 1.5 m above ground level, are often used. To avoid larger objects (insects, parts of leaves or needles, etc.) from falling into the collector and contaminating the sample, a polyethylene net mesh width of 1 mm is recommended (Hansen et al., 2013) to be placed at the top of the neck of the collector. Two weeks or monthly time for sampling is recommended. After each sampling period, the volume of each throughfall and precipitation sample is determined. Each sample can be analysed separately, or a mixed sample can be formed. The samples are kept in a refrigerator (0-4°C) for pre-treatment to prevent chemical and biological sample degradation. The samples are filtered through a 0.45 µm membrane filter to remove any solid material and microorganisms for the subsequent analyses (Clarke et al., 2022).

The following characteristics and analytical methods are determined in the laboratory. pH is measured potentiometrically using a pH meter (ISO 10523:2008). Metals: sodium (Na⁺), potassium (K⁺), magnesium (Mg²⁺), calcium (Ca²⁺), lead (Pb²⁺), and cadmium (Cd²⁺) are determined by Atomic absorption spectrometry (ISO 8288:1986) or ICP MS spectrometry (Wilschefski & Baxter, 2019). Nitrogen-containing ions: NH₄⁺ and NO₃⁻ were measured by Kjeldahl analysis (ISO 1871:2009). Sulfates (SO₄²⁻) are measured spectrophotometrically – Thorin method (ISO 7934:1989). The deposition rate of the ion of interest is calculated by multiplication of solute concentration with relevant precipitation amount and summed for the entire year (Clarke et al., 2022).

Future directions

Future research is needed to offer additional indicators in the biosphere, such as flying insects, birds, bats, frogs, and mammals. Moreover, there

is a need to analyse relationships among all observed ecosystem components for a long-term period by comparing the results of individual measurements and observations.

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