

ACTA SILVATICA  
&  
LIGNARIA  
HUNGARICA



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HUNGARICA

AN INTERNATIONAL JOURNAL  
IN FOREST, WOOD  
AND ENVIRONMENTAL  
SCIENCES

VOLUME 16, NR. 1  
VOLUME 16, NR. 2  
2020



ACTA SILVATICA ET LIGNARIA HUNGARICA  
AN INTERNATIONAL JOURNAL IN FOREST, WOOD AND ENVIRONMENTAL SCIENCES  
*issued by the Forestry Commission of the Hungarian Academy of Sciences*

*The journal is financially supported by the*

*Hungarian Academy of Sciences (HAS),  
Faculty of Forestry, University of Sopron (FF-US),  
Simonyi Karoly Faculty of Engineering, Wood Sciences and Applied Arts, University of  
Sopron (SKF-US),  
National Agricultural Research and Innovation Center, Forest Research Institute (NARIC-FRI),  
Sopron Scientists' Society of the Hungarian Academy of Sciences (SSS).*

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HU ISSN 1786-691X (Print)

HU ISSN 1787-064X (Online)

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*Information and electronic edition:* <http://aslh.nyme.hu>

The journal is indexed in the CAB ABSTRACTS database of CAB International; by SCOPUS, Elsevier's Bibliographic Database and by EBSCOhost database.

*Published by* UNIVERSITY OF SOPRON PRESS,  
BAJCSY-ZS. U. 4., H-9400 SOPRON, HUNGARY

*Cover design by* ANDREA KLAUSZ

*Printed by* LÖVÉR-PRINT KFT., SOPRON

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## Management of *Robinia pseudoacacia* cv. ‘Üllői’ – ‘Üllői’ locust

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**Abstract** – Black locust (*Robinia pseudoacacia* L.) is the most widespread introduced tree species in Hungary. Though it covers 24% of the country’s total forest area, the wood industry has difficulties processing large quantities of this poor quality wood. To address this issue, the Hungarian Forest Research Institute (FRI) initiated a selective breeding program designed to improve black locust wood quality. The breeding was based mainly on the small, elite breeding populations of the so called “ship mast” locust, which possess solid, straight, fork-free stems. Mono- and multi-clonal cultivars were developed and cultivar comparative and growing trials were established. Among the selected cultivars, the cultivar ‘Üllői’ locust (*Robinia pseudoacacia* cv. ‘Üllői’) proved one of the best. As a result, a comprehensive review on the management of ‘Üllői’ locust in Hungary was compiled. This study provides a contribution to the improvement of growing technology used for selected black locust cultivars.

***Robinia pseudoacacia* / ‘Üllői’ locust / selection / growing**

**Kivonat** – Az ‘Üllői’ akác (*Robinia pseudoacacia* cv. ‘Üllői’) termesztése: áttekintés. Magyarországon a fehér akác (*Robinia pseudoacacia* L.) az egyik legelterjedtebb exóta fafaj. Az ország erdőterületének 24%-át foglalja el, azonban a faipar nem képes az alacsony minőségű akác faanyagot nagy mennyiségben feldolgozni. Ebből következően, a honi Erdészeti Tudományos Intézet (ERTI) egy szelekciós nemesítési programot indított néhány évtizeddel ezelőtt a faminőség javítása érdekében. Egy- és többklónú fajtákat hoztak létre, valamint fajtaösszehasonlító és termesztési kísérleteket létesítettek. A kiválasztott fajták közül az ‘Üllői’ akác (*Robinia pseudoacacia* cv. ‘Üllői’) fajta bizonyult az egyik legjobbnak. Ezt a tényt figyelembe véve, átfogó áttekintés készült az ‘Üllői’ akác magyarországi termesztéséről. A tanulmány hézagpótlólag járulhat hozzá a szelektált akácfaajták termesztési technológiájának fejlesztéséhez.

***Robinia pseudoacacia* / ‘Üllői’ akác / szelekció / termesztés**

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## 1 INTRODUCTION

Selecting new clones and cultivars able to provide industrial wood of good quality and volume were the main objectives of the first black locust breeding programme in Hungary in the 1960s. Superior tree groups have been identified in some seed origin stands. Graft material was taken from the plus trees and planted in test plots at Gödöllő (the experimental station of Forest Research Institute (FRI)). *Mono- and breeding populations* were developed and a seed orchard was established from the selections (Keresztesi 1983, Rédei et.al. 2001, 2002).

FRI coordinated the research programme. Results showed the ‘Jászkiséri’, ‘Kiscsalai’, ‘Nyírségi’, ‘Üllői’ and ‘Szajki’ cultivars proved the best in terms of volume expected at felling age (Keresztesi 1988).

Several countries have also started research programmes to improve black locust wood quality and/or increase the production of biomass for energy purposes. Black locust has also been considered a promising tree for animal feed, nectar production, and the re-cultivation of dry and devastated lands. At present, black locust breeding and improvement research is being undertaken in the United States (Bongarten et al. 1991), Greece (Dini-Papanastasi – Panetsos 2000), Germany (Liesebach et al. 2004), Poland (Kraszkievicz 2013), Turkey (Dengiz et al. 2010), India (Sharma et. al. 2006), China (Dunlun et al. 1995), and South Korea (Lee et al. 2007). Countries are increasingly interested in black locust improvement and management with a focus on the species’ response to climate change effects.

The main goals of this paper are as follows:

- Bringing together researchers and forestry professionals who are interested in all aspects of black locust improvement.
- Documenting available knowledge about ‘Üllői’ black locust.
- Facilitating future information exchanges on black locust clones and cultivars.

## 2 ORIGIN AND TREE CHARACTERISTICS OF THE EXPERIMENTS

‘Üllői’ locust (*Robinia pseudoacacia* cv. ‘Üllői’) was bred by B. Keresztesi and his co-workers Z. Marjai, J. Fila, and Z. Bujtás at FRI in the middle of the 20th century (Keresztesi 1983, 1988). The cultivar was registered in 1982. The origin of ‘Üllői’ locust is related to J. Fila who called the attention to the occurrence of this cultivar. In March 1966, plus trees were selected from the forest sub-compartment Üllő 10D on rusty-brown forest soil developed on sand, deposited on meadow forest soil. Collecting scions was difficult because climbers could not establish a safety station on the tree due to their tapering stem and narrow crown (Keresztesi 1988).

The characteristics of ‘Üllői’ locust are as follows: Pinnata type, trunk is vigorous, cylindrical and straight to the top of the crown. A greenish-brown field with many light brown lenticels between two linear stripes are visible on its bark. We noticed many bark plates on old trees. Spines are tiny; circa 10 mm long. The foliage is erect and the short leaf-stalk has 17–19 leaflets. These are oval-shaped and widest in the middle part, while the tips are blunt at the end with small awns. Leaflets on the underside are glaucous. The largest leaflets are in the middle part of the compound leaf. The tree has short-bodied white flowers that produce variable amounts of bloom that follow the same blossom period as common black locust. It has average nectar production and very poor seed-binding; moreover, it provides a medium to low value bee pasture (Keresztesi 1988).

‘Üllői’ black locust is susceptible to late and early frosts; therefore, it is not recommended for sites in higher hilly zones and in areas where frost hollows are present.

Good results can be attained in regions where the mean annual temperature is above 8°C. Fine sands and light loamy soil types are good for these black locust cultivars, provided sufficient soil depth. Shallow soils, soils with a poor water regime and coarse sand, or soil containing many stones are unfavourable. Clay texture is also unfavourable due to its poor aeration and compact condition.

### 3 STUDY SITES

The first experimental stand of the 'Üllői' cultivar was established in 1967 at the FRI Gödöllő Arboretum. Successful vegetative propagation led to further field cultivation experiments with this cultivar, experiments which have been conducted in various parts of the country ever since. This study executed evaluations in 21 experimental forest subcompartments. These are located at Tét, Gödöllő, Isaszeg, Pusztavacs, Helvécia, and Szentkirály. The trial plots are located in either a Turkey oak -sessile oak forest climate or a Forest-steppe climate (according to the Hungarian climate classification categories). The ages of the 'Üllői' black locust stands range from 6 to 35 years. Research site locations are presented in *Figure 1*.



*Figure 1. Location of the research sites*

*Table 1* lists the site description including location (forest subcompartment), site type, and the most important dendrometric characteristics (age, H, DBH, V, N, G, mean tree volume).

Table 1. Location, site type, and stand characteristics

Location, subcompartment	Climate	Hydrology	Genetic soil type	Depth of productive layer	Soil texture	Age	H	DBH	DBH/H* 100	V	N	G	Mean tree volume	Yield class (Rédei, 1984)
						(yr)	(m)	(cm)	(%)	(m³/ha)	(tree/ha)	(m²/ha)	(dm³)	
Isaszeg 7D	3	1	46	4	3	6	5.5	4.9	89.09	23.00	2555	4.82	9.00	III.
Gödöllő, Arboretum	3	1	46	4	3	10	13.3	10.4	78.20	100.03	1672	14.20	60.25	I.
Szentkirály 40 F	3	1	15	3	3	14	14.9	12.2	81.88	133.52	1320	15.43	101.15	III.
Gödöllő, Arboretum	3	1	46	4	3	15	15.6	12.7	81.41	171.18	1672	21.18	102.38	I.
Helvécia 67B	4	1	15	4	3	19	17	18.7	110.00	283.10	950	26.09	298	III.
Gödöllő, Arboretum	3	1	46	4	3	20	19.7	19.7	100.00	182.34	1095	33.38	166.52	I.
Szentkirály 46 G	4	1	15	3	3	20	18.5	15.4	83.42	218.00	1200	22.32	181.67	III.
Szentkirály 47 H	4	1	15	3	3	20	17.5	15.3	87.27	225.10	1300	23.85	173.15	III.
Tét 16 K-I	3	1	46	3	3	21	17	17	100.00	162.27	752	17.07	215.78	III.
Tét 16 K-II	3	1	46	3	3	21	17.7	17.3	97.74	131.39	573	13.47	229.3	III.
Tét 16 K-III	3	1	46	3	3	21	17.5	18	102.86	149.67	606	15.42	246.98	III.
Pusztavacs 212 A	4	1	15	3	3	31	15.5	15.7	101.90	169.03	930	18.10	181.75	V.
Pusztavacs 213 B	4	1	15	3	3	33	18	20.6	114.85	196.95	540	18.03	364.72	IV.
Pusztavacs 213 C	4	1	15	3	3	33	18	18.2	100.90	164.99	620	16.04	266.12	IV.
Gödöllő 5G (137D) - 5/26	3	1	46	3	4	35	22	21.6	98.18	199.80	448	16.42	445.98	II.
Gödöllő 5G (137D) - 5/32	3	1	46	3	4	35	19.4	20.6	106.19	229.80	674	22.46	340.95	III.
Gödöllő 5G (137D) - 5/48	3	1	46	3	4	35	20.4	22	107.84	218.90	546	20.76	400.92	III.
Gödöllő 5G (137D) - 5/44	3	1	46	3	4	35	22.4	23.4	104.46	335.40	690	29.67	486.09	II.
Gödöllő 7 B-I	3	1	46	4	3	35	18.9	21.6	114.29	235.46	625	22.90	376.74	IV.
Gödöllő 7 B-II	3	1	46	4	3	35	20.7	22.1	106.76	237.15	567	21.75	418.25	III.
Gödöllő 7 B-III	3	1	46	4	3	35	20.8	22.2	106.73	192.75	451	17.46	427.38	III.
Gödöllő 7 B-IV	3	1	46	4	3	35	18.4	22.3	121.20	235.81	596	23.28	395.65	IV.

**Climate:** 3 Turkey oak – sessile oak, 4 Forest steppe.

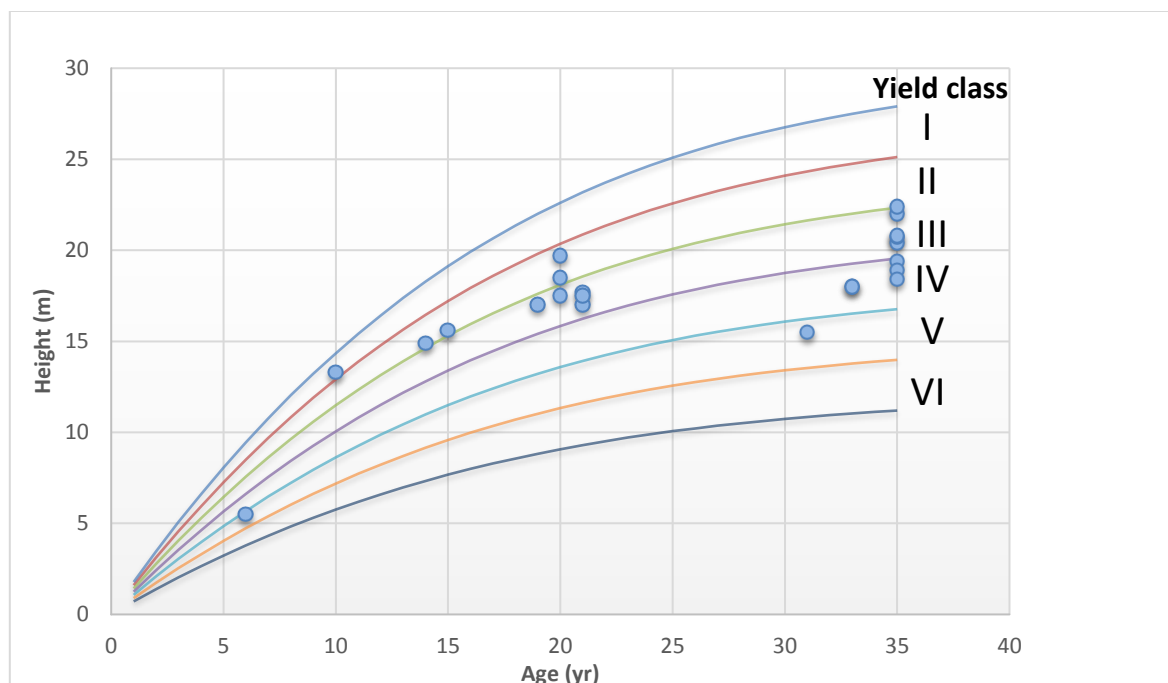
**Hydrology:** 1 No additional water supply.

**Genetic soil type:** 15 Sand with humus, 46 Rusty brown forest soil.

**Depth of productive layer:** 2 shallow, 3 medium deep, 4 deep.

**Soil texture:** 3 sand, 4 sandy loam.

Distribution of the sample plots in the site index curves of the black locust yield table are presented in *Figure 2*. As the figure shows, most plantations belong to yield class I to III. This means 'Üllői' black locust can produce relatively high volume on good sites where the objective is the production of sawlogs. A high proportion of poles and props can be expected from yield IV plantations.



*Figure 2. Experimental plots in the site index curves of black locust yield table (Rédei, 1984)*

#### 4 VOLUME EQUATIONS

Volume equations based on a single variable of DBH may be constructed from existing multiple-entry volume tables or from the scaled measure of standing or felled trees. Such equations are particularly useful for quick timber inventories because height and form estimates are not required and trees can be tallied by species and DBH only.

Volume equations based on DBH alone are sometimes compiled for inventories of relatively small areas, but this is not an essential condition; in some instances, "local" equations may be as widely applicable as "standard" equations. From 30 to 100 samples are usually considered a minimum number for small tracts, depending on the range of diameter classes to be included in the equation.

*Figure 3-1* and *3-2* provide relationships of tree volume to DBH, and the same relationship transformed to a straight line, based on measurements of 55 'Üllői' trees at Pusztavacs region. The tree volume equations are subsequently used to estimate the average tree volume in each diameter class.

*Figure 4* provides the relationship of mean tree volume ( $v$ ) and DBH based on measurements of 22 'Üllői' black locust plantations (see *Table 1*). Multiply the mean tree volume by the number of trees per hectare to give the total volume per hectare. Multiply the volume per hectare by the stocked area of the plantation to obtain the total volume of the plantation.

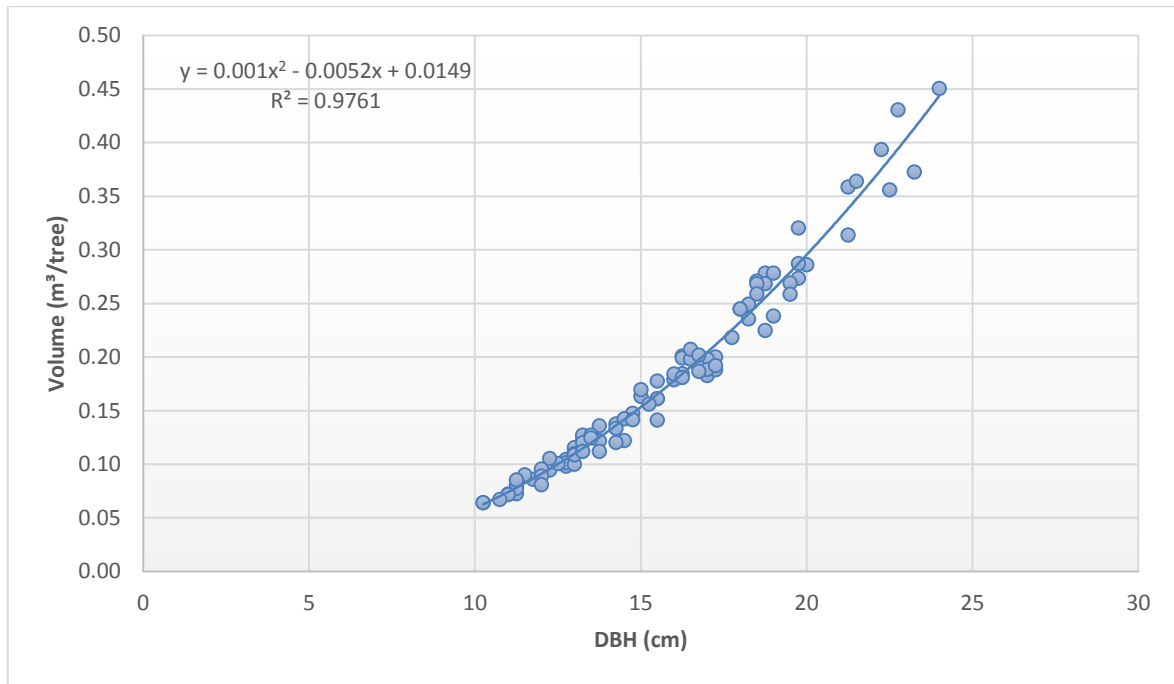


Figure 3-1. Curvilinear relationship of tree volume to DBH  
(based on measurements of 31 years old 'Üllői' black locust trees in Pusztavacs 212 A)

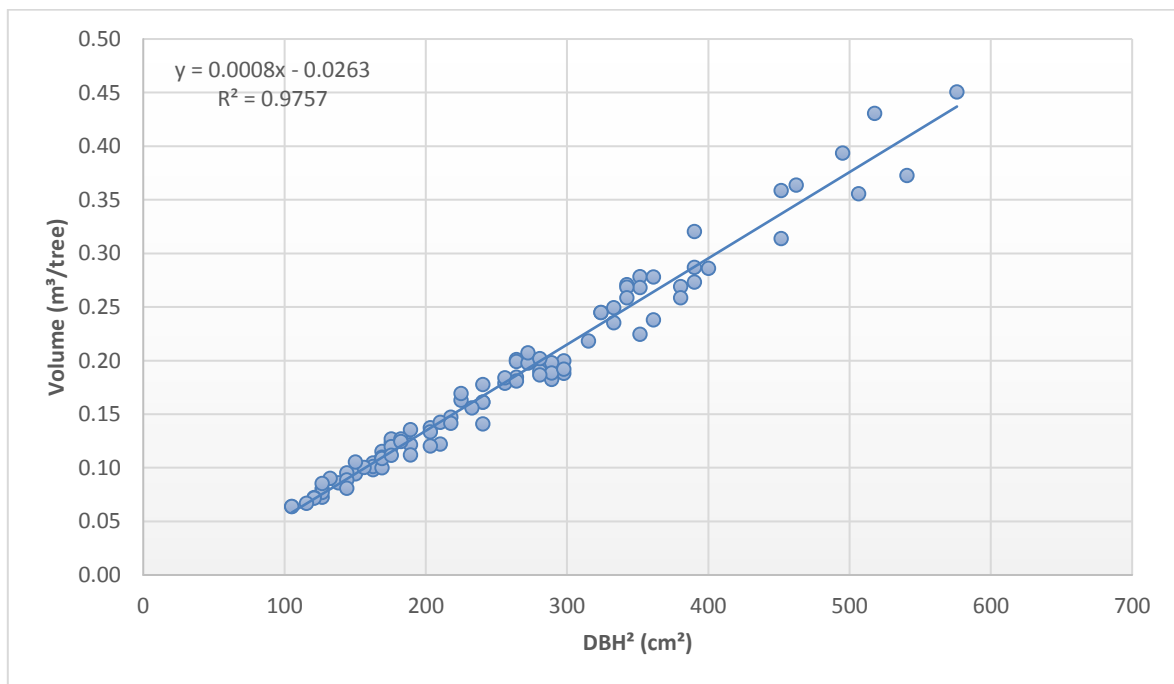


Figure 3-2. Straight line relationship of tree volume to single tree basal area of single trees  
(based on measurements of 31 year old 'Üllői' black locust trees in Pusztavacs 212 A)

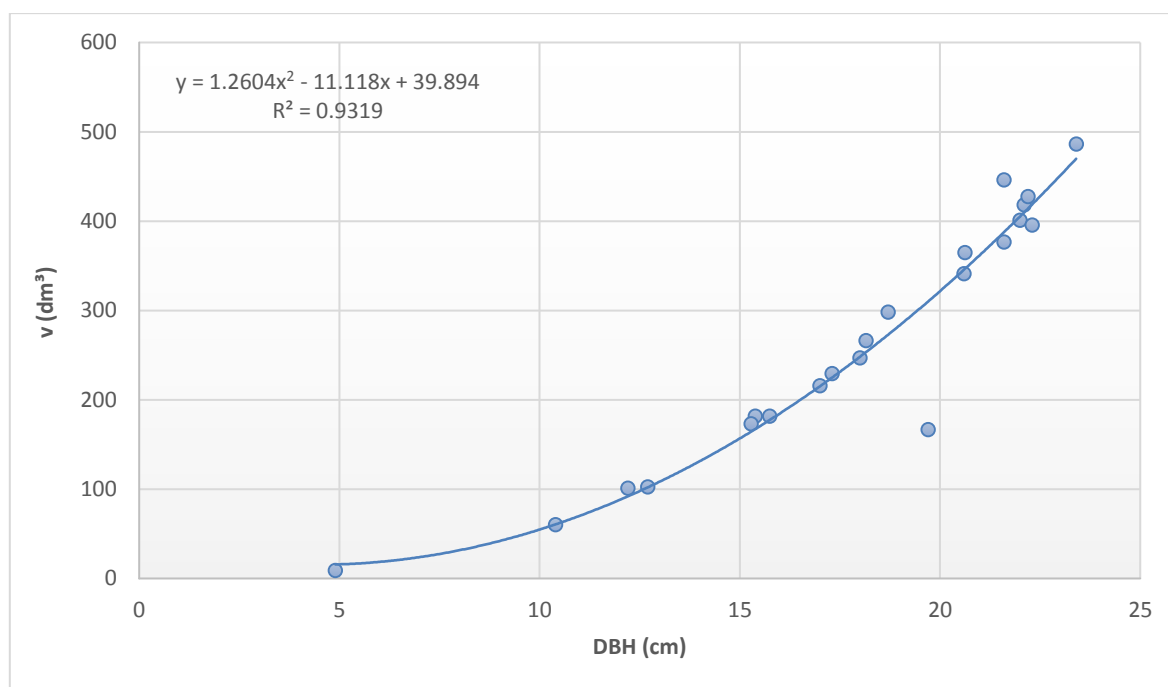


Figure 4. Curvilinear relationship of mean tree volume to DBH (based on measurements of 22 'Üllői' black locust plantations)

## 5 TENDING CUTTING MODELS

Tending techniques for black locust have been developed as a result of both advances in research and practical experience. A grouping of forest tending operations to form a tending regime can be made on the basis of results of long-term stand structure and forest yield trials. This is a great help for planning, prescribing, and controlling tending operations. The number of forest tending operations can be reduced by an effective forest tending regime and, at the same time, cleaning and thinning intensity can be increased. Introducing new cultivars as an alternative to commercial black locust growing has opened new perspectives for further development.

Tending principles for black locust stands established with cultivars are in some ways different from those established with common black locust seedlings or regenerated by coppicing. As in this case where stands are established with genetically uniform plants, initial spacing can be wider theoretically if there is no risk of game damage. Instead of a number of selective tending cuttings, a single comprehensive operation could rationalize the whole growing process (Rédei 2013).

### Recommendations for tending cuts (enlargement of growing space) for 'Üllői' black locust plantations

Tending guidelines of stands established with selected black locust cultivars are different from stands established by seedlings or regenerated by coppicing. Tending phases typical for multiclonal common black locust stands with similar growth conditions (cleaning, thinning) are more difficult to separate for the "Üllői" black locust because the growth properties of monoclinal cultivars are theoretically identical. The particular aim of tending cuttings is to form the growing space for the optimal growth of the trees. When designing the ages and

intensities of cutting, we relied on both the results of thinning experiments and the experience of forest managers working with this specific clone.

On good and excellent sites, altogether two enlargements of growing space are applicable to produce raw material for the sawmilling industry in stands planted in a 2.5×2.0 m spacing (5 m<sup>2</sup>/tree growing space) (Table 2.). During the first enlargement of growing space (at the age of 9–10), tree number reduction is approximately 50%, so spacing will be 2.5×4.0 m (10 m<sup>2</sup>/tree growing space) after the tending. The second enlargement of growing space (at the age of 16–17) also reduces the number of stems by 50%. During this process, the greater part of the yield is already suitable for industrial utilization. Hence, this growing technology can be considered economically profitable.

Prospective tree plantations of selected black locust cultivars tended according to the demonstrated model in Table 3 are profitable only on excellent and good sites. If reduction of the rotation ages (20–25 years) is planned, the growing aims can be the production of poles, or saw logs of a lower size limit.

Table 2. Models of enlargement of growing space of selected black locust cultivars.  
Aim of growing: sawlog. Initial spacing: 2.5 x 2.0 m.  
Initial number of seedlings: 2000 plants/ha.

Label	Age	Height	Diameter at breast height	Number of trees	Expected total volume
		H	DBH	N	V
	(yr)	(m)	(cm)	(tree/ha)	(m <sup>3</sup> /ha)
Yield Class I					
1. Enlargement of growing space	9–10	14	13	1000	90
2. Enlargement of growing space	16–17	20	18	500	130
3. Harvest cutting	30	25	25	450	270
Yield Class II					
1. Enlargement of growing space	9–10	13	11	1000	90
2. Enlargement of growing space	16–17	18	16	500	120
3. Harvest cutting	30	23	23	450	220
Yield Class III					
1. Enlargement of growing space	9–10	12	10	1000	55
2. Enlargement of growing space	16–17	17	15	500	80
3. Harvest cutting	30	21	21	450	170

It is also important that *pruning* should be done on time and with skill in stands established with ‘Üllői’ cultivars. At a mean crop height of 2.5–3.0 m, all branches in the first 1 m of stem should be removed as well as any others that reach into the space between rows and hinder cultivation. Form pruning of the crown should also be done at this time. The second pruning is carried out when height is 5–6 m. Only rows remaining after the first cleaning need be pruned. The third pruning, to a height of 3–4 m, is due after the cleaning and is limited to final crop trees. The final pruning, up to a height of 5–6 m, is done after thinning.



Table 3. Models of enlargement of growing space of plantations established by selected black locust cultivars. Aim of growing: poles, prospectively sawlogs. Initial spacing: 3.0 x 3.0 m. Initial number of seedlings: 1100 pieces/ha

Label	Age	Mean height	Mean diameter	Number of trees	Expected volume
		H	DBH	N	V
	(yr)	(m)	(cm)	(tree/ha)	(m <sup>3</sup> /ha)
Model I					
Before enlargement of growing space	10	13	10	1100	60
After enlargement of growing space	10	14	11	700	50
Harvest cutting	20	20	18	700	180
Model II					
Before enlargement of growing space	8	10	8	1100	35
After enlargement of growing space	8	11	9	750	30
Before enlargement of growing space	15	17	14	750	105
After enlargement of growing space	15	18	15	500	85
Harvest cutting	25	22	20	500	180

## 6 CONCLUSIONS

Common black locust may – to varying degrees – have negative properties such as warping and twisting, forked stems, and low industrial wood yield, which are all disadvantageous for cultivation.

Therefore, from the second half of the 20th century, the staff of the Hungarian Forestry Research Institute (FRI) has been engaged in the improvement of black locust cultivation technology, including the selection and cultivation of selected black locust cultivars. The primary purpose of these initiatives is to improve stem quality and increase wood and nectar yields. In the case of 'Üllői' black locust, the aim was to improve stem quality (Keresztesi 1988).

Even though black locust cultivars possess better qualities than common black locust, they are not widespread in the afforestation practice of forest enterprises. The reason is the relatively high costs of cultivar propagating material. Consequently, it is cheaper for forest managers to apply common black locust instead of cultivars. Hopefully, EU subsidies and local/national funding for the forest sector will change this situation in the future. The 'Üllői' cultivar is one of the most cultivated varieties, having about 15 thousand rooted cuttings at the Nyírerdő State Forest Shareholders Company in Nyíregyháza.

For some decades black locust has garnered greater attention in an increasing number of countries due to global climate change and the energy crisis, which have stimulated research on relatively rapid growing, nitrogen-fixing trees such as black locust. This short review posits the following conclusions:

- (1) selected black locust cultivars like 'Üllői' can be grown well under semi-marginal site condition as well;
- (2) vegetative propagation method – root cuttings – have proved to be as a suitable means in black locust clonal selection;
- (3) by growing selected black locust cultivars, it is possible to increase the stem quality significantly by 12–25% on average (Rédei et. al. 2017).

**Acknowledgements:** The research was made in frame of the “EFOP-3.6.1-16-2016-00018 – Improving the role of research, development, and innovation in higher education through institutional developments assisting intelligent specialization in Sopron and Szombathely”. We express our gratitude to Imre Bíró, the Director of Baktalórántháza Forest Estate, Nyírerdő State Forest Shareholders Company for providing information on ‘Üllői’ black locust practice-oriented management.

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## The Multifunctional Role of Shelterbelts in Intensively Managed Agricultural Land – Silvoarable Agroforestry in Hungary

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**Abstract** – The use of shelterbelts as windbreaks to protect and increase field productivity has a long history in Hungary. Nevertheless, when shelterbelts began to wane, many environmental problems such as soil drying, deflation, and erosion began to occur, which in turn led to economic difficulties. Earlier field experience supported by new results indicates that shelterbelts are beneficial for intensively-treated fields, this despite the space shelterbelts require. Our research study aims to summarize the information available in Hungarian and international literature regarding the most effective shelterbelt structure. In addition, the study supports the design of multipurpose tree plantations with recommendations to mitigate climate change impacts and minimize the negative effects of intensive agricultural technology. In this article we would like to draw attention to the fact that shelterbelts can serve as effective tools in agroforestry and can be regarded as a means of ensuring economically and environmentally sustainable methods for agriculture. Below, we summarize how shelterbelts can help with adaptation to coming global and local challenges; we also describe why and how shelterbelts can be renewed and implemented in a reasonable way.

**shelterbelt / productivity / green infrastructure**

**Kivonat** – Az erdősávok szerepe intenzíven művelt mezőgazdasági területeken - szántóföldi agrárerdészet Magyarországon. Egyes európai országokban komoly hagyományokkal rendelkezik a mezővédő erdősávok telepítése az épített környezet, a szántóföldek védelme, a termelékenység növelése érdekében, a szélerősség csökkentése és a klíma szabályozása által. Ahol ezeket az erdősávokat felszámolták, komoly környezeti problémák merültek fel, mint például erózió, a talaj kiszáradása, defláció, amelyek gazdálkodási nehézségeket okoznak. A korábbi szakirodalom és jelenlegi európai kutatási eredmények alapján úgy tűnik, a területfoglalással együttvéve is előnyösek az erdősávok az intenzíven művelt területeken. Kutatásunk célja, hogy a magyar és nemzetközi szakirodalom alapján összefoglaljuk a mezővédő erdősávok leghatékonyabb felépítéséről rendelkezésre álló információkat, és ajánlásokkal támogatjuk a többcélú erdősáv rendszerek tervezését a klímaváltozáshoz való alkalmazkodás és az intenzív mezőgazdasági technológiák kedvezőtlen hatásainak csökkentése érdekében.

**erdősáv / termelékenység / zöld infrastruktúra**

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## 1 INTRODUCTION

Extreme weather, droughts, and the increased frequency of flooding have negative impacts on natural vegetation as well as the quantitative and qualitative parameters and safety of agricultural production (Akpoti et al. 2019, Luetzenburg et al. 2019, Wiréhn 2018). Erosion, pollution, snowdrift, frost, and drought can all cause problems for infrastructural facilities (e.g. roads), and for quality of life as well. (Echavarren et al. 2019, Khavarian-Garmsir 2019). Natural or anthropogenic impacts can be reduced through technological solutions, but living plant organisms may replace these or increase their efficiency when biologically active areas are developing, which can favourably affect the quality of the environment (e.g. protecting species and soils, climate conditioning.) Targeted usage of appropriately planted vegetation (including non-forest plantations<sup>1</sup> as well) can significantly contribute to the supportable execution of ecological needs, and the requirements of environmental management and nature protection. In that way, the development of shelterbelt systems could strongly contribute to the EU Strategy on Green Infrastructures (GIs), which promotes the deployment of GIs across Europe (EC 2013).

The first shelterbelt data in Hungary is connected to a windbreak established in 1802. The aim of planting 10 rows of willow trees was to settle drifting sand and facilitate agricultural production (Danszky 1972). The first purposeful establishment of forests were in the 1950s, which resulted in the shelterbelts reaching their maximum length in Hungary in this period (Négyesi 2018). Agricultural techniques developed by leaps and bounds until the 1970s, causing a demand for large-scale farming. This in turn led to a decrease in shelterbelts as the space reserved for them were absorbed into farming (Takács – Frank 2008). In addition to this, shelterbelt ownership became unclear after communism ended; therefore, many remaining plantations were simply abandoned. Nowadays, the common European Union agricultural policy supports establishment of shelterbelts, and they are intensively researched alongside economic interests and ecosystem services.

The significance of this study is that it evaluates the effects of shelterbelts in a multifunctional way based on the results of studies from different perspectives accumulated over decades. The collected data indicates that, aside from their land requirements, the most significant disadvantage of shelterbelts is the decrease in yield caused by competition. A properly planned and planted shelterbelt comprises a very small portion of agricultural land, and its advantages are many times greater than its disadvantages (Mize et al. 2008). Moreover, root pruning effectively reduces the competition with the crop (Kort 1988, Kowalchuk et al. 1995).

## 2 THE EXTENT OF SHELTERBELT SYSTEMS IN EUROPE

Hedgerows and shelterbelts are grouped together as windbreak tree plantations in European surveys and project reports; therefore, separate data for each is currently unavailable. These agricultural protection plantations in some European countries are examples of the integration of trees with farming systems. Herzog (2000) defines hedgerows as structures comprised of trees or bushes that separate land parcels of different owners. Data from 2001 estimated the area of hedges to be 117,174 ha for England, Scotland, and Wales (den Herder et al. 2015). Hedgerows and windbreaks cover about 12,400 ha in Belgium (den Herder et al. 2017, based on Etat de l'Environnement en Wallonie 2010). In Hungary, a shelterbelt system of around 16,400 ha could be found in 2001 (Takács – Frank 2008), which is only half of the area occupied

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<sup>1</sup> non-forest plantation: planting trees on areas, on which agriculture is unprofitable, or along streets, irrigation canals, cisterns, watercourses, or in the surroundings of settlements or monuments (Gál et al. 1960).

by these agroforestry systems in the 90s. According to the estimation, between 40-80% of the hedgerows have disappeared in Europe since the end of 1960s (Herzog 2000). The policy in itself was not the only cause; the use of land solely for production was also a factor (Baundry et al. 2000).

Though many traditional agroforestry systems have disappeared with the intensification of agriculture since the 1960s, a revived interest in integrating trees with agricultural production systems has occurred. "This interest comes from farmers who can see benefits in terms of increased and more diversified production" (den Herder et al. 2015).

The authors of this article would like to draw attention to shelterbelts as effective instruments for agroforestry and for economically and environmentally sustainable agriculture. The study provides a summary of how shelterbelts can help the adaptation to coming global and local challenges through ecosystem services, and offers clear guidelines on the reasonable implementation of shelterbelts.

### 3 WINDBREAKS

Shelterbelts decrease harmful effects mainly by reducing wind speed. The reduction of wind speed through shelterbelts of appropriate structure and direction may generate micro and mezzo climate changes that are advantageous for cultivated crops. Furthermore, these plantations can reduce accident risk on motorways by eliminating snowdrifts caused by crosswinds along roads, as well as limit the spread of pollution, dust, and erosion on bare surfaces. They can also reduce the spread of foul smells. The establishment of a shelterbelt is a relatively cheap solution for protecting agricultural land. The efficiency of reducing wind speed is about 10–15% on the windward side, and can reach 60% on the leeward side (Boskovic et al 2010).

The effectiveness of shelterbelts as wind speed reducers can be best described by the openness factor, which is the ratio of wind speeds measured on the protected side behind the belt and those measured in open areas. The openness factor depends on the "porousness" of the shelterbelt's structure measured in its leafy condition. The most effective are the so-called fretwork or porous-structured shelterbelts. In these belts, the gaps that let the moving air through add up to 10 to 30% to the lateral surface, creating an openness factor of between 0.35 and 0.70. This means the wind speed on the protected side of the shelterbelt will generally be reduced by 50%. Behind closed plantations (without gaps or at less than 10% of the lateral surface), turbulence, heat pockets, and frost corners may develop. Open belts (with a gap ratio of more than 30%) are ineffective at reducing winds and may even increase wind speed through the so-called echelon-effect (Dömsödi 2010, Gál 1972).

Regarding the widths of shelterbelts, these can be categorized into three main groups. Narrow belts are 6 to 11 meters wide and contain 3 to 7 rows. Medium belts are 12 to 20 meters wide containing 8 to 13 rows, while wide belts with widths of 20 to 30 meters and 14 to 20 rows belong to the category of protecting forests (Gál 1972).

The results of Gál (1961), which assessed the wind-reducing effects of different shelterbelt structures in relation to their height, are summarized in the chart below (the extension of protective effects is given by a multiplier to the height of the trees). At lower wind speeds, the effectiveness of dense shelterbelts decreases, whereas that of porous ones increases.

Based on research results and economic aspects that were also taken into consideration, the deployment of mainly narrow – 12 to 15 meter wide and 7 to 9 row – porous belts is recommended (Gál 1961).

Recent researches have introduced the concept of total area density ( $Ad$ ), which is obtained by dividing projected area of leaf, branch, and stem per unit ground area, by the average crown

length, because most of the total area of vegetation is in the crown mainly.  $Ad$  multiplied with the width of the shelterbelt ( $Ad \times W$ ) is considered to be a measure of the total surface area per unit length of the shelterbelt obstacle. Torita-Satou (2007) found a significant positive correlation between the sheltered area and  $W \times Ad$ .

*Table 1. Efficiency of different shelterbelt structures,  $h$  = tree height (based on Gál 1961)*

Effect		Distance		
		closed	porous	open
Windbreak	front side	5 – 22h	3 – 17h	5 – 10h
	behind/protected side	15 – 49h	15 – 51h	11 – 20h
Highest protection		1 – 5h	1 – 5h	1 – 10h
Practically important (min 50%) wind decrease		10h	10h	–

Curiously, Hungarian experiments showed that snow stopping properties of shelterbelts are not influenced by porousness, but rather by tree height, the geographic structure of the belt, and the surrounding surfaces (Takács 2008). The more complex the obstacle we set up perpendicularly to the wind direction is, the better the expected result should be. A complex 4-row plantation alongside the road at a minimum distance of 20 meters, where the line of trees is combined with an edge of shrubs (e.g. articulated in two parts), can be more effective than a conventional 8 to 10 row protective belt. The reason may be that the articulated structure of the 4 row belt and the turbulence created by it can change the direction of the wind vectors and the energy of the particles conveyed by the wind. Thus, the particles settle along the wind-exposed side of the belt in a strip about 20 meters wide in the uncovered area between the belt and the line of trees (shrub) as well as on the embankment between the road and the line of trees.

As described above, in addition to appropriate orientation, the most important factor is to shape the structure so that it is suitable for the purpose of protection. Experience shows that it is unnecessary to plant 15 to 20 row-wide shelterbelts since the first couple of rows of trees can break the strength of winds insofar as it does not endanger the protected area or project.

Model experiments show that the length, height, width, and cross-sectional shape have an effect on the aerodynamic features of the shelterbelt, as well as on the internal structural components, such as the amount and arrangement of its vegetative surface area and volume, as well as the geometric shape of individual vegetative elements (Brandle et al. 2004, Zhou et al. 2004).

#### 4 MITIGATION, CLIMATE ADAPTATION AND PRODUCTIVITY

Transpiration and assimilation are much higher in forests than in other forms of vegetation due to the high leaf-surface index, which has a cooling effect on the environment. Thus, the carbon sequestration of the forested areas coupled with agricultural systems may dampen global warming, while enhancing productivity (Amichev et al. 2016, Mátyás (ed.) 2005).

The effects of shelterbelts that influence the micro-climate (e.g. windbreak, increasing the relative moisture of air, decreasing evaporation, promoting the formation of dew and homogeneous blankets of snow), manifest themselves in increasing agricultural productivity.

Decreased air movements help reduce plant and soil evaporation. This leads to an improved water balance and hydration and, thereby, lower energy requirements to compensate dehydration. Stomas do not close in lighter winds, enabling undisturbed ventilation. Reduced air movement reduces the chilling of the environment; thus, soil and air temperatures increase, which is favourable for germination, the function of plant cells, and soil microbes, too (Szarvas

2010). The physical damage (twisting by the wind or sandblasting by eroding particles is smaller in the protection of a shelterbelt (Boskovic et al 2010).

Summarizing the results of several researchers (Kölös 1979, Takács 2008, Abdalla – Fangama 2015, Zheng et al. 2016), we can conclude that a 10–12m wide, articulated and at least 20m high, but young shelterbelt, supplemented with a shrub zone to break wind and snow, can have a positive effect on crop yield up to a distance of 300 m for a wide range of crops: groundnuts, cotton, vegetables, cereals, maize.

A statistically evaluated yield analysis was carried out in the 1960s for the seven most important crops (winter wheat, winter and spring barley, alfalfa, maize, carrot, pasture grass) in 18 selected areas in Hungary (Gál 1963). The conclusion was that the production-increasing effect is demonstrable on both sides of the shelterbelt, regardless of their compass orientation. The best result is achieved if shelterbelts are situated perpendicularly to the typical direction of wind. In Hungary, shelterbelts positioned in an east-west direction are the most effective since protection against wild northerly winds and dry southerly winds is extremely important. The danger of drought occurs mainly with winds with a temperature higher than 25 °C / 77 °F, and relative moisture lower than 35%.

Concerning the wind-breaking and snow-catching properties of shelterbelts, the width of the protected zone for increased yield is influenced mostly by the height and structure of the shelterbelt; the width has no significant influence.

The width of the effective zone can be 6 to 15 times bigger than the height of trees on the northern and southern side of the shelterbelt, while on the eastern and western side, this is limited to 8–10 times. The biggest rise in crop yield has been experienced in a strip 3–10 times wider than the height of the trees.

The favourable effects on climate and yield are more apparent in shelterbelt sites situated in locations that experience weather extremes and drought; the more extreme the conditions, the more apparent the favourable effects become.

Table 2 shows the extra yield on shelterbelt-protected areas, compared to samples taken from unprotected control plots.

*Table 2. Extra yield in shelterbelt-protected plots (Based on Gál 1963)*

Plant species	Extra yield (%)
winter wheat	9.8 – 26.8
winter barley	1.7
spring barley	6.1 – 33.5
alfalfa	20.3 – 22
maize	2.9 – 28.7
carrot	6.2
pasture grass	15.3

In comparison, Nuberg (1998) found similar values of the weighted mean yield increase except for alfalfa, which reached 99% in Australia.

However, as stated in both research studies, somewhat weaker crop yield has been found in areas close to the shelterbelts –a distance ranging from 5–60 m –than in the middle of the plot. In Hungary on the southern edge of the shelterbelt, this negative effect is less significant.

The effect of shelterbelts on crop yield depends on the sensitivity of the crop against wind (Gál 1963).

Nevertheless, shelterbelts increase the overall safety of yield due to the protection they offer against drought and wind damage.

## 5 SOIL PROTECTION

In agricultural plant producing systems, irrigation alone cannot prevent drought or solve water supply needs; this is particularly true on sites stricken by extreme weather. Irrigation influences soil water balance only. Combating atmospheric drought requires the reduction of dry winds. In the absence of this, the wind continuously replaces the moist air layers that result from evaporation and transpiration. As a result, the need for irrigation increases, and the requirement of secondary salinization in soil occurs. The favourable micro-meteorological effects of shelterbelts result not only in improved productivity of non-irrigated agricultural sites, but also play an important role in increasing irrigation efficiency and soil protection. Beyond that, based on an examination of microflora and microfauna in soil profiles, 8–10-year-old shelterbelts also have a favourable effect on deeper soil layers. Beside soil ventilation, life in the soil is also positively influenced by plantations of mixed stands. Deeper soil layers also have the opportunity to unfold nutrients, which is beneficial for tree growth (Gál et al. 1960, Carnovale et al. 2019). Regarding carbon sequestration, several research studies (Saha et al. 2009, Nair et al. 2010, Lorenz – Lal 2014) reveal that tree plantations on agricultural land can significantly increase soil organic carbon (SOC) content. Long term managed plantations such as shelterbelts can store SOC in the upper soil level similar to adjacent semi-natural forests (Lorenz – Lal 2014). Tree species richness increases the amount of stored SOC.

Establishing shelterbelts can provide solutions for damaged areas such as industrial sites, landfills, and sludge reservoirs that cannot be afforested due to their toxicity. In such cases, the area surrounding the contaminated site should be afforested in the interests of environmental protection. Over a longer time period, conditions at these contaminated sites can improve through the benefits shelterbelts provide (windbreak, flue-dust, lixiviation of toxic material), which first enables the settling of natural grass and, later, the growth bushes and trees (Dömsödi 2010).

Shelterbelts also can be a solution for gully erosion, as mentioned in Deng et al. (2015). They recommend an optimal planting density of farmland shelterbelts for the prevention of gully erosion at 1100–1300 m/km<sup>2</sup>.

Examining the annual water budget on soils, well-shelterbelts can lead to a favorable process in protected areas: atmospheric precipitation rises, physical evaporation from the soil surface decreases, and the accumulation of considerable water reserves in the soils occurs (Lazarev 2006).

## 6 NOISE AND AIR PROTECTION

Due to their effectiveness and limited space requirements, technological solutions are the most commonly used methods for noise reduction near motorways. Nevertheless, building noise barriers can be disproportionately expensive in cases involving longer road sections or a diffuse noise source with a large extension. In addition to their windbreak function, shelterbelts can also serve as effective noise reducers when the distance of the sound traversing the plantation (so the width of the belt) is a minimum 30–50m. In this case, noise is reduced up to 3–4 m height from the surface, but the noise reduction is not more than 10–15 dB (Islam et al. 2012). However, the literature also refers to the so-called screen-noise created by the whispering leaves of trees, which can have a soothing, relaxing effect.

Plant usage, mainly with sufficiently wide tree or bush rows, has many additional favourable aspects that artificial technical solutions do not provide. For example, in contrast to walls, plants absorb the sound of vehicles rather than reverberate and increase the noise. In



addition, they also provide all the added benefits green spaces provide, ranging from carbon sequestration to making a microclimate more pleasant (Palotás 1985, Barótfi 2000).

The many problems associated with air pollution validate the air-purifying function of shelterbelts. Tree stands increase the roughness of the surface and cause vortexes in streaming air. The leaves catch not only the precipitation, but also filter out dust, heavy metal, sulphur-dioxide, freon, etc. As well as improving the CO<sub>2</sub> balance of our atmosphere, forests stands have a significant filter effect against trace gases and aerosols; however, this environmental influence can be fatal for tree stands in extremely polluted areas. Under the effect of vortexes, the transported particles deposit on the surface of leaves, herbaceous plants, and soil (Heath et al. 1999, Islam et al. 2012). The scale of turbulence depends principally on scragginess of crown storey, while adhesion of aerosol particles is influenced by leaf area index (LAI), leaf surface features, and crown structure (Mátyás (ed) 2005). Prominently high deposition values were measured in spruce (*Picea*) stands, with high surface roughness (Takács 2008).

According to the measurements of Kölüs (1979), the 15–25 ppm CO concentration near the motorway is not demonstrable at the opposite side of the shelterbelt, while 11ppm concentration in areas without shelterbelts decreases only to 1.2ppm at a distance of 50m from the motorway. A consistent tree plantation at a width of 10–12m can catch the deposition of smut as well as gaseous, small-sized particles that may be harmful to respiratory organs. An increasing number of researchers agree (Fórián – Hagymássy 2009, Chen et al. 2015, Amadi et al. 2016, Amadi et al. 2017) that wayside hedgerows or tree plantations can play a significant role in suspending air pollution and salty sprinkle, the contaminated rainwater sprayed by vehicles. Another point of view is that tree stands act as complex “filters” and play an important role in the natural accumulation of pollutants. The typical air drifts connected to afforestation work very much like a conveyor belt as they transport the air moisture, carbon-dioxide, and other gaseous components of metabolism (Willis et al. 2017). The relatively lower temperature in the forest processes an intensive air transport between the atmosphere of the forest and the layer above, which dissolves pollutants into the stand. Forest stands also have their own air circulation, and this helps the pollution engaging effect of the forest.

## 7 ECOLOGY

The wildlife of many areas is affected by human establishment and activity. Human activity and construction alters natural areas, disrupting the contacts between certain wildlife populations, migration opportunities for some individual wildlife species and, finally, affecting the sum of natural living conditions for wildlife. Coherent non-forest tree stands, for example shelterbelts, which can serve as ecological corridors, are suitable for limiting the impacts human activities can have on a landscape (Barna 2004). These ecological corridors, together with protected areas and other semi-natural sites, can create a network of biotope systems, and as “green corridors” can ensure the variegation of sites, life circumstances, communication, and the spread of interconnected plant and animal species. In addition, shelterbelts are ecological systems that can contain significant wildlife; several species within these are natural predators of pests, which can have a beneficial impact on agriculture through pest reduction (Szarvas 2010, Todd et al. 2018, Gontijo 2019). Furthermore, by reducing wind speed, shelterbelts can significantly prevent the spread of some wind-carried pests and aphid-transmitted viruses (Mize et al. 2008).

Though farmers generally consider ecological issues to be of lesser importance than economic factors, they do experience and appreciate the strong correspondence between ecology and crop productivity and sustainability. The influence that the presence of pollinators has on different production systems is a good example of the relationship between ecological and economic factors.

For example, bees not only directly create food by converting nectar to honey, but more importantly support agriculture through their pollination activity. According to URL1, it is commonly understood that bees are responsible for at least one-third of all global food supplies and billions of dollars of agricultural production. Bee-dependent crops include the majority of tree fruits and berries, several vegetables, and some important forage species such as alfalfa, clovers, and legumes. Having a diverse population of pollinators is even better as this can ensure crops receive some pollination and fruiting even if honeybee populations fail.

Bee keeping is promoted by CAP Pillar II in the European Union, which is the world's second largest honey producer. (Santiago-Freijanes et al. 2016). By providing pollens and nectars for bees, shelterbelts can play a significant role in domestic honey production as well (Donkersley 2019). The wind speed reducing effect of shelterbelts causes a higher amount of pollinating insects on the protected field compared to the open areas. Honey bee (*Apis mellifera*) flight is inhibited at wind speeds of 6.7–8.9 m/s (Mize et al. 2008). By providing pollen and nesting resources for honey and wild bees, shelterbelts positively affect the diversity of the pollinator fauna (Hass et al. 2018). The intensity of the management – both for shelterbelts and adjacent crops – also affects bee diversity (Wu et al. 2019) and total gamma diversity (Duflot et al. 2015).

The changes in agricultural practices and the movement away from diverse landscapes in the past 50 years, has caused a significant decline in pollinator species (Odanaka – Rehan 2019). Multi-canopy layouts with permanent herbaceous soil cover provide year-round benefits to bees and agricultural systems as a whole by lengthening the available term of pollen starting early in the year with willow (*Salix sp.*) and hazel (*Corylus sp.*) and then ending with chestnut (*Castanea sp.*) and English ivy (*Hedera helix*). Other species are a source of nectar, including maple (*Acer sp.*), mountain ash (*Sorbus sp.*), blackthorn (*Prunus spinosa*), quince (*Cydonia oblonga*), and elderberry (*Sambucus sp.*), or honeydew, e.g. poplar (*Populus sp.*), beech (*Fagus sp.*) or propolis, as oak (*Quercus sp.*) and certain conifers. These effects can nearly double honey harvest volumes (Clément et al. 2016). Though honey bees are a focus of research because of their dominance in pollinator communities, wild bees and other pollinators can actually be more effective pollinators due to their higher frequency of contact with the flowers (Földesi et al. 2016).

Hundreds of useful wild bee species and subspecies are unique pollinators of many seed crops, including rapeseed for oil, legumes as secondary crops, cultivated fruit plants etc. Open areas may be left inside or on warm margins of larger patches in net nodes when designing shelterbelts to make feeding or nesting areas for game and thermophilous wild bees, spiders, and other insects (Zajäckowski 2016, Morrison et al. 2017).

Semi-natural habitats like shelterbelts also promote the appearance of generally forest-related spider (Araneae) and beetle (Staphylinidae) species that do not occur or are only occasionally found in intensive cultivation areas (Szél – Kádár 2012, Li et al. 2018). Similarly, the species richness and diversity of springtails (Collembola) also highlights the importance of shelterbelts in agricultural environment (Winkler – Traser 2012, 2017). With bird communities, species specific to agricultural fields and specific to forests appear and nest in the shelterbelt system. Also, species that rarely nest in closed forests can often be found in shelterbelts (Jánoska 2011). Similarly, special temporary mammal communities appear in shelterbelt-protected agricultural areas. In addition to the common rodent species and communities of cultivated areas, there is a steady population of generalist rodents of European temperate forests (Németh 2014).

Although amphibians and reptiles are not typical animal communities on farmland, species occurring only in wooded areas also occur in shelterbelts. This underlines the role of such tree plantations as an ecological corridor by facilitating the migration and spread of amphibians (Winkler 2012).

## 8 SOCIO-ECONOMIC ASPECTS

In recent years, the role of landscapes has significantly changed. Attention is mainly directed to those areas that have been attributed to a single destination such as conventional monoculture agricultural areas. Nowadays, the process of transformation into multifunctional landscapes, where people living in a region rely on a higher variety of resources, can be observed (Schaller et al. 2018). In a multifunctional landscape a typically agricultural area is not only the scene of agricultural production, but also a biological and social living space. Agroforestry systems, including windbreaks and shelterbelts, are necessarily part of these multifunctional landscapes due to their complexity, diversity, and valuable ecosystem services. These services bring benefits to both landowners and society. Landowners benefit from shelterbelts in several ways. While most environmental services such as soil improvement and increased biodiversity effects cannot be estimated due to a lack of information and data, other benefits such as yield increment and energy conservation are measured private benefits of shelterbelts supported by a number of evidences. Society as a whole also benefits from shelterbelts in terms of climate regulation through carbon sequestered in the system, and improvements in water quality and biodiversity (Grala 2004, Kulshreshtha et al. 2018). In addition, the utilization of locally produced biomass brings significant energy savings at the regional or national level and contributes to the achievement of renewable energy targets.

Even though they offer many benefits for farmers, landowners, and society as a whole, shelterbelts have been removed from many livestock farms, croplands and farmsteads worldwide. The reasons for their removal include the following (Grala 2004, Tyndall 2009, Pisanelli et al. 2012, Kulshreshtha et al. 2018, Amichev et al. 2020):

- space needed for buildings, equipment and other infrastructure
- weather damage (flood, storm, fire, etc.)
- damage by human activity (chemical or mechanical effects)
- age of shelterbelt (tree degradation, structure disintegration)
- change of land size and technology (intensification, larger machines, aerial spraying etc.)
- poor market facilities
- labour and time requirements for planting and maintaining trees and shrubs
- the economic consequences of all aspects listed above
- less experience and/or lack of knowledge on behalf of land users and landowners

The use of other agro-ecologically advantageous microclimate and soil improvement land use methods (e.g. no tillage or reduced tillage, mulching, growing cover crops) is another possible factor; the farmers may not perceive the benefits of shelterbelts and thus remove them.

According to some research examining farmer motivations, farmers who decided to maintain or establish shelterbelts did so for a variety of reasons including snowdrift control; dust, sand, noise, spraying, and wind protection; yield increment; livestock protection; firewood production; aesthetic reasons; wildlife habitat; product diversification; and the mitigation of livestock emissions (Dix 1976, Brandle et al. 1984, Vernon et al. 1991, Mertia et al. 2006, Tyndall 2009, Kulshreshtha et al. 2018, Rois-Díaz et al. 2018). Having shelterbelts in arid and environmentally sensitive areas is even more important as shelterbelts play a significant role in optimising yield; furthermore, in certain places and growing seasons, shelterbelts are essential for crop production (Mertia et al. 2006, El Amain – El Madina 2014, Li et al. 2020).

Kulshreshtha et al. (2018), points out that the decisive factors in decisions to remove shelterbelts are the educational attainment long-term planning of landowners.

A number of estimates have been calculated to examine whether it is worth keeping shelterbelts and similar green linear infrastructure elements. The results are wide-ranging; some show little benefit while others estimate a significant impact (Dix 1976, Vernon et al. 1991,

Mertia et al. 2006, Tyndall 2009, Kulshreshtha et al. 2018, Pisanelli et al. 2019). In a U.S. survey Brandle et al. (1992) concluded that windbreaks are an economically attractive investment over a wide range of conditions. Analysis by Grala (2004) reveals that additional crop yields necessary to break even vary significantly across windbreak scenarios, lifespans and lengths of the protected zone. According to Tyndall (2009), 75 % of Iowa hog producers who believe shelterbelts help to physically and social-psychologically mitigate odours would be willing to pay to plant and maintain shelterbelts. Livestock farmers likely see more direct benefits of shelterbelts than crop producers do, primarily from the social-psychological aspects stemming from public relations related to matters of odour control.

The economic and social value of natural assets can be measured by the sum of all the benefits provided by their ecosystem functions. However, when examining economic aspects, it is very important to emphasize that the large number of factors makes each system special; hence, judging the benefits of maintaining shelterbelts requires a unique calculation tailored to local circumstances. No universal method has been developed to calculate the value of positive externalities due to positive environmental effects, which may be significant. Therefore, the benefits shelterbelts extend to society are not considered by most producers in their management decisions as they offer no compensation for the producers themselves. In contrast, Rempel et al. (2017) found that producer costs were easily identifiable and that these strongly influenced management decisions. Shelterbelt timeframes also complicate the issue. The economic benefits of shelterbelts are only realized after 10-15 years, which is beyond the annual timeframe by which agricultural producers typically operate. This contributes to increased uncertainty that further discourages agricultural producers (Grala, 2004). Moreover, in many cases, available subsidies provide little motivation for farmers to install or maintain shelterbelts.

The positive perception of farmers is a very important step in the adoption of agroforestry practices (Mertia et al. 2006, Pisanelli et al. 2012, Kulshreshtha et al. 2018). Results of a survey undertaken to determine farmers' perceptions of silvoarable agroforestry across Europe in 2003 and 2004 suggest silvoarable agroforestry would become a more common feature of the European landscape if it were provided with appropriate promotion and support (Rigueiro-Rodríguez et al. 2009). CAP should support this type of farming by mutually reinforcing measures rather than through exclusive measures, which should also be thoroughly explained and encouraged by experts. Due to the lack of awareness and practical knowledge, Pisanelli et al. (2012) and Rois-Díaz et al. (2018) highlights the importance of promotion at the institutional level through training and extension activities with the aim of raising awareness of available support in addition to practical knowledge of farming and alternatives. In order to promote agroforestry, it is also essential to draw consumer attention to the quality of agroforestry products and the ecosystem services provided by agroforestry systems.

## 9 POSSIBLE NEGATIVE EFFECTS OF SHELTERBELTS ON ADJACENT CROP

Though shelterbelts have many advantages, they do have some disadvantages as well. The shade of the trees, the competition, and the spread of invasive plants have a negative effect on crop yield. The most common mentioned handicap of the plantations in agricultural land is the competition between woody vegetation and the adjacent crops, especially under conditions of limited moisture (Brandle, et al. 2004, Jørgensen 2009). Generally, the competitive zone is 1-2 h (h is the height of the trees), where the yield loss can reach 49% (Nuberg 1998). Although competition is mainly for soil water content (Kowalchuk et al. 1995), tree shade reduces opportunities for photosynthesis, and the roots increase demand on soil nutrients. The allelopathic effect of litter also causes yield loss (Nuberg 1998). Theoretically, without wind, the effects of shelterbelts would be negative; on the other hand, the trees reduce evaporation

and maintain moisture by decreasing wind, which is a positive effect for crop yield (Vandermeer 1989).

Shelterbelt competition can be decreased by root-pruning. The effectiveness of this depends on the rooting characteristics of the trees/shrubs. A root cut in the top 60 cm of the soil at 0.5 h distance from the trees eliminates the crop yield reduction for three years (Kort 1988, Kowachuk et al. 1995).

The sensitivity of different crops for competition is various. While wheat and oats show a larger loss in the shade of shelterbelts, the reduction in alfalfa and other perennial hay crop yields is smaller. Corn showed no apparent yield loss due to competition (Brandle et al. (ed) 1988).

Although shelterbelts can provide habitat for wildlife, they may also promote the spread of undesirable, for example invasive plant and animal species. The role of valuable or desirable species also can be disadvantageous, particularly when they feed on crops rather than on pests and weeds (Mize et al. 2008).

In addition to the disadvantages of the shelterbelts, the lack of theoretical knowledge and practical experience about managing tree plantations is a great limiting factor for farmers (Stancheva et al. 2006). Based on crop yield measurements, the installation of a shelterbelt will pay off in the long run (Easterling et al. 1997). The protection effect appears after the trees are 6 years old and increases yearly, reaching the full efficiency at the age of 20 (Garrett – Buck 1997).

## 10 GUIDELINES FOR THE ESTABLISHMENT AND MAINTENANCE OF SHELTERBELTS

The positive effects of shelterbelts will only prevail with well-prepared planning, appropriate installation, and targeted usage (Yang et al. 2018). Therefore, some guidelines for the structure and choice of tree species should be taken into consideration during installation. Takács (2008) determines the ideal shelterbelt structure as the following (*Figure 1*):

- an additional line of trees or shrubs should be settled on the windward side, 12-20 meters from the edge of the shelterbelt,
- the windward side of the shelterbelt should be permeable and higher than the accompanying shrub or tree line
- an open area between the shelterbelt and shrub line should be left
- trunk density should be high on windward side and thinning dense towards the leeward side
- tree heights in the interior lines should be diverse (two-storey stands)
- the shape of the protected (leeward) side should be slope or stepped
- leeward edge does not extend beyond the crown projection of the outer tree-line

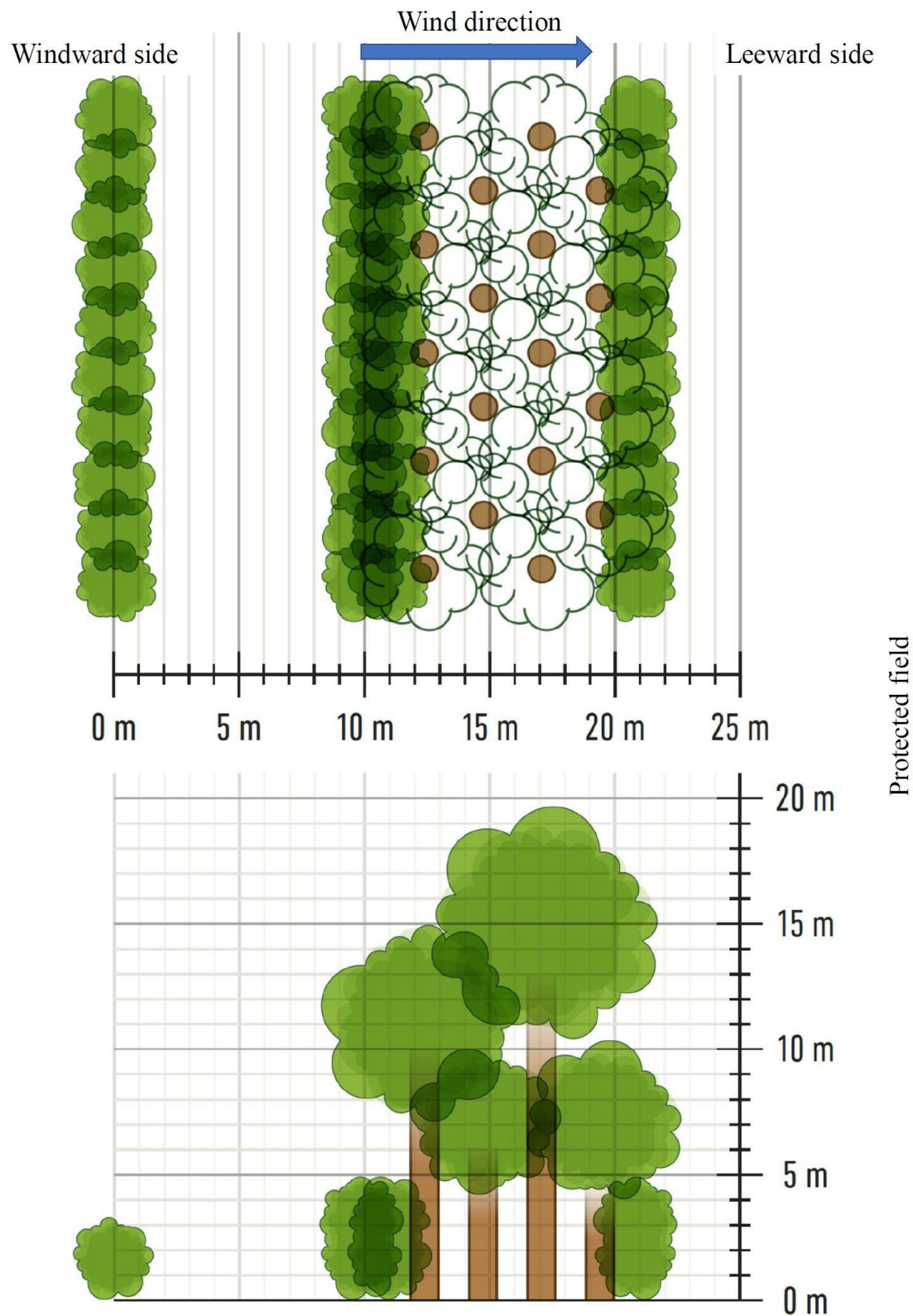


Figure 1. Ideal shelterbelt structure

Starting from the protected site, a two-meter-high bushy strip is settled at 10 meters from the belt. The edge of the belt is 3–4 meters wide and 5–6 meters high; 2 lines, 1 meter line spacing, extending into the trunk space. Distances between the rows of trees are 1–1.5 meters long, the trees are offset from each other. The height of the first line of trees is 15–16 meters,

line 2 is 10–12 meters, line 3 is 18–20 meters and line 4 is 10–12 meters high. The edge on the protected side has a more simple structure and reaches into the stand by 2–3 meters. In this way, the bandwidth can be maximized to 10–12 meters.

When using forest belt to protect against snow drifts the distance from the traffic lane border should be at least 7 meters (10 meters without borders), but should not exceed 20 meters. For this purpose, the use of two lines of shrubs and 2–3 tree lines on the exposed side while one shrub-line on the protected side of the belt is sufficient.

### Road

Besides forming a harmonic relationship between the artificial lines of traffic and landscape, the aim of roadside afforestation is to improve traffic safety. Afforestation is used with the goal of drawing attention to the road, accident prevention (forewarning of dangerous points, end of road, slip road), and as an “optical stopper” effect. Other functions connected with this are optical lead, shadowing the road, and protection against snow and wind. Furthermore, afforestation plays a role in improving aesthetic and landscape values (fine view) (Takács 2008).

### Species

Tree and shrub species that primarily develop the appropriate belt structure providing suitable protective effects are recommended for instillation in shelterbelts. In the site conditions of the protected area, these species are able to grow quickly, form plant communities, and contribute to the preservation of soil fertility. They are resistant to disease and weeds, and less sensitive to chemicals used in agriculture. In addition to giving wood and fruits, they also serve as bee-pastures. Wind resistance is also an important factor of selection (Table 3).

Table 3. Classification of tree species according to their wind resistance (based on Barna 2004)

Wind resistant species	poplars ( <i>Populus</i> sp.), pedunculate oak ( <i>Quercus robur</i> ), lime ( <i>Tilia cordata</i> ), elm ( <i>Ulmus minor</i> ), alder ( <i>Alnus glutinosa</i> ), black locust ( <i>Robinia pseudoacacia</i> )
Moderately wind resistant species	Hungarian ash ( <i>Fraxinus angustifolia</i> ), larch ( <i>Larix</i> sp.), beech ( <i>Fagus</i> sp.), maple ( <i>Acer platanoides</i> ), bigleaf lime ( <i>Tilia platyphyllos</i> )
Physiologically wind-sensitive species	hornbeam ( <i>Carpinus betulus</i> ), birch ( <i>Betula pendula</i> ), pine ( <i>Pinus sylvestris</i> ), red oak ( <i>Quercus rubra</i> ), aspen ( <i>Populus tremula</i> ), spruce ( <i>Picea abies</i> ), white pine ( <i>Pinus strobus</i> ), and common fir ( <i>Abies alba</i> )

In order to design the optimal structure of the upper canopy, main tree species should be selected in line with the specific site conditions. By using the proper species, the maintenance of the shelterbelt will be sustainable for a long time. It is also important that a resistant tree community with spread crown can be developed. The upper level is complemented by filling tree species of the second level. These may already be shade-tolerant tree species, but utilizing the given habitat conditions in the best possible way is necessary to help the growth of trees in the upper level. The shrub layer is also a structuring element whose main task is to protect the soil of the shelterbelt beyond forming its edge. Evergreen pines, juniper, thuja, and thick-branched shrubs can be considered for the purpose of protection against winter and spring winds. Thus, forest belts will not become open in winter; their snow-retaining ability will grow and they may serve as winter shelter for wild animals.

The design of shelterbelts requires simplicity. Excellent combinations can be developed by the use of one or two main tree species, one to two complementary tree species, and one-to-three shrub species. By contrast, the aim in the proximity of protected areas is to develop a diverse combination of species providing stability. Tree and shrub species having advanced root systems that can compete with agricultural crops e.g. *Salix alba*, *Tilia cordata*, *Fraxinus pennsylvanica* (Gencsi – Vancsura 1992) are not recommended for installation. In addition, those species that have strong root-shooting abilities, are wind or frost sensitive, and are less resistant to disease or potential intermediate hosts of insects damaging crops, should also be avoided (Table 4) (Gál – Káldy, 1977).

Table 4. Attributes of shelterbelt types (Gál – Káldy, 1977)

Type	Complementary tree species	No. of rows	Width (m)	Soil condition
giant poplar ( <i>Populus x canadensis</i> "Robusta")	maple ( <i>Acer platanoides</i> )	5	9	loamy agricultural soil, alluvial soil, peat soil, sandy soil
Italian poplar ( <i>Populus italica</i> )	bingleaf lime ( <i>Tilia platyphyllos</i> )	4	7,5	farmland with good nutrition supply, peat soil of better quality
giant poplar ( <i>Populus x canadensis</i> "Robusta") + pedunculate oak ( <i>Quercus robur</i> )	large leaved lime ( <i>Tilia platyphyllos</i> )	8	12,5	nutrient-rich alluvial soils, heavy clay soil, humus sand, improved saline soil
black locust ( <i>Robinia pseudoacacia</i> )	oleaster ( <i>Elaeagnus angustifolia</i> )	7	11	sandy soils (not applicable in heavy soils)
pine ( <i>Pinus sylvestris</i> ), red oak ( <i>Quercus rubra</i> )	–	5	8	not too heavy clay, loamy- or nutrient-dense sandy soil
lime ( <i>Tilia cordata</i> ) + sessile oak ( <i>Quercus petraea</i> )	maple ( <i>Acer sp.</i> ), elm ( <i>Ulmus sp.</i> ), alder ( <i>Alnus glutinosa</i> )	10	15,5	dry, slightly acidic soil with thin topsoil

Beyond planning and creating the structure, proper maintenance is critical to keeping the integrity of the shelterbelt spatially and through time. This is more difficult in systems where all the individual trees and shrub components have been planted at the same time. However, the management techniques of shelterbelts can be similar to general forest management, the purpose is different. In case of shelterbelts, the aim of the management is to maintain their effectiveness. The activities begin soon after planting with weed control till the canopy layer closure (Zhu 2008). Later, the goal of management is to maintain the diverse structure and porosity of the shelterbelt (Takács 2008).

Consequently, in the absence of regeneration, effectiveness will decrease due to natural mortality. It is therefore important to use a diversity of species in protective plantations and inspect them regularly in order to identify and restore vulnerable parts of shelterbelts (Xie et al. 2018, URL2). A properly planned and planted shelterbelt comprises a very small portion of the agricultural land, and their advantages are many times greater than their negative effects (Mize et al. 2008).



The current Hungarian support system does not give detailed guidelines for implementing a shelterbelt. The number of trees in an agroforestry system is limited to 200–250 pieces/ha. The width of the shelterbelt is defined from 15 to 20 m, and a 1 m wide shrub belt must be planted in both sides. In order to reach the maximum efficiency and ecological benefits, the available research results on the appropriate structure and species composition should be taken into account in the future support regulation.

**Acknowledgements:** The project was supported by EFOP-3.6.2-16-2017-00018 in University of Sopron project.

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## Comparative Local Case Study of Coniferous Forest Litter of the "*Pinus halepensis* Mill" in Arid and Semi-arid Areas of Western Algeria

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**Abstract** – Forest tree species produce litter, which is the plant/soil interface that ensures the maintenance of soil fertility whose properties depend on the botanical species considered. The differences of properties are marked in the nature of the decomposition processes and the forms of humus which result from it. In this study, the physicochemical characteristics and biological activity of litter were compared in coniferous plots located in the semi-arid and the arid zones of western Algeria. The objective of this work was to characterize and compare the physical-chemical properties and microbiological characteristics of softwood forest litter in the semi-arid and arid areas of western Algeria. We analyzed the properties of 50 samples of Aleppo pine litter collected from five stations in each zone. Analysis results show a highly significant difference ( $p < 0.05$ ) in the physical-chemical properties between the semi-arid and arid zone: humidity (20.7% – 6.51%), pH (5.98 – 6.14), conductivity (0.42 mS/cm – 0.65 mS/cm), carbon (45.74% – 73.42%), nitrogen (1.17% – 0.86%) and C/N ratio (37.47 – 73.42). A comparison of the mean of microbial biomass and their efficacy reveals what is homogeneous in both zones, with a small difference in basal respiration.

The heterogeneity of these results indicates that such observations still need to be made in other forests of the Algerian territory in order to better understand the functioning of forest ecosystems and the effect of climate on these compartments, especially soil.

**decomposition / physicochemical properties / biological parameters / aridity / Aleppo pine**

**Kivonat** – Az Aleppó-fenyő erdei avarjának összehasonlító vizsgálata Nyugat-Algéria száraz és félszáraz területein. Az erdővel borított területek talaj/növény rendszerében a talaj termékenységének fenntartását az erdei fafajok avarprodukciója biztosítja. A termőrétegtépződés folyamatának tulajdonságai jelentősen függenek a fajfajösszetételtől, ebből eredően pedig különbségek jellemzik a bomlási folyamatokat és a keletkező humuszformákat. Jelen tanulmányban avarminták fizikai-kémiai tulajdonságait, valamint a bennük lezajló biológiai aktivitást hasonlítottuk össze nyugat-Algéria félszáraz és száraz övezeteiben fekvő túlevelű állományokban. A vizsgálat fő célja a kutatási területekről származó fenyőavar fizikai-kémiai és mikrobiológiai tulajdonságainak jellemzése és összehasonlítása volt. A kutatás során 50 Aleppó-fenyő avarminta tulajdonságait vizsgáltuk minden mintaterületről 5 mintát gyűjtve. Az eredmények szignifikáns ( $p < 0,05$ ) eltérést mutatnak a félszáraz és száraz övezetek mintáinak fizikai-kémiai tulajdonságai között: nedvességtartalom (20,7% – 6,51%),

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pH (5,98 – 6,14), vezetőképesség (0,42 mS/cm – 0,65 mS/cm), szén (45,74% – 73,42%), nitrogén (1,17% – 0,86%) és C/N arány (37,47 – 73,42). A mikrobiális biomassza átlagának és hatékonyságának összehasonlítása azt mutatja, hogy mindkét zónában homogének a folyamatok, csak kis különbség van az alaplégzésben. Az eredmények alapján Algéria egyéb erdeiben is vizsgálatokat kell végezni az erdei ökoszisztémák működésének és az éghajlati hatások jobb megértése érdekében, különösen a talajra nézve.

**lebontás / fizikai-kémiai tulajdonságok / biológiai aktivitás / szárazság / Aleppó-fenyő**

## 1 INTRODUCTION

Considering the bioclimatic criteria, Algeria includes all the Mediterranean bioclimates from moist to dry. Forest formations are found on virtually all bioclimatic stages. This allows the presence of a great diversity of biotopes occupied by an important floristic richness especially in the forest ecosystems that are found at almost all stages of bioclimatics. Today the Forestry Directorate-General (DGF 2018) estimates this forest heritage at 4.1 million ha, of which 1.420.000 ha consists of forests, 2.410.000 ha of Maquis shrubland, and 280.000 ha of young reforestation. The main tree species are Aleppo pine (*Pinus halepensis*) (69%) and cork oak (*Quercus suber*) (21%). In smaller areas, cedar (*Cedrus satlantica*), maritime pine (*Pinus pinaster*), oak species (*Quercus ilex*, *Q. faginea*, *Q. suberet* *Q. afares*), and eucalyptus are predominant (DGF 2018).

The Aleppo pine forests are mostly present at the semi-arid level, content with 350 mm of annual precipitation, and adapting to any type of soil. Occupying the highest area in Algeria, they are essentially confined to the east and west of the country. The areas of Aleppo pine are found on the coastline, the Tell, the Saharan Atlas, and the Aures Nememcha. In Algeria, the forest has social and scientific functions and, to a lesser degree, an economic function especially in relation to cork oak (*Quercus suber*) (Louni 1994). Algeria is characterized by very diverse and fragile forest ecosystems, incumbent on its geographical position and the significant variations of its climate.

In Algeria, conifers include the majority of forest and pre-forest formations. These are very important economically and ecologically, particularly through their role of protecting the soil from the processes of desertification and erosion, which are very dynamic in the semi-arid and arid regions (Benabadji et al. 2007). These areas are among the most fragile ecosystems in the world due to recurrent droughts and the growing overexploitation of scarce resources. Arid and semi-arid areas occupy about one-third of the earth's land surface and account for roughly one billion human inhabitants, who are often among the poorest in the world (Malagnoux et al. 2007). Forests, trees, and herbaceous plants are essential components of arid-zone ecosystems. At the level of these semi-arid and arid areas, vegetation is continually struggling against harsh climatic factors, and nutrient-poor soil and organic matter (Borsali 2013).

Among various factors, forest/soil relationships can be addressed through the impact of litter on fertility (Dupuy 1998). Litter is the superficial layer that covers the soil. It constitutes the vegetal mass from the leaves (70 to 94%), branches, and stems and forms all the organic matter (Rapp 1969, Mangelot 1980). Many factors may be involved in litter decomposition; physicochemical properties play an especially important role (Lossaint 1959). Indeed, litter plays an important role in soil protection, the storage of mineral elements, and the restitution of these minerals to the soil. The disappearance or destruction of litter is accompanied by a sudden fall in the stock of available mineral elements, which may be a limiting factor for plant growth (Dupuy 1998). The suppression or decrease of protective layers represented by litter and vegetation after a fire subjects the ground to direct sunlight and raises its general



temperature (Raison et al. 1986) because litter plays the role of a sponge that protects the ground and keeps it moist (Faurie 2011).

Through temperature and humidity, the climate directly influences the decomposition of plant debris; however, the climate can also affect the physicochemical and biological properties of litter through its influence on plant community composition and litter quality (Lavelle et al. 1993, Aerts 2006, Pérez et al. 2007). Our objective was to characterize and compare the physical-chemical and microbiological properties of softwood forest litter in semi-arid and arid areas of western Algeria to see if the arid gradient has an effect on these characteristics in order to better anticipate the future of litter in the semi-arid zone due to climate change. The sites were chosen to cover the panel of pedoclimatic conditions corresponding to the semi-arid and arid climates of the western Algeria.

## 2 MATERIALS AND METHODS

### 2.1 Study areas

#### 2.1.1 Semi-arid area

The Jebel Sid Ahmed Zeggai forest is located 4.5 km west of Saida province; it is part of the mountains of Saida, which are the eastern extension of the mountains of Dhaya, which belong to the Atlas Tellian (*Figure 1*). This forest covers an area of 2232 hectares on a limestone brown soil dominated by 90% Aleppo pine. Other plant species present are: are lentisk (*Pistacia lentiscus* L), cade juniper (*Juniperus oxycedrus*), evergreen oak (*Quercus ilex*), and esparto grass (*Stipa tenacissima*). This forest is extremely dense (2000 plant ha<sup>-1</sup>) and has significant regeneration. Aleppo pine has an average age of 50 years with an average height of 6 to 8 m. From a climatic point of view, the forest benefits from a semi-arid climate ( $T_{min} = 3\text{ }^{\circ}\text{C}$ ,  $P = 344.6\text{ mm}$ ) located on superior stage of the Mediterranean vegetation ( $T_{min} > 3\text{ }^{\circ}\text{C}$ ,  $200 < P < 400\text{ mm}$ ); the seasonal regime of the zone is of the HAPE type (Winter, Autumn, Spring, Summer) and has 6 months of drought (Zouidi et al. 2019).

#### 2.1.2 Arid area

The Jebel Antar Forest is located in the commune of Mecheria in the east of Naama province (*Figure 1*). This forest is a piedmont area of Jebel that plays a protective role against the desertification of the area. It is a mass afforestation with an area of 1000 ha on a calcimagnesian soils. Aleppo pine is used as the main species at a rate of 95% with a density of 1600 plant/ha. Cypressus (*Cupressus*), betoum (*Pistacia atlantica*), white retem (*Retama raetam*), esparto grass (*Stipa tenacissima*), and white wormwood (*Artemisia herba-alba*) are also found in this forest. Today, Aleppo pine trees have an average height of between 3 and 5 m. The area has recently encountered several factors of degradation due to desertification and urbanization with the consequence of a radical transformation of the affected plant formations. The forest benefits from an arid climate ( $T_{min} = 2\text{ }^{\circ}\text{C}$ ,  $P = 203.5\text{ mm}$ ) located on a superior stage of the Mediterranean vegetation ( $T_{min} > 2\text{ }^{\circ}\text{C}$ ,  $100 < P < 300\text{ mm}$ ). The seasonal regime is type APHE (autumn, spring, winter, summer) with 8 months of drought (Zouidi et al. 2018).

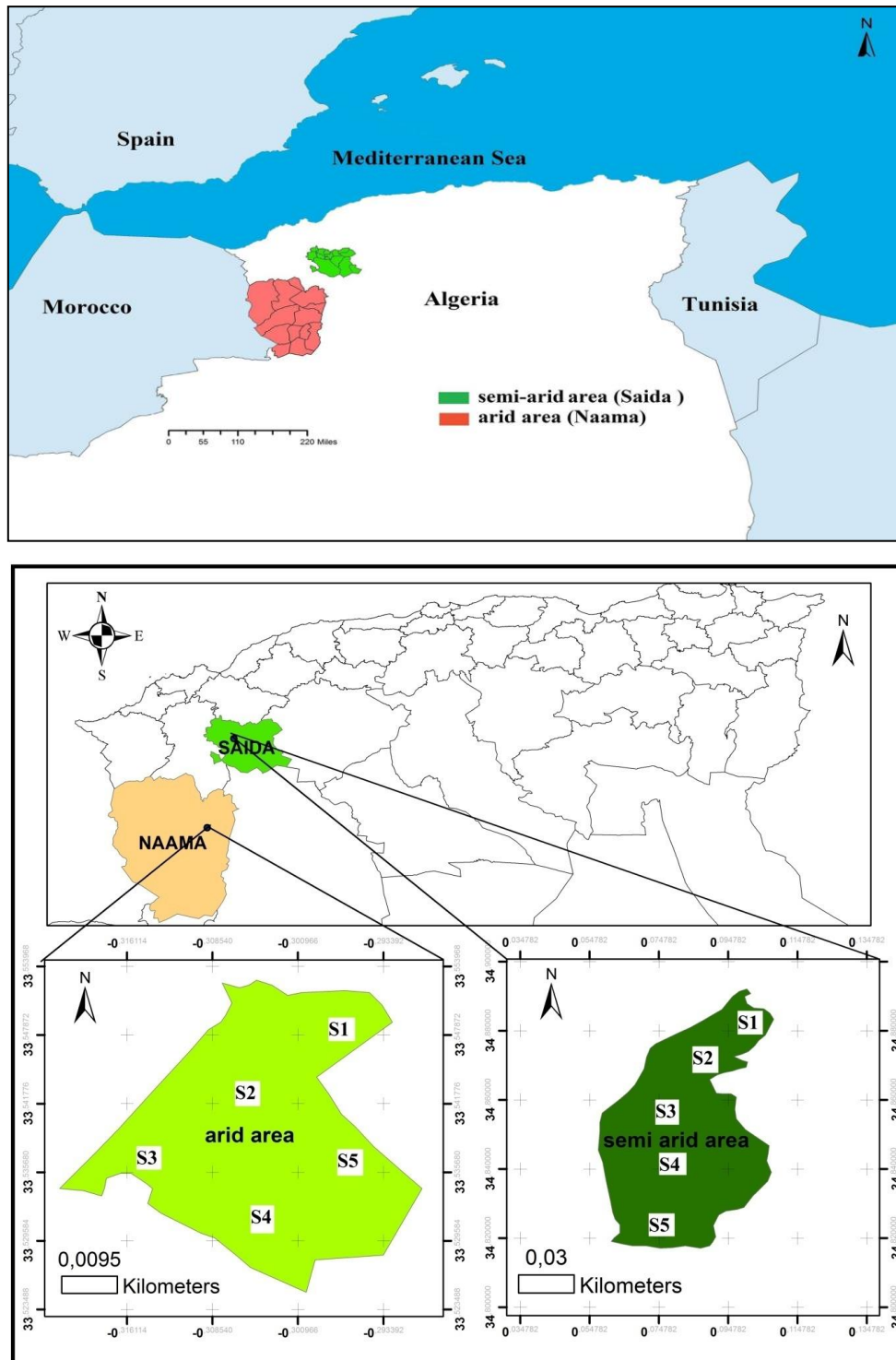


Figure 1. Geographical situations of study areas

## 2.2 Litter Sampling

Five sampling stations were selected for each zone (*Table 1*). Five samples were randomly collected at each station. All stations are located at altitudes between 970 and 1280 m with a similar exposure (N). Samples of approximately 1kg of litter (OL horizon) were collected under the canopy of *Pinus halepensis* Mill. in March 2016. Each sample was sorted manually to remove any shellfish shells, pebbles, or twigs.

Table 1. Geographical and characterizations of the study stations

Areas	Station	Altitude (m)	Latitude	Longitude
semi-arid area	S 01	975	34°52'13.7" N	00°05'09.5" E
	S 02	1067	34°51'22.3" N	00°04'40.9" E
	S 03	1146	34°50'29.5" N	00°04'57.6" E
	S 04	1160	34°49'31.8" N	00°05'22.6" E
	S 05	1081	34°49'23.7" N	00°04'33.8" E
arid area	S 01	1080	33°32'02.3" N	00°19'13.1" W
	S 02	1140	33°32'23.7" N	00°18'25.1" W
	S 03	1119	33°32'51.6" N	00°17'55.0" W
	S 04	1085	33°31'52.7" N	00°17'55.3" W
	S 05	1108	33°31'52.7" N	00°18'25.6" W

### 2.3 Physicochemical Analyses

Litter water content was determined by measuring fresh weights and weights after oven-drying (80°C) for 24h (Alarcón-Gutiérrez 2007). The pH and conductivity of the samples were measured on a litter suspension obtained by mixing 5 g of litter with 100 mL of distilled water. The measurement was carried out 2 h after using a pH meter (Métrohm, Herisau, Switzerland) (Alarcón-Gutiérrez 2007). Total organic carbon (COT) and total nitrogen (TN) were measured as follows: kiln-dried initial litter subsamples and each microcosm litter were sprayed in a ceramic mortar and analyzed by combustion in an analyzer Elemental, FlashEA 1112, Thermo Fisher; the calculated C/N ratio then presents a chemical character that may show the decay rate of plant debris (Gloaguen –Touffet 1982).

### 2.4 Biological analysis

Basal respiration ( $\mu\text{g C-CO}_2/\text{g dry litter}$ ) was measured according to the protocol described by Anderson and Domsch (1978) to assess the physiological state of the microbial communities of litter; 3 grams (dry equivalent) of fresh litter stored at 4 °C were weighed in a glass vial (117 ml). The vials were closed with a hermetically sealed plug immediately after the replacement (4 minutes) of their internal atmosphere via a stable  $\text{CO}_2$  concentration atmosphere, and incubated 4 hours at 25°C. After incubation, an aliquot of atmosphere of the vial (1 ml) was injected using a syringe into a gas chromatograph (Chrompack CHROM 3 – CP 9001). The chromatograph was equipped with a TCD detector and a filled column (Porapack) in which helium circulates at a flux of 60 mL/h. The values obtained were adjusted to 22°C according to the law of the gases perfect at  $Q_{10} = 2$ . Ambient  $\text{CO}_2$  concentrations were subtracted from the  $\text{CO}_2$  concentrations measured after incubation to obtain the amount of  $\text{CO}_2$  produced by the heterotrophic microorganisms contained in the sample. Microbial biomass was estimated by the glucose-induced respiration method (Anderson – Domsch, 1978). A mixture of talc and glucose (1 000  $\mu\text{g carbon/g}$  of litter) was added to the three grams (dry equivalent) of litter. An incubation of 100 minutes was performed to achieve a maximum rate of induced respiration. The vials were closed with an airtight stopper immediately after the replacement (4 minutes) of their internal atmosphere by an atmosphere of stable  $\text{CO}_2$  concentration, and then incubated for 90 minutes at 22°C. The  $\text{CO}_2$  concentration of the vials was analyzed with gas chromatography and corrected in the same way as previously described for basal respiration. Induced respiration rates were converted to microbial biomass values using the equation given by Beare et al. (1990). The metabolic quotient ( $q\text{CO}_2$ ) was calculated as the ratio of basal respiration/microbial biomass to Anderson and Domsch (1985).

## 2.5 Data analysis

The student *t*-test was used to compare the results of the physicochemical and microbiological properties of litter between the semi-arid and the arid areas using Sigmaplot 14 software.

## 3 RESULTS

### 3.1 Physicocemical characteristics

The evaluation of litter quantities of Aleppo pine litter taken from one square meter shows a good production of litter with high moisture in the semi-arid zone (1493 gr/m<sup>2</sup>; 20.70%) compared to the litter in our arid zone, which presents quantities (906.4 gr/m<sup>2</sup>) with low moisture (6.51%). The comparison of the averages reveals this difference is significantly high between the two zones ( $p < 0.001$ ). Conductivity and pH are elevated in the arid zone (6.14 for the pH and 0.65 mS/cm for the conductivity). The pH is low acid (pH greater than 5) in our semi-arid zone with a low conductivity (0.42 mS/cm) and presents a significant difference between the two zones ( $p < 0.001$ ). On the basis of the results, a carbon concentration and a high C/N ratio were recorded in the arid zone (73 for the C/N ratio; 73.42% for the carbon) in contrast to the concentration of nitrogen, which presents a significant average in our semi-arid zone (1.17%) more than the arid zone (0.86%). The statistical study based on the comparison of the means (student's *t* test) shows a highly significant difference ( $P < 0.001$ ) of these parameters (*Figure 2*).

### 3.2 Microbial properties of litters

The microbial parameter averages of litters are recorded in table 02. Based on the comparison of biological parameter averages of litter, we recorded a high basal respiration in the semi-arid zone (97.78 µg de C-CO<sub>2</sub> /h/g) compared to the arid zone, which presents an average of 85.42 µg of C-CO<sub>2</sub> /h/g. Statistical analysis of the results shows a notably small difference ( $t = 2.14$ ;  $p < 0.05$ ) of this microbial basal respiration (BR) between these two zones. It should be noted that the average of microbial biomass (BM) and metabolic quotient ( $q\text{CO}_2$ ) are high in our semi-arid zone (BM - 4.31 µg of carbon microbial/g ;  $q\text{CO}_2$  - 23.67 µg of C-CO<sub>2</sub>/h/g). However, the bacterial biomass and the metabolic quotient did not show any noteworthy difference between the two zones ( $p > 0.05$ ).

Table 2. Microbiological properties of forest litter in arid and semi-arid areas.

Microbial analysis	Student <i>test-t</i>	Semi-arid area	Arid area
Basal respiration at 22°C (µg of C-CO <sub>2</sub> /h/g)	2.14*	97.78 ± 17.39	85.42 ± 22.98
Microbial biomass (µg of Carbon microbial/g)	1.73ns	4.31 ± 0.90	3.94 ± 0.54
Metabolic quotient ( $q\text{CO}_2$ ) (µg of C-CO <sub>2</sub> /h/g)	0.753ns	23.67 ± 6.51	22.17 ± 7.57

This table records the average values ± deviation; Microbial properties of soils; the *p* value of independent test is presented with its threshold of significance (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; ns: not significant).

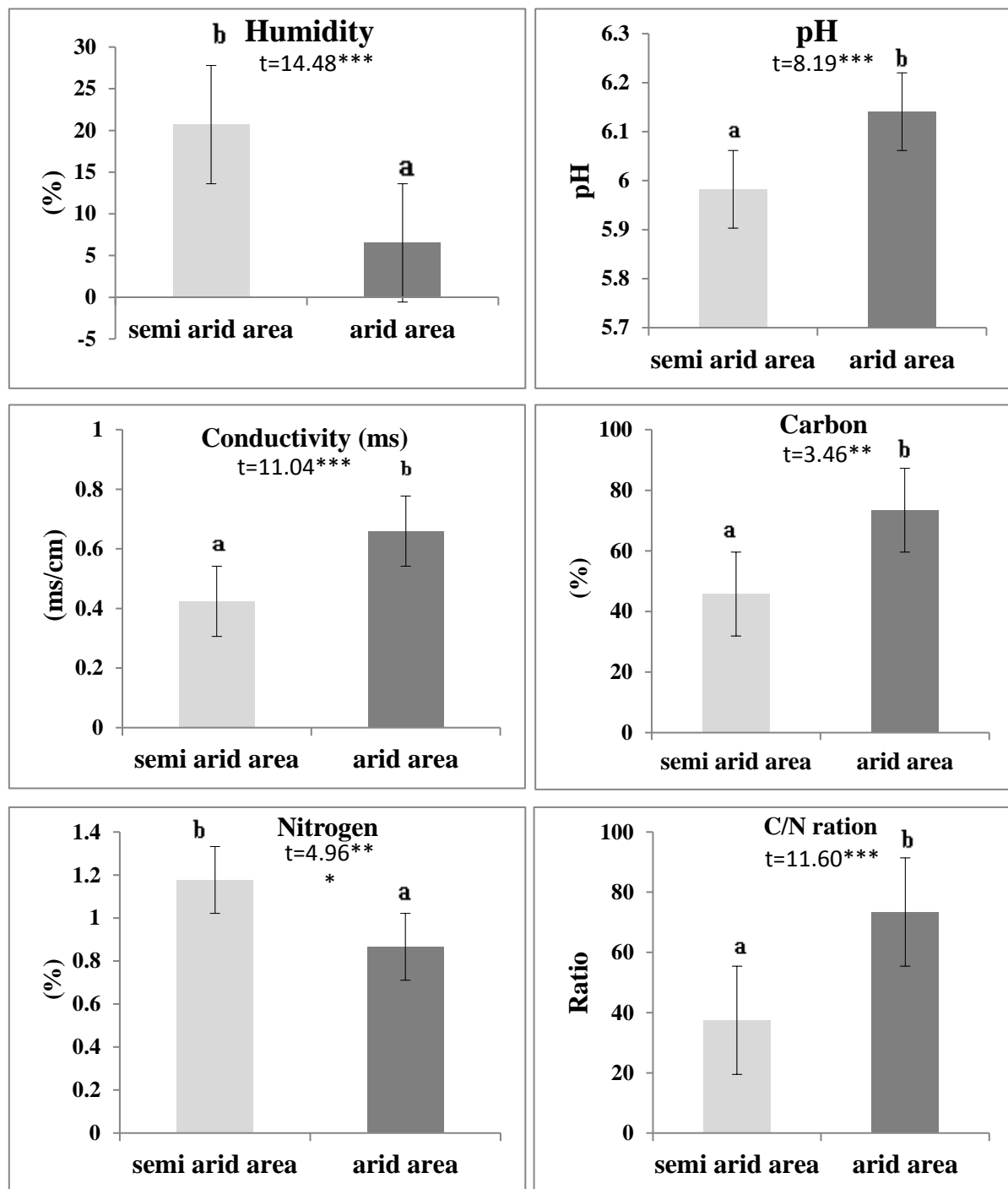


Figure 2. Physicochemical parameters of litter in semi-arid and arid areas. Averages  $\pm$  standard deviations. The t-value of the Student's test is presented with its significance threshold (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , ns: not significant)

#### 4 DISCUSSION

Forest litter is mainly composed of softwood leaves, needles, and dead wood. It forms a source of energy and essential elements for the metabolism of microbial communities. Our study reveals there is an effect of the arid gradient on the production of *pinus halepensis* litter, which is more important in the semi-arid zone compared to the arid zone. This is certainly due

to forest density as well as climatic and edaphic conditions specific to each area. Several scientists have shown that litter production is controlled by climatic and edaphic factors that regulate production and forest stand density (Puig – Delobelle 1988, Mutabesha 2009). Litter in arid forests is susceptible to winds, and according to Kumada et al. (2008), it is natural that this weather effect removes a significant amount of litter.

The work presented in this research concerns the quality of litter in two areas on two different bioclimatic stages (arid and semi-arid). The moisture measurements highlight the footprint of the climatic stage on each zone. In fact, litter in the semi-arid zone has a more significant proportion of moisture than litter in the arid zone, which indicates that the wilted leaves of *Pinus halepensis* retain more water in the semi-arid zone. Air and rainfall are more important in this area when it comes to soil moisture (344 mm/year) than it is in the arid area where there is less rain (203 mm/year) and, therefore, less water in the soil and litter (Zouidi et al. 2018, Zouidi et al. 2019). It should also be stressed that water evaporation of leaves in the semi-arid zone is low as it is in the arid zone where temperatures and periods of drought are longer and more pronounced. In addition, the low density of species trees and herbaceous species are non-existent in the arid zone, which facilitates the loss of water from the litter and soil. Consequently, the water content of the litter and the dead plants depend solely on physical phenomena such as exchange by capillarity with the soil and in equilibrium with the moisture content (in vapor form) of the atmosphere located in immediate contact with the litter (Trabaud 1976). High temperatures with reduced plant cover in the arid zone also reduce soil moisture by increasing evaporation (soil and litter) and perspiration (Tardif 2013).

The pH of litter in both the semi-arid zone (5.98) and the arid zone (6.14) are low in acid ( $\text{pH} > 5$ ). Softwoods, and especially pine, are considered acidifying species (Gobat et al. 2003, Lagacé 2009); in fact, the litter acidifies during decomposition and its pH gradually rises to 6 after 3 months (Lossaint 1959). The pH is more acidic in the semi-arid zone because there is less leaching that will limit the pluviolessivats loaded with phenols (which can deproton and therefore generate a higher acidity) (Bernhard-Reversat 1972). On the other hand, this is also probably due to the higher  $\text{CaCO}_3$  content in the arid zone, which acts like a ‘tampon’ and, therefore, causes a small increase the pH (Zouidi et al. 2018). Litter in the arid zone may remain saline compared to litter in semi-arid areas. This result is probably related to the nature of soil and the presence of minerals in soils in this arid zone (Zouidi et al. 2018). The difference in carbon levels between the two zones can be explained in the following manner: the high percentage in the arid zone can be explained by the pedoclimatic variation between the two study areas, which influences the significant photosynthetic activity of the conifers, especially in the presence of solar radiation in the arid zone that lasts all year as shown by Puig and Delobelle (1988). Changes in carbon levels, therefore, reflect the climatic or edaphic variations of an annual cycle with a lag of a few months. Forest litter fallout and decomposition are key processes in the formation of carbon (C) and nutrient cycling in terrestrial ecosystems. These processes determine the amount of carbon stored in the humus (Berg et al. 2001, Sabine et al. 2014). Carbon stocks will increase if litter production (carbon input) increases. With regard to litter production, it is closely related to the rainfall regime. The lack of rainfall in the arid zone (8 months dry) is accompanied by falling leaves, which translates into increased litter production (Paul – Clark 1996, Dupuy 1998). This also explains the significant amount of carbon in the arid zone, which has a seasonal rainfall regime that is less than that of the semi-arid zone. This causes pine trees to shed their needles and increases litter production. The accumulation of organic carbon in the humus of the closed conifer formation can be explained by the quality of the litter composed of recalcitrant materials to the microbial decomposition such as tannins and polyphenols (Berg 2000, Prescott et al. 2000). Nitrogen levels remain low, especially in arid zones. This can be explained by the very slow decomposition of the Aleppo pine litter; indeed, several studies have confirmed that

decomposition is influenced by the initial concentrations of mineral nitrogen (Aerts 1997, Kaspari et al. 2008, Wieder et al. 2009) and total litter nitrogen, which decreases with decomposition (Gloaguen – Touffet 1982, Qasemian et al. 2012). According to Salleles (2014), the source of nitrogen for plants in low-input (unfertilized) ecosystems such as arid zones is mainly derived from litter decomposition and the mineralization of soil organic matter. As a result, litter plays an essential role in the recycling of nitrogen in the forest ecosystem (Salleles 2014). When litter is subjected to favorable climatic conditions (temperature and humidity), it has a high initial nitrogen content (Kurz-Besson 2000). This is one of the key factors that regulate the decay rate of plant debris, as pointed out recently (Taylor et al. 1989). Conifers are characterized by acidifying litter which, due to their composition, cause a slowing of the biodegradation of humification with a C/N ratio generally greater than 50 (Duchaufour, 1980). The C/N ratio in the semi-arid zone translates into the capacity of a litter to be decomposed more or less rapidly in the arid zone. This shows a very slow decay. This report is only a general indication of the potential of litter to decay (Taylor et al. 1989). A strong C/N ratio of the initial litter was correlated with a low rate of decay and increased with the age of the needles in place, corresponding to nitrogen depletion and lignin enrichment (Gloaguen – Touffet 1982, Lagacé 2009).

The results showed that microbial biomass remains homogeneous and low in both the arid and semi-arid zones as a result of lack of water and high temperatures. As some authors have reported in their work (Sabaté et al. 2002, Papa et al. 2008), the most important factors affecting soil microbial biomass are precipitation and temperature. In addition, studies on forest ecosystems have shown significant decreases in fungal and bacterial biomass during drought periods (Krivtsov et al. 2006, Borsali et al. 2017). Salinity is a factor influencing the activities of microorganisms, particularly in arid and semi-arid areas (Toberman et al. 2008). Basal respiration remains weakly variable between the two zones and depends on water availability, temperature, and biochemical composition of litters such as lignin, cellulose, hemicellulose, and C/N ratio (Arunachalam et al. 1998).

## 5 CONCLUSION

Forest litter is the plant-like interface in the forest that protects the soil and ensures that fertility is maintained through the production of nutrients. The aim of this study was to demonstrate the differences between litters in semi-arid and arid areas and to determine any imprint of exposure to bioclimatic stages on the physicochemical and biological properties of resin litter. The results showed a significant difference in all physicochemical parameters ( $p < 0.05$ ) and particularly in moisture where a 14.19% difference between the two areas was recorded. The differences for carbon and nitrogen, both of which promote decomposition and ensure the life of decomposing organisms, were 27.68% and 0.49%, respectively. The pH of the two semi-arid and arid zones shows that acidifying litters are mull or mild humus ( $\text{pH} > 5$ ); this acidity is a character of conifers and in particular Aleppo pine. The decrease in the moisture content of the arid forest litter (6.51%) caused an increase in carbon (73.42%) content and, consequently, the elevation of the C/N ratio (73%) and the slow decay of the litter. Litter degradation in the arid zone is slower than in the semi-arid zone. This is due to the pedoclimatic factors of the arid zone (mother rock nature, precipitation erosion, drought, and salinity). The forests dominated by Aleppo pine from both zones produce poor quality litters that are difficult to degrade. In these zones litter possess low activity and low microbial biomass, with an average of 4.12  $\mu\text{g}$  of carbon microbial/ g.

**Acknowledgements:** We would like to thank the Mediterranean Institute of Biodiversity and Marine and Continental Ecology, University of Aix-Marseille, France, for the chemical and biological analyses of forest litter.

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## Comparison of Physical, Chemical and Biological Soil Properties under Norway Spruce, European Beech and Sessile Oak – a Case Study

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**Abstract** – This study examined the interaction of tree species and soil development in litter and the 0-10 cm mineral topsoil layer in European beech, Norway spruce, and sessile oak forests. It also compared the main soil chemical, physical, and selected microbiological indicators as well as the microbial biomass, basal and substrate induced respiration, lipid phosphate content, phospholipid fatty acid profiles (PLFA), and respiratory quinones (RQ). With Norway spruce, soil pH, clay, and silt content were significantly lower, while exchangeable acidity was higher. This leads to a major loss of exchangeable cations of the upper soil layer resulting in lower base saturation. The microbial metabolic activity and microbial biomass of deciduous forest soils were significantly higher. The respiratory quotient (q) was highest in spruce, indicating disadvantageous circumstances for microbial activity. Our results demonstrate the importance of a complex study of physicochemical and biological soil parameters when investigating the impact of forest management on soil by, for example, providing data for the development of forest condition monitoring activities.

**tree-soil interaction / soil acidification / microbial soil indicators**

**Kivonat** – Fafajok erdőtalajra gyakorolt hatásának összehasonlítása fizikai, kémiai és biológiai talajtulajdonságok alapján. A fafajok és a talajképződés összefüggését vizsgáltuk az avarszintben és a 0-10 cm-es felső ásványi talajrétegben európai bükk (*Fagus sylvatica*), közönséges lucfenyő (*Picea abies*) és kocsánytalan tölgy (*Quercus petraea*) főfafajú erdőkben. Összehasonlítottuk a talaj fő kémiai, fizikai és egyes mikrobiológiai paramétereit, a mikrobiális biomasszát, az alap- és szubsztrát-indukált légzést, lipid-foszfát tartalmat, foszfo-lipid zsírsav profilokat (PLFA) és a respirációs kinonokat (RQ). A talaj pH, az agyag- és iszap% szignifikánsan alacsonyabb volt, a kicserélhető savasság magasabb volt a lucfenyő esetében, ami a kicserélhető kationok nagymértékű kimosódását mutatja a felső talajrétegben, alacsony bázistelítettséget eredményezve. A mikrobiális metabolikus aktivitás és a mikrobiális biomassza értéke a lomblevelű erdők talajában szignifikánsan magasabb volt. A respirációs kvóciens (q) értéke a legmagasabb a lucfenyő alatt volt, a mikrobiális lebontás kedvezőtlen feltételeire utalva. Eredményeink bizonyítják a fiziko-kémiai és biológiai talajparaméterek komplex vizsgálatának fontosságát az erdőgazdálkodás talajra gyakorolt hatásának vizsgálatában, adatokat szolgáltatva például az erdőállapot-monitoring tevékenységek fejlesztéséhez.

**fa-talaj kölcsönhatás / talajsavanyodás / mikrobiális talajparaméterek**

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## 1 INTRODUCTION

Climate change and forest management impacts present severe challenges for forestry. The decline in vitality of important tree species such as Norway spruce has accelerated in recent decades (Mátyás et al. 2010). Site conditions essentially limit the range of tree species used in forestry. Climate, hydrology, and soil together determine the development of the soil-plant system. Expected climate change induced shifts of forest communities will also be linked to soil indicators, emphasizing the importance of the interaction between tree species composition and soil indicators (Führer et al. 2010, Bartha et al. 2018).

Slowing microbial degradation processes can disrupt forest ecosystems with severely-reduced buffer capacities caused by (partly anthropogenic) acidification and nutrient cycles (Ca, Mg, and K). This leads to a gradual loss of biodiversity, which can lead to the reduction of several important ecosystem processes in the soil (Borken – Brumme 1997).

Katayouan and Kooch (2019) compared four different forest types with respect to the effects of tree species composition on nutrient-cycling and soil-related processes. Their results proved that changes in litter quality had subsequent negative effects on soil fertility, as described by physicochemical and biological soil indicators, thereby emphasizing the importance of soil quality maintenance in silviculture.

Recognizing the factors that influence soil microbial communities is important for understanding how human activities, such as forest management and tree species selection, may impact ecosystem functioning (Bahnmann et al. 2018). Numerous methods to assess biological processes in soils exist. Oxygen-based respiration is a common way to measure the *metabolic activity of microbes*. This measurement effectively quantifies the respiratory activity of microbes living in soils (Anderson – Domsch 1978, Dilly 2003). In situ measurements of the biochemical determination of microbial cell components (e.g. phospholipids) can *determine the microbial biomass*. The structure of the *microbial community* is also of great importance: analysis of specific biochemical cell components, the so-called *signature molecules*, can provide important information (Hiraishi 1999, Kandeler 2007, da Costa et al. 2011, Birgander et al. 2014).

Our former site investigations around the research area in the Sopron Mountains revealed that high litter accumulation, surface soil acidity, and the presence of a leached upper E-horizon was connected to spruce monoculture forest stands. These findings suggest that replacing ancient broadleaved species with conifers can affect the physicochemical and biological soil properties of the upper layers.

Our case study investigated the roles of three different forest types including European beech (*Fagus sylvatica*), sessile oak (*Quercus robur*), and Norway spruce (*Picea abies*) on an O and A horizon quality, described by physicochemical and biological indicators. Under natural conditions in Hungary, these tree species often compete with each other to occupy sites. The currently unfavourable ecological conditions for spruce are deteriorating further due to climate change. In addition, the expected processes are also endangering the living conditions of beech. That is why it is crucial to study the forest-scale replacement of one tree species for another and how these species replacements interact with soil processes and nutrient cycles.

We hypothesized that Norway spruce forest cover is less favourable for soil biological activity than broadleaved tree species forests are, and that this results in differences of soil biological activity. Our objectives were, therefore, to find and assess soil physicochemical and biological indicator differences between conifer and broadleaved stands.

## 2 MATERIALS AND METHODS

### 2.1 Study area

The chosen study areas were a European beech (*Fagus sylvatica* L.; 41 years old; Lat: 47°39'19.7"N; Lon: 16°27'16.9"E, Sopron 171/G forest comp.) stand, a Norway spruce (*Picea abies* L.; 54 years old; 47°39'26.1"N; 16°27'16.1"E, Sopron 171/F forest comp.) stand, and a sessile oak (*Quercus petraea* Liebl.; 46 years old; 47°39'33.4"N; 16°28'14.3"E, Sopron 163/D forest comp.) stand in the Sopron Mountains near the Hungarian-Austrian border. The region is located in the warm temperate forest zone (yearly average temperature 9.5-9.8 °C), and is dominated by deciduous broadleaf tree species. Elevation is between 500-550 m; average yearly precipitation is about 800-850 mm. The yield classes of all three stands are similar, measured as 4 (on a scale with decreasing quality from 1 towards 6).

According to the Hungarian forest climate classification, the climate of the area is beech because the 50-year (1961-2010) average of the Forestry Aridity Index (FAI) interpolated to the area is 3.65, which is the typical value of a beech climate, i.e. 3.5 and 4.75 (Führer 2010, Führer et al. 2011). Conditions below an FAI value of 3.5 are more favourable for spruce and those above an FAI value of 4.75 are more favourable for sessile oak. Further description of the area is provided by Gribovszki et al. (2006).

Parent material is unclassified tertiary (Miocene) fluvial sediment, on which a loamy soil containing coarse gravel formed. The soils in all three sampling areas belong to the WRB soil reference group *cutanic luvisols* and possess similar basic reference group properties.

### 2.2 Sampling and measurements

A soil pit was opened in all three stands and soil samples in 6 replicates were taken from each horizon respectively. These replicates were immediately transported to laboratory for storage at 4 °C until analysis. Soil microbial investigations were completed for litter samples and mineral soil samples from 0-10 cm depth. Laboratory analyses of physical and chemical soil characteristics were measured according to the Hungarian standards (MSZ-08-0205-2: 1978; MSZ-08-0206-2: 1978; MSZ-08-0215: 1978; MSZ-08-0452: 1980; MSZ-08-0480-2: 1982), summarized by Buzás et al. (1993). Soil microbial investigations: soil and litter samples were divided into subsamples to perform parallel microbial activity and microbial biomass measurements.

*Microbial metabolic activity:* the mineralization rate of dead organic matter accumulated at soil surface (litter layer and 0-10 cm layer of the mineral soil) was determined with 6 replicate measurements via the "basal respiration" (BAS) method (Heilmann – Beese 1992).

*Microbial biomass:*

a) *Substrate-induced respiration* (SIR) method SIR tests were conducted in 6 replicates of soil samples from each stand to determine the microbial biomass. The SIR tests were performed according to the modified method of Anderson – Domsch (1978) as described by Heilmann – Beese (1992). From the basal respiration and the microbial biomass, the respiratory quotient (q) was calculated as a simple quotient. This value provides information on the effectiveness of the decay.

b) *Lipid phosphate measurements:* Soil samples taken from the 0–10 cm mineral soil layer were placed into a Bligh and Dyer's solution (Bligh – Dyer 1959) in sterilized glass jars, and cooled and stored at –20 °C until processed. Further processing included the chemotaxonomic measurements completed by the modified method of Findlay by Tóth et al. (2004).

*Respiratory quotient* (q-CO<sub>2</sub>): From the basal respiration and the microbial biomass, the respiratory quotient (q) was calculated as a simple quotient of basal respiration divided by

microbial biomass. This value provides information on the effectiveness of the decay (Dilly, 2003).

*Comparison of microbial communities:* Cultivation independent chemotaxonomic methods were applied to study microbial communities.

*Examination of the respiratory quinones (RQ):* The organic components cleaned from mineral soil particles were evaporated under vacuum, and the lipid soluble materials (PLFA and RQ) were solved with chloroform. A silica-based octadecyl column was employed to separate lipid soluble components. The purification of the chloroform fraction containing quinones was performed with thin-layer silica gel. High pressure liquid chromatography was used for the instrumental analyses of the purified filtrate of quinones. Detection is based on specific light absorption observed at 270-nm wavelength. Test samples were compared with quinone profiles of pure cultures of bacterial strains.

*Determination of phospholipid fatty acids (PLFA):* Glyco- and phospholipids were washed from the chromatographic column using acetone and methanol, respectively. The methanol-phase was – likely to the preceding – concentrated in vacuum evaporation at 37 °C. The dried phospholipid content was dissolved in 0.5 ml methanol: toluol (1:1) mixture. After separation of the two phases, the fatty acid analysis was performed by gas chromatograph (Tóth et al. 2004).

## 2.3 Statistical analysis

For data analysis, we used standard statistical tools (Statistica and Syntax 2000 statistical programs): quantitative data of physicochemical and microbial soil indicators were expressed with mean values and standard deviation. Paired sample t-tests were applied to compare physical and chemical soil indicators. Principal component analysis was used to describe the microbial community differences in the studied stands via chemotaxonomic markers (PLFA and RQ). Differences were considered significant at  $P < 0.05$ .

# 3 RESULTS AND DISCUSSION

## 3.1 Soil physical and chemical indicators

The mean values of physicochemical test results of the upper 0–10 cm mineral soil layer of the three forest stands are shown in *Table 1*.

*Soil pH* ( $H_2O$ ) and exchangeable acidity values ( $y_2$ ) were significantly lower for spruce when compared to both deciduous stands, while hydrolytic acidity values ( $y_1$ ) were significantly higher for spruce only in comparison to oak.

In reaction to the alkaline hydrolysis (in case of  $y_1$ ), all three stands soil colloids showed an acidic-highly acidic nature. For soils – under agricultural use – having hydrolytic acidity values ( $y_1$ ) greater than 8, liming is classified as unconditional (Buzás et al. 1993).

*Humus- and nitrogen contents* were significantly higher under sessile oak, compared to the other stands. The nitrogen content of the oak forest's soil was medium, and the other two had a low nitrogen supply. In contrast, plant-available phosphorus and potassium were both lowest in the soil of the spruce stand.

*The Carbon/nitrogen ratio* of soil organic matter was 18, 15, and 14 for oak, beech and spruce, respectively. All three values – which are favourable in terms of degradability – were lower than C/N values detected by Joergensen and Scheu (1999) in a beech and spruce forest in the Solling Mountains in Lower Saxony. Similar to their findings, the difference of C/N ratios between deciduous and coniferous stands was also small in our study. The C/N rates are generally believed to be inversely proportional to the breakdown intensity, but some studies

have shown that microbial degradation in forest soils cannot be described as a function of this single parameter, as other factors have an influence on their context (Spohn – Chodak 2015).

*Table 1.* Physical and chemical soil indicators in 0-10 cm depth of the mineral soil of three forest stands (Standard deviation in parentheses).

Main tree species	<i>Quercus robur</i>	<i>Fagus sylvatica</i>	<i>Picea abies</i>
pH (H <sub>2</sub> O)	4.9 (0.18)	4.6 (0.11)	4.3 (0.08)
pH (KCl)	4.0 (0.22)	3.6 (0.07)	3.4 (0.09)
y <sub>1</sub> (hydrolytic acidity)	28 (2.6)	36 (3.0)	35 (1.9)
y <sub>2</sub> (exchangeable acidity)	3 (1.4)	9 (1.0)	13 (0.6)
Humus content (%)	4.85 (0.20)	2.26 (0.06)	2.43 (0.14)
N <sub>total</sub> (%)	0.16 (0.02)	0.09 (0.02)	0.10 (0.03)
C / N	18 (1.15)	15 (3.98)	14 (5.79)
AL-soluble P <sub>2</sub> O <sub>5</sub> (mg * 100 g <sup>-1</sup> d.s.)	13.2 (2.32)	22.5 (3.08)	11.4 (2.34)
AL-soluble K <sub>2</sub> O (mg * 100 g <sup>-1</sup> d.s.)	16.1 (2.48)	19.8 (2.32)	9.9 (1.83)
Cation Exchange Capacity = T-value (mmol IE * 100 g <sup>-1</sup> d.s.)	30.0 (3.16)	41.5 (3.33)	39.3 (3.72)
exchangeable Ca <sup>2+</sup> , Mg <sup>2+</sup> , K <sup>+</sup> és Na <sup>+</sup> = S-value (mmol IE * 100 g <sup>-1</sup> d.s.)	5.5 (1.64)	7.0 (2.10)	6.3 (0.52)
Base saturation% (V%)	18 (6.6)	17 (4.29)	16 (1.63)
hy% (higroscopicity)	1.99 (0.09)	2.04 (0.18)	1.83 (0.24)
K <sub>A</sub> (plasticity index acc. to Arany)	54 (3.25)	55 (3.95)	50 (2.80)
5 hours capillary rise (mm)	150 (7.73)	113 (9.68)	141 (5.56)
Particle size distribution			
Sceletts (>2 mm)%	0 (0)	0 (0)	0 (0)
cS% (coarse Sand% 2-0.2 mm)	31 (2.79)	24 (5.35)	21 (5.13)
fS% (fine Sand% 0.2-0.02 mm)	23 (3.37)	33 (2.94)	54 (2.59)
Si% (Silt% 0.02-0.002 mm)	26 (3.13)	21 (3.01)	7 (2.07)
CL% (clay% <0.002 mm)	20 (5.61)	22 (6.02)	18 (4.76)

*Total adsorption capacity of the soil* (T-value according to the modified method of Mehlich MSZ-08-0215: 1978 = CEC<sub>i</sub>) was lowest in the oak forest soil; slightly higher quantities were obtained under spruce and beech. The latter two are average values found in Cutanic Luvisol soils in Hungary, but the value under oak is below average.

*S-values* (sum of “basic” or “alkaline forming” cation in Eq according to the modified method of Mehlich) and *V-values* (base saturation in %) all displayed low base saturation for oak, beech, and spruce respectively. This indicates that negative surface charges of soil particles are mostly neutralized with Al<sup>3+</sup> and H<sup>+</sup> ions. Even if differences were not significant, the soil of the spruce stand once again had the most unfavourable properties (V=15%), increasing the risk of clay-mineral destruction with negative effects on nutrient and hydrological cycles of the ecosystem.

The oak and beech forest soils had a loamy texture according to the *particle size distribution* (Si+Cl=46%, 43%) (*Table 1*), while the texture of the spruce stand is a poorer (Si+Cl=25%) sandy or sandy loam texture, which could possibly indicate the removal of clay from this horizon, resulting in a weaker expression of structure, reduced pore space, and water holding capacity of the upper mineral soil over a longer term. Nevertheless, this would need to be proved by an additional texture analyses of underlying B-horizons.

### 3.2 Soil biological indicators



The results of metabolic activity measurements are shown in *Figure 1*. Microbial respiration intensity of the beech litter was the highest. The basal respiration of spruce litter was significantly ( $p = 0.05$ ) less. Values of oak leaf litter did not differ significantly from that of the other two stands.

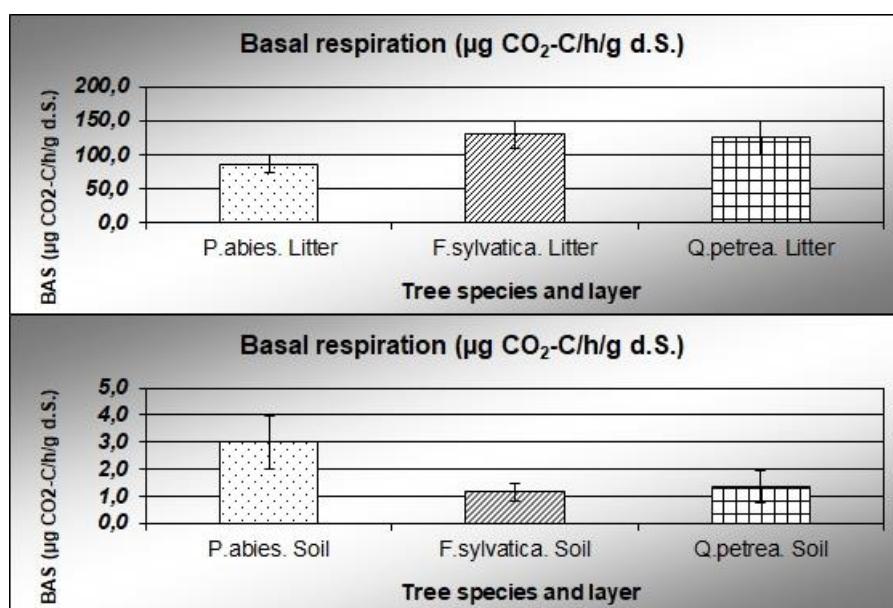


Figure 1. Basal respiration (with standard deviation) in the litter and the 0–10 cm mineral soil layers in the spruce (*P. abies*), beech (*F. sylvatica*) and sessile oak (*Q. petrea*) forests

We detected a reversed situation regarding the microbial respiration of the uppermost 0–10 cm mineral soil layer: soil carbon mineralization was highest under spruce. The two deciduous stands did not differ from each other in this respect.

*Microbial biomass from substrate induced respiration (SIR):* the SIR-value was lowest under spruce (*Figure 2*), while microbial biomass stocks were more than twice as high in deciduous leaf litters. The highest microbial biomass in the mineral soil was found under spruce. The microbial carbon stock of the upper mineral soil layer of the beech forest was significantly lower. The value under oak is very similar to this, but due to the high standard deviation, it is not significantly different from the other two stands.

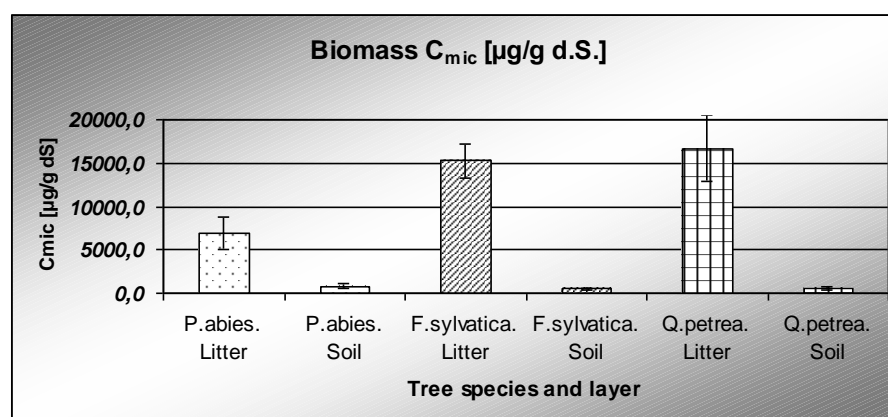


Figure 2. Microbial biomass ( $C_{mic}$ ) (with standard deviation) measured with the SIR method in the litter and the 0–10 cm mineral soil layers in the spruce (*P. abies*), beech (*F. sylvatica*) and sessile oak (*Q. petrea*) forests



In the end, the microbial biomass concentration found in the litter and upper mineral soil was largest in the oak forest. The concentration was only slightly smaller in the beech forest, but it was only half of the level under spruce.

Similar to microbial biomass values derived from SIR, according to lipid-PO<sub>4</sub> measurements, the soil under spruce had the highest microbial biomass (Table 2.) and were roughly equally low in the soils of the two deciduous stands. Values found were only about 1/3 of those measured with the SIR method.

Table 2. Microbial biomass calculated from the lipid phosphate content of the 0–10 cm mineral soil layer in the spruce (*P. abies*), beech (*F. sylvatica*) and sessile oak (*Q. petrea*)

Forest stand	<i>P. abies</i>	<i>F. sylvatica</i>	<i>Q. robur</i>
Microbial biomass $\mu\text{g C}_{\text{mic}} \cdot \text{g}^{-1} \text{ d.s.}$	291	166	157

The respiratory quotient for each of the three stands is significantly lower in the mineral soil than in the leaf litter (Figure 3). The value of both layers of spruce was significantly higher compared to the deciduous stands.

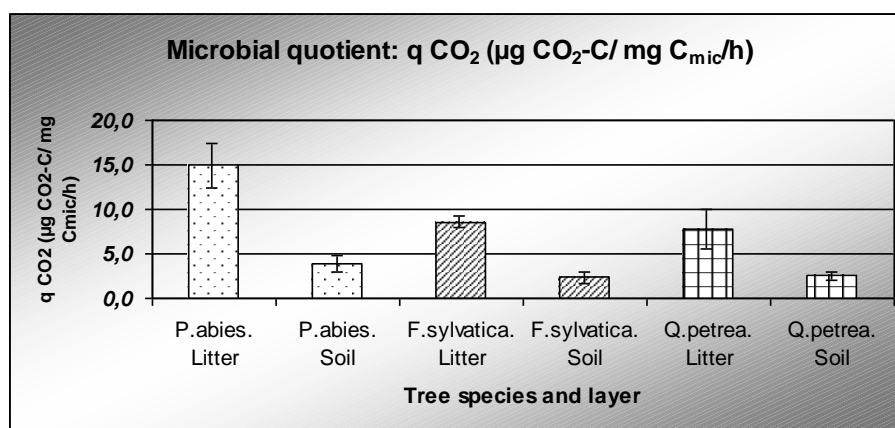


Figure 3. Respiratory quotient ( $q\text{-CO}_2$ ) (with standard deviation) in the litter and the 0–10 cm mineral soil layers in the spruce (*P. abies*), beech (*F. sylvatica*) and sessile oak (*Q. petrea*) forests

In the soil microbial community investigations, the fatty acid patterns of each sample contained compound (C16: 0, C14: 0, Q10), which can be found in a wide range of microbes. In all samples, the highest amounts found were of plant fatty acids (C18:1 t9c11, C18: 2 c9-12) and of the very common Q-10 molecules (main or secondary quinones of numerous bacteria, occurring in the mitochondria of many eukaryotic cells, in plants and protists as well). MK-7 occurred in all three stands, branched iso-, anteiso-, 16–19 carbon atoms containing fatty acids, these compounds formed the dominant fraction of the compounds in each case.

Compounds characteristic for micro-fungi occurred in larger quantities (Q-11H<sub>2</sub>, Q-10H<sub>2</sub>); these did not emerge in the beech stand under oak and under spruce. In addition, these samples contained higher amounts of poly-unsaturated menaquinones (MK10H<sub>4</sub>, MK10H<sub>6</sub>). C20:2 and C22:3 fatty acids were also found.

Figure 4 illustrates the differences of soil chemotaxonomic markers between the upper soil layers of the three forests. Those components occurred in each sample and, in order to prevent interference with the variance of the samples, are not included in the figure.

The principal component analysis of soil samples from three forest stands brought a clear separation of the stands (figure not shown). The soil of the spruce forest differed most from the deciduous stands with regard to its soil microbial characteristics. More specifically, the

lower BAS, the lower SIR and lipid-phosphate derived microbial biomass as well as the highest RQ brought a statistically clear separation for spruce.

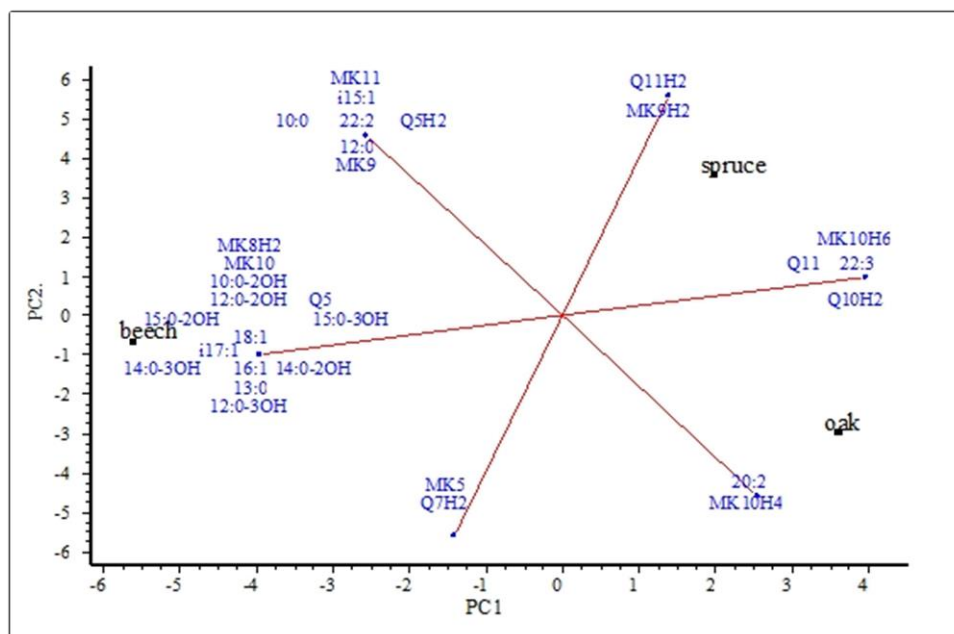


Figure 4. Main component analyses of the microbial communities described by chemotaxonomical markers (PLFA and RQ) in the spruce (*P. abies*), beech (*F. sylvatica*) and sessile oak (*Q. petraea*) forests soils

The abovementioned results indicate that acidification of the upper soil was greatest in the coniferous stand. Considering the highly acidic range and the high level of hidden-acidity, inherent small differences in pH can seriously impact the flora and fauna of the ecosystems, as observed with  $\text{pH} < 4.2$  for soil biota by Mössmer (2001), or as described in a long term study by Oulehle et al. (2007).

Forest stands usually need to restore mineral phosphorus daily because the plants can completely take up the available amount in a short time. The different order in comparison of humus contents vs. readily available phosphorus is partly justified by the slightly more intense microbial degradation measured in the beech forest, as suggested by BAS values.

Based on the concentrations of soil organic matter and, thus, the important macro-nutrients, oak and beech stands experienced more favourable conditions than the spruce forest. Lower soil pH-values and low availability of nutrients may result in reduced plant biomass production, assuming slower microbial turnover by spruce (Gisi 1990; Blume et al. 2016).

As expected, beech and oak litter proved to be more easily biodegradable than spruce needles (Gencsi – Vancsura, 1997). However, the accumulation of a thick layer of spruce needles has not yet been found, likely due to the short history of the conifer stands at the site. Although we expected lower microbial metabolic activity in the upper mineral soil under spruce, results showed an opposite trend, with lower metabolic activity in the deciduous stands. On one hand, it must be noted that the level of metabolic activity in the mineral soil of all stands was almost two orders of magnitude smaller than measured in the litter layer, which still indicates that decay of organic matter is altogether faster in the deciduous stands overall. The more intense breakdown in the upper mineral soil of the mostly acidic soil under spruce can perhaps find its causes in the slower transformation taking place in the litter layer, which leads to a higher transport of weakly transformed, easily degradable compounds to the upper mineral soil layer. This could be also indicated by the closest C / N ratio under spruce as well.

Similar results were found in the literature. Joergensen and Scheu (1999) studied soil chemical and microbial gradients with depth under beech and spruce forests.

Microbial biomass values delivered from SIR were very high according to the data recorded in Dilly (2003). This is probably the result of the less intense soil acidification and the higher average temperatures of the Sopron sites. A comparison of data to a Hungarian study (Molnár et al. 2016) on arable land proved our values to be higher, probably as forest litter remains on soil surface as opposed to the annual removal of biomass in agriculture.

Similar to decay activity, microbial biomass was much smaller in the mineral soil compared to the litter in all three forests respectively. A slight shift of microbial decay from the litter layer to upper mineral soil is again indicated in the case of spruce with high microbial stocks at the upper 10 cm of the mineral soil. Our data matches those in Raubuch and Beese (1999), who found a clearly marked gradient down from fresh litter throughout to mineral B horizon regarding microbial biomass and the  $SIR-C_{mic}$ .

The metabolic quotient ( $q$ ) was higher for spruce both for the litter and the mineral soil layer. Scheu and Parkinson (1994) studied the microbial biomass, BAS, RQ, and  $C_{mic}/C_{org}$  ratios in different soil layers of a poplar and of a pine forest in Alberta, Canada. The  $q$ -ratio decreased strongly with increasing soil depth, from which the authors concluded that the use of the carbon content of the substrate by microorganisms (i.e. assimilation!) becomes more efficient at the later stages of decomposition in both types of forests.

Similar to findings of Djajakirana et al. (1996), our PLFA results showed a higher proportion of fungi in the microbial communities under spruce than in the deciduous stands. A higher RQ experienced in the litter and in the mineral soil of spruce indicates that the fungal/bacterial ratios are increased with progressing soil acidification, while the carbon originating from available organic material sources can be assimilated less efficiently by the microbes. This results in higher proportions of  $CO_2$  released during decay of the same amount of organic compounds. This assumption could be controlled with the use of the selective inhibition method.

Lower biomass values derived from lipid-phosphate are probably explained by the increased number of active microbes after the addition of glucose in the SIR method. However, there was a good correlation of microbial biomass values in terms of comparison of the three forest stands.

It should be also noted that the results of the PLFA analysis detected a large amount of plant-characteristic compounds, so the lipid phosphate biomass values may not only characterize the microbial biomass, but are also influenced by the remains of fine roots in the soil samples. Consequently, these results should be treated with some reservations.

The above-described biological indicators confirm that the microbial processes suffer inhibition in the mostly acidified forest soil under spruce and even the effectiveness of microbial metabolism was reduced.

Among the chemical characteristics of the soil, highest latent acidity ( $y_2$ -value), and the lowest clay+silt contents separate the soil under spruce statistically again. The deciduous beech and oak stands showed higher mean values of pH, higher nutrient contents and base saturation as well as higher proportions of fine particles, indicating more stable soil structures. These results are consistent with the phenomenon described in literature, that deciduous stands tend to build in higher amounts of the so-called “basic” or “alkaline forming” cations, repeatedly resulting in higher proportions of these cations in their litter, which then get incorporated into the humus compounds of the upper mineral soil (Blume et al. 2016).

More favourable soil chemical and physical characteristics lead to better overall nutrient and hydrological cycling. Also, metabolic activity values and microbial biomass values are higher, and the breakdown is more efficient in the deciduous stands than it is in coniferous stands.

Similar correlations were shown by Bååth and Anderson (2003) who studied 53 deciduous forests soils for soil fungal/bacterial ratios in the microbial biomass, combining the SIR method with the selective inhibition of respiration (SI) technique. Strong linear correlation was found between the total microbial biomass calculated from the SIR- and PLFA-methods. Both biomass values were positively correlated with soil pH. The fungal/bacterial ratio value specified with selective inhibition was significantly decreased with increasing soil pH.

Several compounds found in our samples can be characteristic of a wide range of microbes, some for plants and others only generally for bacterial and plant mitochondria. These had little taxonomic value for our study. Nonetheless, it is not surprising to find these compounds in large quantities in forest soils.

Similarly, compounds specific for common Gram-positive soil bacteria – e.g. *Bacillus*, *Agromyces*, *Actinomyces*, *Aureobacterium* genus (MK-7, branched iso-, anteiso fatty acids with 16-19 carbon atoms) – were found. These formed the dominant fraction in all three soils.

Chemotaxonomic markers separate the beech forest soil clearly. On the one hand, this is due to the presence of large amounts of short-chain hydroxy-fatty acids, which are mainly characteristic for lipopolysaccharide layers in Gram-negative bacteria. The diversity of these compounds is conspicuous in the soil of the beech stand, and some are even specific for a narrower range of taxa, e.g. C12:0-2OH - *Alcaligenes*, *Chitinomonas*; C14:0 - *Alcaligenes*, C15:0-2OH - *Burkholderia*.

Gram-negative soil bacteria belong to a diverse, wide range of multiple bacterial genera, which are often heterotrophic, breaking down their carbon sources by respiration or fermentation. Among these chemoautotrophic bacteria (e.g. *Nitrosomonas*, *Nitrobacter*, *Thiobacillus*), there are those for which the source of carbon is atmospheric CO<sub>2</sub>. Their total absence in the soil of oak and spruce is unlikely, but their presence may be “masked” by other common compounds.

The Q-5 compound appeared only in the beech soil. Although we do not consider it as a main quinone of any bacteria, it is a secondary quinone in a number of Gram-negative, facultative anaerobic organisms (e.g. *Escherichia*, *Klebsiella*, *Proteus*, *Aeromonas* genera). Some of these can carry out very intense mineralisation (“rot”) even in the absence of O<sub>2</sub>, without producing harmful by-products.

The *K. pneumoniae* found here is a common nitrogen fixing representative of this genus. Species of *Aeromonas* are capable of butylene glycol fermentation, while species of the *Proteus* genus mix with acidic fermentations. These organisms are largely not soil bacteria; however, it is conceivable that they could have found their way into the soil via animal faeces. Otherwise, members of the *Enterobacteriaceae* family cannot survive long in this environment.

The C18:1 fatty acid appears also only under beech. Besides plants, this may be characteristic for mycorrhizae as well. Iso branched C17:1 and C15:1 fatty acids are characteristic markers of *Cytophaga* genus and of some sulphate-reducing bacteria. The largest amount of the MK5 compound, which is specific as a main-quinone for *Campylobacter* species and as a side-quinone for *Flavobacterium*, was found in the soil of the beech stand. These are typical residents of root surfaces of various plants.

Other compounds were detected in the soil of the beech stand: MK-9, MK-10, MK-11 can be at first quinones of some common soil bacteria (*Agrobacterium*, *Aureobacterium*, *Rathayibacter*, etc.). On the other hand, these act as main- and secondary quinones of the *Bacteroides* genus. The presence of the *Bacteroides* genus is probable. Other fatty acids characteristically found in anaerobic bacteria were detected in addition to these quinones. The species of the genus *Bacteroides* – together with other anaerobes (e.g. *Clostridium*) – are characterized by their role in causing “soil sickness” in badly aerated soils.

Compounds specific for microfungi were found in soil samples from the oak stand, and even more typically, in the samples from the spruce stand. Besides these, we found greater quantities of polyunsaturated menaquinones (MK10H<sub>4</sub>, MK10H<sub>6</sub>) in both soils, which suggests the presence of Actinobacteria, a very common group of Gram-positive bacteria living in soils. In terms of their metabolism, Actinobacteria are obligate aerobic saprophytes, and comprise an average of about 1-10% of the total soil bacterial population. They are completely absent in the litter layer and usually have their largest numbers in 5-10 cm depth of the mineral soil. They grow on multicomponent substrates, attack mostly hardly-degradable materials, like lignin, chitin, and starch. Several Actinobacteria (specifically *Streptomyces* species) produce antibiotics such as streptomycin, chloramphenicol, or tetracycline, and they are partly responsible for the typical scent of the soil, which is due to the release of gaseous Terpenoid derivatives (e.g. geosmin) due Gisi (1990). In the same samples, the C20:2 and C22:3 compounds suggest the presence of protozoa and cyanobacteria. In the soil, these spend the active phase of their lives in the water-filled soil pores and in surface water films on soil particles and roots.

Regarding the physical and chemical indicators as well as microbial activity and biomass patterns, the results of the microbial community investigations show a different distribution of the three forest stands. While soil acidification under spruce resulted in more unfavourable conditions compared to those of the nearly similar deciduous forests soils, the microbial community compositions were closer to each other in the spruce and the oak stands, and we saw a more distinct, specific microbial soil community under beech. This separation of beech originated partly from the presence of microbes, suggesting anaerobic conditions (facultative and obligate anaerobes) compared with the aerobic conditions indicating microbes of the other two forest soils. A reason for this could be the close parallel layering of the beech litter leaves, which, in contrast to spruce needles and curvy oak leaves, seal the soil surface during precipitation.

Microorganisms are critical in mediating C- and N-turnover in soils. Yet, a recent study (Zheng et al. 2019) has shown that soil-C processes were only directly affected by the soil environment, but not affected by microbial community composition. In contrast, soil-N processes were significantly related to bacterial/archaeal community composition and bacterial/archaeal/fungal richness/diversity, but not directly affected by the soil environment.

All of this demonstrates that the ecological assessment of forest soils should never be conducted solely by physical-chemical characterization. It is equally important to perform additional microbiological studies, and vice versa.

**Acknowledgements:** This work was supported by EFOP-3.6.1-16-2016-00018 project of the University of Sopron.

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## Effects of Meteorological and Site Parameters on the Health Status of Beech (*Fagus sylvatica* L.) Forests in Hungary

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**Abstract** – The influence of meteorological parameters on the health status of beech (*Fagus sylvatica* L.) was analyzed using long term datasets (1989-2010) collected in 15 sample plots located in Hungary's main beech regions. Leaf loss values were correlated with different meteorological parameters as explanatory variables. Analysis was performed by the CReMIT (Cyclic Reverse Moving Intervals Techniques) method. Weather, stand, and site parameters were also examined with PCA for comparison. Leaf loss levels showed stronger correlations with maximum monthly temperatures than with monthly precipitation sums. The monthly number of summer days and monthly number of hot days displayed a similar correlation to leaf loss as the maximum monthly temperature did. The correlations were regularly stronger and more frequent on more arid sites where the climate is less favorable for beech. Temperature affected leaf loss more than precipitation did. Our results show that beech forests may suffer heavy damage if climate change continues as projected.

**beech / *Fagus sylvatica* L. / leaf loss / climate change / CReMIT / Hungary**

**Kivonat** – Meteorológiai és egyes termőhelyi tényezők hatása a bükk (*Fagus sylvatica* L.) egészségi állapotára Magyarországon. A meteorológiai tényezők bükkösökre gyakorolt hatásait 15 mintaterületen gyűjtött hosszútávú (1989-2000) adatsorokon vizsgáltuk. A lombvesztés értékét különböző meteorológiai tényezőkkel korreláltattuk, a CReMIT (Cyclic Reverse Moving Intervals Techniques) mozgó időablakos módszer segítségével. Ezután az időjárási, termőhelyi és erdőállomány-jellemzőket is bevonva PCA analízist is végrehajtottunk. A lombvesztés erősebb kapcsolatot mutatott a havi maximum hőmérséklettel, mint a havi csapadék-összeggel. A havi nyári napok és hőségnapok összegei a maximum-hőmérséklethez hasonló összefüggéseket mutattak. A korrelációk erőssége nagyobb volt a szárazabb klímájú, bükknek kevésbé alkalmas mintaterületeken. Eredményeink alátámasztják, hogy a jövőben a bükkösök súlyos károknak lehetnek kitéve, ha a klímaváltozás az előrejelzett forgatókönyvek szerint alakul majd.

**bükk / *Fagus sylvatica* L. / lombvesztés / klímaváltozás / CReMIT / Magyarország**

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## 1 INTRODUCTION

Although the ratio of European beech (*Fagus sylvatica* L.) forests is relatively low in Hungary (112,603 ha; 6.0% of total forested area – NFK 2020), the species still possesses ecological and economic importance. Hungarian beech forests are situated on low to mid-mountain elevations, and on extrazonal occurrences in lower, hilly areas. Due to its distinct climatic needs, beech is considered a forest-climate indicator species in Hungary. Optimal montane/submontane sites with preferable climate for beech have a restricted extent in Hungary; hence, beech forests often grow in suboptimal sites.

Beech has significant precipitation needs and weak drought tolerance (Arend et al. 2016), which makes it vulnerable to climate change (Geßler et al. 2007). This is especially true in conditions and sites where beech forests thrive in Hungary today. Nonetheless, climate change appears to have the opposite effect in other north-eastern European provenances where suitable areas for beech are expanding (Augustaitis et al. 2015).

Climate change scenarios project worsening conditions for beech forests in Southern and Eastern Europe, particularly in the Carpathian Basin (Berki et al. 2007). Some researchers suggest beech may even disappear from Hungary altogether in the next century (Mátyás et al. 2010, Führer et al. 2011). Central and Southern European beech stands have suffered incremental reduction during drought and warm years since 1980 (Jump et al. 2006, Zimmermann et al. 2015, Giagli et al. 2016). A reduction in suitable habitat for beech due to climate change is also projected for the Iberian Peninsula (del Río et al. 2018).

Hungarian forests already face direct or indirect damage related to extreme weather conditions, particularly drought damage (Csóka et al. 2009, Lakatos – Molnár 2009, Rasztovits et al. 2014, Janik et al. 2016). In Hungary, beech is close to its xeric limits, which makes it more susceptible to climate change.

As in many other European countries, severe beech declines were recorded at several locations in Hungary in the late 1980s. This the FRI (Forest Research Institute) to launch the beech health monitoring plot network in 1989 (Koltay 2004). The initiative was based on the experiences of the ICP-Forests network (Michel – Siedling 2014), and its aim was to closely monitor the health trends of Hungarian beech stands and, subsequently, analyze the collected data. In this paper we processed the dataset on beech monitoring plots, and used statistical methods to identify correlations between meteorological, forest site, forest stand, and leaf-loss parameters.

Our starting hypothesis predicted direct positive correlations between temperature-related parameters and leaf loss, and negative correlations between precipitation and leaf loss. We also expected delayed and/or cumulative effects of weather from previous years.

## 2 MATERIALS AND METHODS

### 2.1 Location and main parameters of the sample plots

Altogether, 32 beech monitoring plots have been established in Hungary since 1989. Individual sample trees were marked and numbered on the monitoring plots. The stands in which the plots are located were managed by state forest companies, according to normal management plans. All the plots were established with 100 sample trees; over time, the number of trees gradually decreased due to mortality, intermediate cutting, etc. In our present analysis, we selected 15 plots that fulfilled the criteria listed below:

- The time series was at least 15 years in duration,
- The number of sample trees in 2010 was at least 50,
- The decrease of the number of sample trees during the examined period did not exceed 30%.

## 2.2 Site and stand parameters

The ages of the monitored stands varies between 64 and 135 years. *Table 1* provides other basic information about the monitored stands. The sample trees were surveyed once a year, in early September. NARIC FRI researchers completed the assessments. The visual survey was executed according to ICP methodology, and the surveyors regularly participated in international calibration workshop events (ICP Forests.net). For this study, we used the assessed leaf-loss values of the sample trees within 5% accuracy, but we scored several other parameters as well. Leaf loss is an important health indicator, and it is also comparable to other results. Only sample trees in Kraft classes 1 (outstanding/dominant) and 2 (codominant) (Kraft 1884) were included in the analysis since the trees in other social classes are too strongly influenced by competition for light.

Forest stand data involving our plots were obtained from the “Hungarian forest inventory”, from the National Land Center, Department of Forestry. This database contains detailed information (including site and stand parameters) about the location of the monitoring plots in the forest compartments.

*Table 1. Basic data about the 15 selected monitoring plots included in the study*

Forest subcompartment	Coordinates	First year	N (pcs) (first year)	N (pcs) (2010)	Age (2010)	Altitude (m)
Bőszénfa 12E	N46 15.727 E17 47.470	1992	100	98	128	300
Felsőtárkány 140D	N48 02.394 E20 28.986	1994	100	98	101	600
Felsőtárkány 55A	N48 01.515 E20 24.938	1994	100	99	95	700
Füzér 86F	N48 34.248 E21 25.785	1992	97	81	86	700
Füzér 86G	N48 34.123 E21 25.707	1992	99	80	81	700
Gyöngyössolymos 41B	N47 52.918 E19 57.480	1992	100	98	108	700
Kislőd 4B	N47 11.512 E17 38.402	1996	90	61	117	500
Kőszeg 43H	N47 22.103 E16 27.902	1992	76	64	133	800
Nagyhuta 10C	N48 28.157 E21 25.580	1995	89	66	89	400
Orfű 21B	N46 07.610 E18 10.292	1992	69	62	62	500
Répáshuta 11C	N48 03.288 E20 32.060	1994	100	100	83	700
Répáshuta 12G	N48 03.135 E20 32.290	1994	99	97	78	800
Szentpéterfölde 20A	N46 36.055 E16 44.973	1989	68	42	119	300
Ugod 31A	N47 17.534 E17 39.765	1992	120	84	101	500
Zselickislak 8E	N46 15.617 E17 48.380	1995	100	99	126	400

## 2.3 Meteorological parameters

Data was provided by the Hungarian Meteorological Service as a 10 km x 10 km grid interpolated data set from standard weather station measurements by the MISH method (Szentimrey et al. 2005). We used the following monthly weather parameters from the data series: maximum monthly temperature; monthly precipitation sum; monthly number of hot days, monthly number of summer days. We chose monthly maximum temperature instead of average temperature because extreme weather events are generally more damaging to tree health. Defined meteorologically, hot days are those in which the maximum daily temperature reaches/exceeds 30 °C. Summer days are those in which the daily maximum temperature reaches/exceeds 25 °C. The meteorological dataset was available between 1961 and 2010.

## 2.4 Statistics

Leaf loss data collected in a long-term monitoring network was correlated with different meteorological indices using CReMIT (Cyclic Reverse Moving Intervals Techniques). This is a special moving-window based method implementing moving averages and moving interval techniques (Pödör et al. 2014). The method makes it possible to systematically increase the number of used dependent or independent variables by creating new, derived time series from the basic dataset in a systematic way. Next, linear regression was used to examine the relationships between the derived meteorological parameters as independent variables, and the health status (indicated by leaf loss) as dependent variables. The significance of the results was examined by Student's t-test. To see the effects of the two previous years' weather on forest health, we examined correlations dating back 36 months. In our CReMIT analysis, the time windows did not extend beyond five months. We examined the year of the leaf loss assessment, the previous year's damage, and the leaf loss assessments from two years ago.

With temperature, precipitation, and number of summer days as parameters, we chose May, June, July, August, and September as the time windows for the three examined years. We omitted winter precipitation amounts since we decided to present results that were comparable to all weather indices.

Only the time windows from June to September were feasible for the number of hot days; thus, the length of the input time series was not exactly even. Nevertheless, through our selection of method and sample plots, we endeavored to ensure the differences would not significantly impact the results.

Forest health data was correlated with all of the derived meteorological parameters. This data was available for all the sample trees, so it was possible to stratify the results into Kraft-classes (1 – predominant, 2 – dominant, 3 – codominant and partially dominated, 4 – overtopped) (Kraft 1884, in Assmann 1968).

The CReMIT method created all of the continuous time series from the previous three years with the above-mentioned different window sizes ranging from one to five months.

Once the CReMIT results were achieved, the sample plots were examined according to forest site, meteorological, and stand parameters to determine if these results were similar to the CReMIT results. Principal Component Analysis (PCA) was chosen to perform this examination, mainly because the method can also be used if the parameters are not (or not fully) independent (Pearson 1901). Forest site parameters were included: in the case of forest soil, topsoil layer thickness and slope are crucial as both affect soil water dynamics. Exposure (0 = north slope; 180 = south slope) and altitude define the mesoclimate.

Forest stand parameters were also used in PCA: species mixture, canopy closure, and social position (Kraft classes). Stem diameter and stand height can be complex variables due to site conditions; hence, these variables were excluded from our examinations. *Table 3* contains the 14 examined environmental parameters.

Basic database management was performed with Libreoffice and Microsoft Office software. We also used R software environment and PAST v.2.17c (Hammer et al. 2001) software for statistical analysis.

## 3 RESULTS

The significant CReMIT results were rather diverse. On some occasions, neighbouring sample plots with extremely similar site conditions (and with the same interpolated meteorological datasets) surprisingly exhibited different responses. Only results with 90, 95, and 99% significance levels of individual time windows were summarized in *Table 2*.

Positive correlations were expected between “maximum monthly temperature” and leaf loss. Four plots gave consistently strong relationships; as a result, the expected correlations appeared. Unexpected negative correlation values emerged for plots located at higher elevations. These results occurred sporadically in six plots, but with one plot (Orfű 21B), the negative correlation was remarkable between the given year’s leaf loss and also with the temperature values from two years prior. In Kraft class 2, the results were usually similar, with the exception of the Kislőd plot.

As expected, “summer days” gave results similar to “maximum monthly temperature”. Although the number of plots with significant correlations were lower, the significance levels were usually stronger. In five plots, unexpected negative results occurred, which were similar to the maximum temperature results.

The number of “hot days” were obviously fewer than the number of “summer days”, but the results were similar. The significant correlations were even stronger, but fewer in number. Negative correlation values occurred only in one plot, in two time windows, in the Kraft class 2.

In the case of “monthly precipitation sum”, negative correlation values were presumed. The expected negative correlations occurred in eight plots; five of these had remarkably strong significant correlations. Unexpectedly, positive correlations occurred in five plots. Orfű 21B had a remarkable and unexpected correlation with the values from two years before the actual leaf loss values. The Kraft 2 class also shows similar but weaker correlations. The main results of the CReMIT analysis are summarized in *Table 2*.

*Table 2. Evaluation of the CReMIT results by scoring the strength according to the frequency of significant (above 90%) time windows (1, 2, 3) and direction (“–” = negative correlation; “+” = positive correlation) of correlations between leaf loss and weather parameters in a given year (0) and the previous two years (–1; –2)*

Parameter	Maximum monthly temperature			Monthly precipitation sum			Summer days			Hot days		
	0	–1	–2	0	–1	–2	0	–1	–2	0	–1	–2
<b>Location/Year</b>	<b>0</b>	<b>–1</b>	<b>–2</b>	<b>0</b>	<b>–1</b>	<b>–2</b>	<b>0</b>	<b>–1</b>	<b>–2</b>	<b>0</b>	<b>–1</b>	<b>–2</b>
Böszénfa 12E	3	0	0	–2	0	0	3	0	0	3	0	0
Felsőtárkány 140D	0	0	2	0	0	0	–1	0	1	0	0	0
Felsőtárkány 55A	0	0	0	0	0	0	0	0	1	0	0	0
Füzér 86F	2	0	0	–3	0	–1	3	0	0	0	0	0
Füzér 86G	2	0	0	–2	0	–2	3	0	0	0	0	0
Gyöngyössolymos 41B	0	0	0	0	–1	0	0	0	0	0	2	0
Kislőd 4B	2	1	0	–2	0	0	2	1	0	3	1	0
Kőszeg 43H	1	0	–1	0	0	0	2	0	0	3	0	0
Nagyhuta 10C	–1	–2	–1	–2	–2	0	–2	–2	0	0	0	–1
Orfű 21B	3	0	–2	0	0	2	3	0	–1	3	0	0
Répáshuta 11C	0	–1	0	0	2	0	0	0	0	1	0	0
Répáshuta 12G	0	–1	0	0	1	0	0	0	0	3	0	0
Szentpéterfölde 20A	1	1	–1	0	1	1	0	0	–1	1	1	0
Ugod 31A	0	0	3	2	1	0	0	–1	3	0	0	1
Zselickislak 8E	3	0	0	–2	0	0	3	1	0	3	0	0

Furthermore, CReMIT results (based on leaf-loss data series) were compared with forest site data collected via PCA analysis, which was performed in 15 (meteorological, site-dependent, and stand-dependent parameters all together) variables. This resulted in four new variables (4 Axes) that together account for 85% of the variation in forest site variables of the

sample plots (Table 3). Meteorological parameters were represented in the PCA analysis by their averages for all sample plots and for the whole examination period. Axis 1 is strongly positively correlated with the topsoil layer thickness and temperature variables, and negatively correlated with altitude. This relationship is obvious since average temperature normally increases as altitude decreases. Similarly, on lower altitudes, topsoil layers are usually thicker. The second component (Axis 2) is negatively correlated with slope, crown closure, and the proportion of Kraft class 1 and 2; it is also positively correlated with precipitation. As can be observed in the variance values of the components, temperature, and topsoil layer thickness are the main parameters. It is also clear that temperature is more relevant than precipitation sum. These support the results derived from time series analysis (CReMIT). Similar to weather, temperature has the most influence on health status at the long term level.

The third component (Axis 3) is determined by mixing ratio, and the fourth (Axis 4) contains exposure/facing and the age of trees. Only the mixing ratio determines the third component.

Table 3. Eigenvalues, proportion of variance and environmental variable loadings for the first 4 principal components in PCA

Axes	Axis 1	Axis 2	Axis 3	Axis 4
<i>Statistical parameters</i>				
Eigenvalue	6.0	2.6	1.8	1.4
Explained variance	43.0%	18.9%	13.0%	10.2%
Cumulated variance	43.0%	61.9%	74.9%	85.1%
<i>Environmental variables</i>				
Altitude	<b>-0.809</b>	-0.070	0.264	0.161
Exposure/Facing	0.324	0.210	-0.510	<b>-0.516</b>
Slope	-0.441	<b>-0.539</b>	-0.447	0.460
Topsoil layer thickness	<b>0.904</b>	0.059	0.250	0.064
Mixture ratio	-0.138	0.357	<b>0.873</b>	-0.094
Age	0.425	0.328	-0.081	<b>0.638</b>
Crown closure	-0.346	<b>-0.636</b>	0.378	-0.483
Average temperature	<b>0.937</b>	0.159	0.068	0.166
Maximum temperature	<b>0.955</b>	-0.206	-0.013	0.104
Sum of hot-days	<b>0.924</b>	-0.068	0.241	-0.151
Sum of summer-days	<b>0.974</b>	-0.126	0.047	-0.047
Precipitation sum	-0.122	<b>0.934</b>	-0.022	-0.014
Precipitation sum between May-Aug.	-0.559	<b>0.581</b>	0.213	0.208
Proportion of Kraft class 1/2	0.088	<b>-0.601</b>	0.447	0.412

The PCA detected three groups among the sample plots (Figure 1). These groups are colored red, yellow, and green. The red group contains the sample plots that had the strongest correlation between leaf loss and weather parameters according to CReMIT. The other plots in PCA also show an order similar to the results of CReMIT.



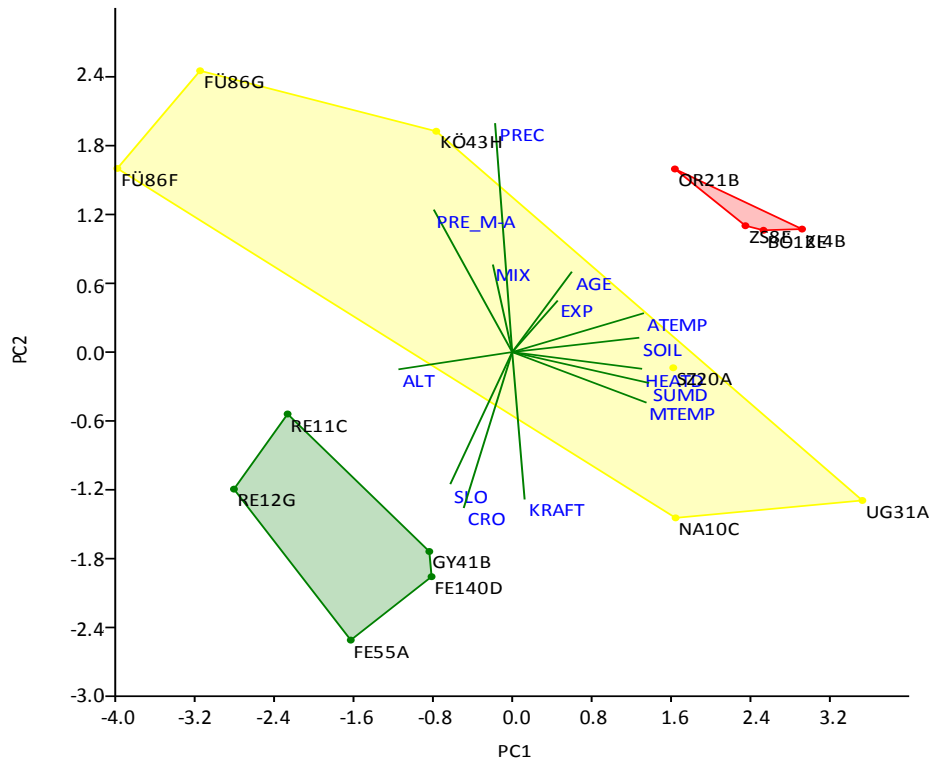


Figure 1. Scatter biplot of PCA. The diagram displays both the loadings (correlations between the original environmental variables and the components) as labelled vectors and the component scores of survey plots as labels and colored points. Survey plots with similar parameters are grouped together and linked with colored convex hulls. Plot names are abbreviated with the first 2 and the last 3 characters.

#### 4. DISCUSSION

In this paper we processed a previously gathered dataset on beech health, and searched for statistically detectable correlations with weather indices (in an available meteorological dataset). Our methods successfully detected known connections and demonstrated that these methods are serviceable even in extremely complex systems. That temperature affects leaf loss much more than precipitation sum is an important result from a forest protection perspective. If higher mean temperatures do occur (as they have in recent years), neither the same amount nor a slightly higher amount of precipitation will prevent beech trees from declining. We also could statistically support that beech forests in less suitable climatic conditions are more susceptible to weather extremities.

More specifically, most of the results supported our preliminary hypotheses: temperature and precipitation-type weather parameters have a consequential effect on beech health. Both of the used statistical methods supported these presumptions.

As we assumed, the CReMIT method resulted in a relatively low number of significant correlations. Since forest ecosystems are extremely complex, we could not expect simple linear correlations, as mentioned by Berki et al. (2014). Detecting statistical patterns in forests is often difficult (Granier et al. 1999). Even so, significant results could be detected with weather parameters two years before the actual leaf loss. Time windows in the damage year gave the strongest significant results. Additionally, as was the case in our work, studies examining the weather dependence of beech growth with different running window statistical methods also

detected correlations with various drought indexes (Manninger et al. 2011, Garamszegi – Kern 2014).

Monthly maximum temperature showed frequent and remarkable positive correlations with leaf loss, which is similar to conclusions many researchers have reached regarding average temperature (Siedling 2007, Zimmermann et al. 2015, Popa et al. 2017). In our analysis, this effect could even be detected with temperature values two years before the actual leaf loss. The number of hot days had a high significance in some cases, but the lower occurrence made patterns undetectable.

In our results, the effect of monthly precipitation sums on beech health was weaker than the effect of monthly maximum temperature. As expected, precipitation sums showed mostly negative correlations with leaf loss in our results. Precipitation sum values from previous years had some significant correlations on some time windows, as expected, but the actual year had the greatest effect (in particular sample plots most time windows had significant correlations) on the health status. These findings coincide with results of previous European studies, namely that higher amounts of precipitation have a positive effect on beech health (Meier – Lauschner 2008a, Seletković et al. 2009, Delaporte et al. 2016).

Although our input datasets were not optional (we had to use the available data from our sample plots), the weaker effects of precipitation compared to temperature and the unexpected correlations may raise a potential methodological issue: Using non-locally measured data (10x10 km grid interpolation in our case) may not be reliable enough to reveal the real correlations because the spatial variation of precipitation is very high (Manninger 2017). This may, at least partly, explain the controversial correlations. For every meteorological index, we detected that nearly the same plots had stronger correlations, while other plots had no responses or weak significant responses. The four plots showing the strongest responses kept this feature in almost every parameter and in both Kraft classes.

Sample plots situated in less favorable sites showed consistently stronger correlations with weather parameters. In fact, the plots are all situated in areas where the climate had become suboptimal for beech stands. According to Gálos and Führer (2018), this even applies to beech stands established from 1971-2010. The weather extremes in suitable sites affect health status less intensively. Although our plots are not situated on an ideal altitude for beech, many sample plots may remain relatively healthy. On the lower edge of the climate belt suitable for beech, the health statuses were generally worse.

It is known that many forest stand and forest site parameters impact beech health; for example: stands with higher canopy closure are less susceptible to health problems than those with low canopy closure (Csóka et al. 2009; Bošela et al. 2016). Mixed stands also appear to be more resistant and resilient than artificially grown monoculture beech forests (Mölder – Leuschner 2014, Metz et al. 2016). Nevertheless, seedlings and young trees in mixed forests are still vulnerable to extreme droughts (Lübbe et al. 2015).

PCA detected site and stand parameters, which allowed for the establishment of three identifiable groups among the plots. We could conclude that the differences in the correlations between forest health and weather indices (CReMIT results) in the different sample plots show the same variances, which can be obtained by the PCA examination of climate, forest stand, and site data. This fact implies that differences are caused by these climatic, site, and stand parameters. The importance ranking of these parameters on beech health in our results is monthly maximum temperature, altitude, topsoil layer thickness, monthly precipitation sum, tree social class, canopy closure, slope, species mixture, stand age, and exposure. The patterns of PCA and CReMIT results among the sample plots are almost identical, so the statistical variances caused by leaf loss and site parameters are also almost identical. This implies that the weather effect on beech health is influenced by forest stand and site parameters. Additionally,

it is influenced in the manner PCA calculated (Table 3). This also suggests that beech health risks can be projected based on forest site and stand data.

These results support earlier findings (Neiryneck – Roskams 1999, Stribley – Ashmore 2002, Siedling 2007, Seletković et al. 2009). According to Potočić et al. (2008), during years without weather extremes, the correlation between weather and leaf loss is not particularly remarkable, but a strong connection can be detected in extremely droughty years when the correlation between leaf loss and temperature appears much stronger. Popa et al. (2017) also found different responses of beech health to the weather parameters. In areas where beech occurs naturally, temperature increase has a positive effect on the health status. Wherever beech grows outside this area, precipitation has a moderately positive effect on health, while temperature increase has a negative effect. On the upper edge of the beech area (in high mountains), temperature had a clearly positive effect. On optimal forest sites for beech, both temperature and precipitation had little effect on tree health (Popa et al. 2017). These findings are the logical results of the process of forest acclimatization to weather: the variability of environmental parameters in the long term established a natural range of a certain species that have low impact influence in the “core area”. On the margins, these parameters either open a new frontier for the species, or reduce the area of the species.

Tree-ring analyses provided similar results and correlations between ring width and early summer temperatures than with late summer precipitations in Central Europe. With lower altitudes – which regularly means worsening climate for beech – the effects become stronger (Kolář et al. 2017).

There are many ways to improve the research of weather and forest health relationships. Permanent “on the spot” meteorological parameter measurements would be a major step forward. Applying health indicators other than leaf loss (i.e. measuring photosynthetic activity, sap flow, etc.) could also be a way forward.

Some weather parameters – those not included in our study – could also modify health responses. Cloudy weather (occurring often in mountain valleys) can greatly decrease leaf transpiration (Rozas et al. 2015). Air humidity can also be an important factor. Furthermore, the quantity of soil nitrogen can exacerbate the effects of drought (Dziedek et al. 2016). Measuring these parameters in the future would be extremely useful for understanding beech health responses.

Individual tree level differences in sensitivity and response are also considerable since intraspecific/intrapopulation diversity (i.e. phenological cycle) is typical in all populations and biological processes (Delpierre et al. 2017).

It would be very useful to forecast beech damage – possibly similarly to Italian researchers who found a fungal pathogen’s presence or absence (*Biscogniauxia nummularia*) to be a suitable sign for indicating drought damage in Mediterranean areas (Luchi 2015).

Some opinions suggest that the southern provenances should be more adapted and, therefore, more resistant to a warmer and drier climate (Cavin – Jump 2016, Horváth – Mátyás 2016). Others believe this type of adaptation potential is not strong enough in the case of beech (Knutzen et al. 2015), or that genetic diversity affects the shoot and the leaf growth more than the root growth, which is crucial from a drought tolerance perspective (Meier – Leuschner 2008b).

The predictive site choice of a given tree species (or the tree species choice for a given site) is increasingly important. The climate tolerance of different tree species must be carefully considered since this significantly influences the stability of future forests (Hlásny et al. 2017).

Moreover, if climate change proceeds in the manner some projections predict, more frequent and severe damage events will occur. These events will be far more connected to weather conditions than they currently are (Gálos – Führer 2018). Clearly, a proactive silviculture involving mixed

beech stands, increased structural diversity, and high canopy closure (avoiding large cutting areas) may considerably reduce the risks imposed by the changing climate.

**Acknowledgements:** We would like thank Balázs Nyul, Ervin Rasztoivits, Ernő Führer, Anikó Jagodics, and Levente Szócs for their help and advice.

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## Antioxidant Capacity and Tentative Identification of Polyphenolic Compounds of Cones of Selected Coniferous Species

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**Abstract** – The cones of coniferous species are a waste biomass byproduct that can be potentially utilized for a variety of purposes. One of the many application fields is the extraction of bioactive materials, particularly antioxidant polyphenols. Scientific literature on the antioxidant content of coniferous cones at different ripening stages is limited. In this study, we conducted a comparative analysis of the antioxidant content of selected taxa that are either common in Hungary or that have not yet been investigated in the scientific literature in any great detail (*Cedrus atlantica*, *Larix decidua*, *Picea abies*, *Pinus mugo*, *Pinus nigra*, *Pinus sylvestris*, *Pinus wallichiana*, *Tsuga canadensis*, *Tsuga heterophylla*, *Chamaecyparis lawsoniana*, *Taxodium distichum*, *Thuja occidentalis*, *Metasequoia glyptostroboides*, *Thuja orientalis*, *Cryptomeria japonica*, *Cunninghamia lanceolata*). A comparison of green, mature and opened cones was performed for the assigned taxa. Folin-Ciocalteu total polyphenol content (TPC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to assess the antioxidant contents. Overall antioxidant power was determined by a scoring system that combined the three assay results. In general, best values were found for green cones, followed by mature, and opened cones for each taxon. *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* and *Picea abies* all contained high amounts of antioxidants in both green and mature cones and attained the highest scores. High-performance liquid chromatographic/tandem mass spectrometric profiling of the cone polyphenols was also completed for selected samples. Results provide a basis for future bioactivity testing of these samples.

**coniferous species / cones / antioxidants / HPLC-MS/MS**

**Kivonat** – Tülevelű taxonok tobozainak antioxidáns kapacitása és polifenolos vegyületeinek vizsgálata. A tülevelű fajok tobozai olyan hulladék biomasszát képviselnek, melyeket többféle célra is lehetne használni. Az egyik ilyen felhasználási terület a bioaktív anyagok, például antioxidáns polifenolok kinyerése. A tobozérés különböző fenofázisaiban az antioxidáns tartalomra vonatkozó szakirodalmi adatok hiányosak. Jelen cikkben olyan taxonok vizsgálatát végeztük el, melyek vagy Magyarországon gyakoriak, vagy még nem történt meg a vizsgálatuk (*Cedrus atlantica*, *Larix decidua*, *Picea abies*, *Pinus mugo*, *Pinus nigra*, *Pinus sylvestris*, *Pinus wallichiana*, *Tsuga canadensis*, *Tsuga heterophylla*, *Chamaecyparis lawsoniana*, *Taxodium distichum*, *Thuja occidentalis*, *Metasequoia glyptostroboides*, *Thuja orientalis*, *Cryptomeria japonica*, *Cunninghamia lanceolata*). Elvégeztük a zöld, érett és lehullott tobozok összehasonlító vizsgálatát az összes polifenol tartalom (Folin-Ciocalteu), a FRAP (ferric reducing antioxidant power) és a DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidáns kapacitás meghatározási módszerek segítségével. Az összesített antioxidáns kapacitás kiértékelése a

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három módszer egyesítésével, egy pontrendszer segítségével történt meg. Összességében a legnagyobb antioxidáns kapacitást a zöld tobozokra mértük, a legalacsonyabbat a lehullott tobozokra mindegyik taxon esetében. A legmagasabb pontszámot a *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* és *Picea abies* zöld és értett tobozai kapták. A kiválasztott minták esetében elvégeztük a polifenol készlet profilozását nagyhatékonyságú folyadékkromatográfiás/tandem tömegspektrometriás eljárással. Az eredmények alapját képezhetik ezen minták bioaktivitás-vizsgálatának.

**tűlevelű fajok / toboz / antioxidánsok / HPLC-MS/MS**

## 1 INTRODUCTION

Forestry, logging and timber production wastes (e.g. leaves, wood bark, cones, etc.) can be a rich source of antioxidant compounds (Dedrie et al. 2015, Bouras et al. 2016) with potential utilization fields including the production of healthcare-related products (Packer et al. 1999, Dzialo et al. 2016, Watson et al. 2018), natural food preservatives and ingredients (Coté et al. 2011, Gyawali – Ibrahim 2014, Kobus-Cisowska et al. 2014, Frydman et al. 2005), natural growth bioregulators (Popa et al. 2008, Vyvyan 2002) as well as silver nanoparticles (Fahimirada et al. 2019, Rolim et al. 2019) to name but a few.

As waste biomass basic materials, cones represent a biomass exclusively born by coniferous trees and shrubs belonging to one of the over 615 living species (Auders – Spicer 2012). Conifers bear “seed-cones” and “pollen-cones” out of which the female seed-cones are simply referred to as “cones”; these were the exclusive subject of the present study.

The primary use of forest tree cones has been seed extraction for the production of forestry propagation material. In the Mediterranean region the edible seeds of stone pine cones (*Pinus pinea* L.) are one of the most important tree nuts (Kemerli-Kalbaran – Ozdemir 2019). The empty cones are usually burned (Aniszewska – Bereza 2014) in an uncompressed state or can be converted to briquettes (Gendek et al. 2018). The cones *Juniperus* spp. have traditionally been used for flavouring purposes (Lesjak et al. 2011), while the cone extracts and essential oils of *Pinus*, *Thuja*, and *Cedrus* spp. have been used by traditional medicine for various beneficial (e.g. anti-inflammatory, antioxidant, antiseptic, antifungal, antimicrobial, analgesic etc.) health effects (Watanabe et al. 1995, Lesjak et al. 2011, Süntar et al. 2012, Djouahri et al. 2014). The cone extracts of *Pinus parviflora* Siebold et Zucc. were shown to be very powerful against HIV and influenza viruses (Nagata et al. 1990) and were also shown to possess significant antimutagenic and anticancer effects (Nagasawa et al. 1992). The cone and essential oil extracts of *Metasequoia glyptostroboides* (Bajpai et al. 2014), *Juniperus sibirica* Burgsdorf. (Lesjak et al., 2011), *Tetraclinis articulata* (Vahl) Mast. (Djouahri et al. 2014), *Cupressus sempervirens* var. *pyramidalis* (L.) (Tumen et al. 2012) and of *Pinus* spp. (Süntar et al. 2012, Bradley et al. 2014, Tümen et al. 2018, Wang et al. 2019) were recently shown to have significant beneficial effects on human health. The latest results indicate that pine cone and pine cone extracts can be used for their various useful properties, e.g. being a source as dietary fibre (Kartal – Ozturk 2016), or starting materials for the production of coagulants (Hussain et al. 2019) and adsorbents (Kupeta et al. 2018, Mtshatsheni et al. 2019).

Despite the listed results, the literature lacks systematic research of the antioxidant composition of cones and the assessment of their role as a source of natural antioxidants. Moreover, sample collection times in the presented examples – more specifically, the phenophase of cone maturity – have rarely been documented in the literature. Recently Hofmann et al. (2020) concluded a systematic research using optimized extraction conditions and multiassay evaluation for the assessment of the antioxidant content of coniferous cones while respecting the phenophase of cone maturity; however, this study included only 6 taxa.



The aim of the present research was to extend previous studies (Hofmann et al. 2020) by investigating altogether 16 taxa including Atlas cedar (*Cedrus atlantica* Endl.), European larch (*Larix decidua* Mill.), Norway spruce (*Picea abies* H. Karst.), mountain pine (*Pinus mugo* Turra), black pine (*Pinus nigra* J.F. Arnold), Scots pine (*Pinus sylvestris* L.), Himalayan pine (*Pinus wallichiana* A. B. Jacks.), eastern hemlock (*Tsuga canadensis* (L.) Carrière), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Lawson cypress (*Chamaecyparis lawsoniana* (A. Murray) Parl.), bald cypress (*Taxodium distichum* (L.) Rich.), northern white-cedar (*Thuja occidentalis* L.), dawn redwood (*Metasequoia glyptostroboides* Hu and W. C. Cheng), Chinese arborvitae (*Thuja orientalis* L.), Japanese cedar (*Cryptomeria japonica* (L.f.) D. Don) and China fir (*Cunninghamia lanceolata* (Lamb.) Hook).

Antioxidant properties were assayed by the Folin-Ciocalteu total polyphenol content (TPC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. The evaluation of the overall antioxidant power was accomplished by a scoring system, which combined the results of the TPC, FRAP, and DPPH methods. In this manner a comprehensive evaluation of the results between various samples with potentially different antioxidant compositions was achieved.

The polyphenol profile of most relevant samples with the highest antioxidant potential was also investigated using high-performance liquid chromatography/multistage mass spectrometry (HPLC-MS/MS) in order to identify the structure of major antioxidant compounds, primarily polyphenols.

## 2 MATERIALS AND METHODS

### 2.1 Chemicals and reagents

Double distilled water was prepared for the extractions using conventional distillation equipment. LCMS grade acetonitrile, and acetone was obtained from VWR International (Budapest, Hungary). Gallic acid, ascorbic acid, DPPH, 2,4,6-tripyridyl-S-triazine (TPTZ), iron(III)-chloride, acetic acid, sodium acetate, hydrochloric acid, and sodium carbonate were obtained from Sigma-Aldrich (Budapest, Hungary). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany).

### 2.2 Sample collection and extraction

Sample collection occurred at the Botanical Garden of the University of Sopron in Sopron, Hungary between July-October 2019. Three ripening stages were sampled: green cones (collected in July when cones are green, yet nearly at their full size at the final year of maturation), mature cones (collected in August/September when the cones turned brown in colour and scales began to open) and opened cones (taken in September/October, at a fully opened state having released their seeds and found on trees or to the ground). One healthy individual of each taxon was sampled by collecting a minimum of 10 cones from different parts of the crown at each sampling occasion. Cone samples were put into sealed plastic bags and stored at -20°C until processing. Prior to extraction, samples were thawed and ground. Ultrasonic extraction was performed using an Elma Transsonic T570 ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) as follows: 0.45 g ground sample was homogenized with 45 ml acetone:water 80:20 v/v in a 50 ml centrifuge tube and sonicated for 3 x 10 min as described by Hofmann et al. (2020). One extraction was conducted for each sample.

## 2.3 Determination of antioxidant properties

TPC, FRAP, and DPPH measurements were run in triplicates using of a Hitachi U-1500 type spectrophotometer (Hitachi Ltd., Tokyo, Japan).

### 2.3.1 Total polyphenol content (TPC)

TPC determination was completed by applying the Folin-Ciocalteu assay (Singleton – Rossi 1965) using gallic acid as the standard: 0.5 ml extract solution was mixed with 2.5 ml 10-fold diluted Folin-Ciocalteu reagent. After 1 min, 2 ml 0.7 M Na<sub>2</sub>CO<sub>3</sub> solution was added and the reaction mixture was heated for 5 min in a 50 °C water bath. Reaction was stopped by cooling to room temperature in a cold water bath. Solution absorbance was measured at 760 nm. The results were expressed as mg equivalents of gallic acid/g dry bark units (mg GAE/g d.w.).

### 2.3.2 FRAP antioxidant capacity

The method described by Benzie – Strain (1996) was applied for the measurement of the FRAP antioxidant capacity at 593 nm using ascorbic acid as a standard. FRAP reagent was prepared as follows: 25 ml of 10 mM TPTZ solution (aqueous with 84 µl cc. HCl) was mixed with 250 ml of acetate buffer (300 mM, pH 3.6) and 25 mL of 20 mM aqueous FeCl<sub>3</sub> solution. Fifty µl sample was mixed with 1500 µl FRAP reagent in glass test tubes at ambient temperature and after 5 min reaction time absorbance was measured. Results were given in mg equivalents of ascorbic acid/g dry weight (mg AAE/g dw.).

### 2.3.3 DPPH antioxidant capacity

The slightly modified method of Sharma – Bhat (2009) was used for running the DPPH assay as follows: 2090 µl unbuffered methanol was mixed with 900 µl 2×10<sup>-4</sup> M methanolic DPPH solution and 10 µl extract. After 30 min incubation at room temperature in the dark, the decrease in absorbance was determined at 515 nm. Results were calculated in IC<sub>50</sub> (50% inhibition concentration) values in µg extractives/ml assay (µg/ml) units, representing the amount of extractives which will react with 50% of the added DPPH• radicals in the total assay volume (3 ml).

## 2.4 HPLC-MS/MS analyses

Separation of the cone extracts of Norway spruce and eastern hemlock was achieved using a Shimadzu LC-20 type high-performance liquid chromatograph coupled with a Shimadzu SPD-M20A type diode array detector (PDA) (Shimadzu Corporation, Kyoto, Japan) and an AB Sciex 3200 QTrap triple quadrupole/linear ion trap mass spectrometric (MS) detector (AB Sciex, Framingham, USA). A Phenomenex Synergy Fusion-RP 80A, 250 mm x 4.6 mm, 4µm column was used for the separation with a Phenomenex SecurityGuard ULTRA LC type guard column (Phenomenex Inc., Torrance, USA) at 40°C. The injection volume was 15 µl. The binary gradient of A (H<sub>2</sub>O + 0.1% HCOOH) and B (CH<sub>3</sub>CN + 0.1% HCOOH) solvents was run with 1.2 ml/min flow-rate using the following time gradient: 3% B (0-4 min), 6% B (10 min), 20% B (34 min), 57% B (73 min), 100% B (90-98 min), 3% B (99-106 min). The PDA detector signal (250-380 nm) was recorded to monitor separation of peaks. Negative electrospray ionization mode was used for the MS detector by allowing 0.6 mL/min flow to enter the MS ion source using a split valve. Polyphenols were identified with the Information Dependent Analysis (IDA) scanning function of the mass spectrometer using a survey (Q1) scan between 150-1300 m/z and respective dependent (Q3) product ion scans between 80-1300 m/z. Ion source settings were as follows: spray voltage: -4500 V, source temperature: 500°C, curtain gas (N<sub>2</sub>) pressure: 40 psi, spray gas (N<sub>2</sub>) pressure: 30 psi, drying gas (N<sub>2</sub>) pressure: 30 psi. Chromatographic data were acquired and evaluated using the Analyst 1.6.3 software. Mass

spectra evaluation and compound identification was achieved using the RIKEN tandem mass spectral database (Sawada et al. 2012), via the scientific data found in the literature and by the use of fragmentation rules (McLafferty – Tureček 1993).

## 2.5 Statistics

In order to compare the respective antioxidant capacities of the extracts, ANOVA analysis was run using Statistica 11 (StatSoft Inc., Tulsa, USA) software with the Tukey HSD method.

## 3 RESULTS AND DISCUSSION

### 3.1 Evaluation of the TPC, FRAP and DPPH results

Table 1 includes the TPC, FRAP, and DPPH data of the samples indicating statistical comparison (ANOVA) for the 10 best results within each method. In all of the investigated taxa, the highest TPC was measured in green cone samples, followed by mature and opened cone samples. Overall the highest TPC was determined in the green cones of eastern hemlock ( $157.25 \pm 9.98$  mg GAE/g dw.), Lawson cypress ( $131.68 \pm 4.35$  mg GAE/g dw.), Japanese cedar ( $131.74 \pm 3.00$  mg GAE/g dw.) and dawn redwood ( $113.60 \pm 4.81$  mg GAE/g dw.). Respecting mature and opened cones highest TPC values were determined for dawn redwood (mature:  $91.25 \pm 3.69$  mg GAE/g dw., opened:  $60.16 \pm 8.23$  mg GAE/g dw.), Chinese arborvitae (mature:  $81.22 \pm 5.30$  mg GAE/g dw., opened:  $68.88 \pm 4.91$  mg GAE/g dw.), Japanese cedar (mature:  $74.18 \pm 2.09$  mg GAE/g dw., opened:  $57.41 \pm 2.93$  mg GAE/g dw.) and Norway spruce (mature:  $64.64 \pm 2.68$  mg GAE/g dw., opened:  $46.39 \pm 3.54$  mg GAE/g dw.).

According to literature data, Hofmann et al. (2020) determined high TPC levels for Norway spruce and eastern hemlock green cone samples. Horiba et al. (2016) found  $84.9 \pm 3.3$  mg GAE/g dw TPC in Japanese cedar cones (without seeds), which is comparable to the present results.

The overall highest TPC, determined for eastern hemlock green cones ( $157.25 \pm 9.98$  mg GAE/g dw.) was surprisingly higher than that of the related taxon, western hemlock ( $89.16 \pm 5.51$  mg GAE/g dw.). In fact, Hernes – Hedges (2004) reported the tannin content of western hemlock cones to be 3.13 wt.%; however, the authors did not document either the phenophase of cone maturity or the month of the sample collection. Hernes – Hedges (2004) also found that the bark and green needles contained more tannins compared to cones, yet did not investigate the amount of other types of polyphenols.

The limitation of the Folin-Ciocalteu assay (Singleton – Rossi 1965) is that it is known to interfere with other types of antioxidants (Prior et al. 2005, Everette et al. 2010). In fact, the TPC method is considered one of the >100 different assays currently used for the determination of antioxidant capacity and radical scavenging ability (Cornelli 2009). None of these assays is individually able to measure the total antioxidant power of all compounds in plant extracts. Therefore, the use of multiple assays to estimate the “overall” antioxidant potential of complex extracts is recommended (Ghiselli et al. 2000). The present study used the FRAP and the DPPH methods to provide further results on the antioxidant power of the samples.

Table 1. TPC<sup>1</sup>, FRAP<sup>2</sup>, and DPPH<sup>3</sup> antioxidant capacity of the cones (mean  $\pm$  standard deviation). Different superscript letters indicate significant differences at  $p < 0.05$  (TPC, FRAP, DPPH) between the samples with the 10 best values

	TPC (mg GAE/g dw.)			FRAP (mg AAE/g dw.)			DPPH IC <sub>50</sub> ( $\mu$ g extractives/ml)		
	Green	Mature	Opened	Green	Mature	Opened	Green	Mature	Opened
<b>Atlas cedar</b>	88.41 $\pm$ 1.68	14.96 $\pm$ 2.24	7.46 $\pm$ 0.26	62.08 $\pm$ 3.13 <sup>a</sup>	4.48 $\pm$ 0.11	3.37 $\pm$ 0.10	21.44 $\pm$ 2.94	88.82 $\pm$ 12.86	56.92 $\pm$ 15.87
<b>European larch</b>	83.44 $\pm$ 4.27	25.98 $\pm$ 0.94	17.60 $\pm$ 2.15	55.96 $\pm$ 0.93	14.18 $\pm$ 0.83	4.09 $\pm$ 0.17	9.07 $\pm$ 1.39	12.53 $\pm$ 0.38	28.21 $\pm$ 6.84
<b>Norway spruce</b>	105.58 $\pm$ 7.92 <sup>ab</sup>	64.64 $\pm$ 2.68	46.39 $\pm$ 3.54	72.02 $\pm$ 8.76 <sup>ab</sup>	50.19 $\pm$ 2.08	28.35 $\pm$ 3.37	10.75 $\pm$ 0.32	9.38 $\pm$ 1.14	8.57 $\pm$ 0.17 <sup>ab</sup>
<b>Mountain pine</b>	95.76 $\pm$ 9.48 <sup>a</sup>	22.33 $\pm$ 3.31	15.96 $\pm$ 1.10	60.06 $\pm$ 2.77	9.34 $\pm$ 0.07	7.25 $\pm$ 0.19	7.87 $\pm$ 0.31 <sup>abc</sup>	27.83 $\pm$ 3.73	18.86 $\pm$ 0.14
<b>Black pine</b>	89.22 $\pm$ 4.79	19.70 $\pm$ 3.36	7.08 $\pm$ 0.34	58.21 $\pm$ 2.34	9.55 $\pm$ 0.52	4.50 $\pm$ 0.17	15.33 $\pm$ 1.39	45.90 $\pm$ 2.69	62.32 $\pm$ 1.90
<b>Scots pine</b>	46.30 $\pm$ 1.81	18.99 $\pm$ 1.44	13.19 $\pm$ 1.53	33.42 $\pm$ 3.12	9.41 $\pm$ 0.32	7.26 $\pm$ 0.14	72.40 $\pm$ 21.26	29.32 $\pm$ 1.10	22.88 $\pm$ 0.54
<b>Himalayan pine</b>	62.52 $\pm$ 5.09	17.76 $\pm$ 1.35	8.18 $\pm$ 0.97	38.84 $\pm$ 0.69	8.33 $\pm$ 0.56	3.85 $\pm$ 0.21	25.72 $\pm$ 3.50	54.76 $\pm$ 14.54	72.58 $\pm$ 7.23
<b>Eastern hemlock</b>	157.25 $\pm$ 9.98 <sup>d</sup>	56.13 $\pm$ 4.07	10.57 $\pm$ 1.69	100.11 $\pm$ 0.40 <sup>e</sup>	46.57 $\pm$ 1.02	5.94 $\pm$ 0.25	7.83 $\pm$ 0.29 <sup>abc</sup>	11.37 $\pm$ 0.67	17.74 $\pm$ 1.01
<b>Western hemlock</b>	89.16 $\pm$ 5.51	30.77 $\pm$ 2.22	10.01 $\pm$ 1.77	59.11 $\pm$ 1.73	31.03 $\pm$ 1.55	4.53 $\pm$ 0.09	11.16 $\pm$ 1.37	15.52 $\pm$ 0.84	40.44 $\pm$ 17.94
<b>Lawson cypress</b>	131.68 $\pm$ 4.35 <sup>c</sup>	20.61 $\pm$ 2.27	16.21 $\pm$ 2.11	89.42 $\pm$ 6.82 <sup>cde</sup>	9.18 $\pm$ 0.12	8.36 $\pm$ 0.13	7.23 $\pm$ 0.41 <sup>bc</sup>	22.46 $\pm$ 1.72	30.50 $\pm$ 6.72
<b>Bald cypress</b>	70.99 $\pm$ 4.49	52.20 $\pm$ 1.86	29.53 $\pm$ 3.96	57.34 $\pm$ 1.28	49.69 $\pm$ 5.07	42.42 $\pm$ 3.29	8.45 $\pm$ 0.74 <sup>ab</sup>	13.17 $\pm$ 2.13	13.42 $\pm$ 0.60
<b>Northern white-cedar</b>	93.71 $\pm$ 5.47 <sup>a</sup>	39.96 $\pm$ 2.59	31.38 $\pm$ 2.57	76.46 $\pm$ 3.44 <sup>abc</sup>	49.81 $\pm$ 0.11	18.54 $\pm$ 0.83	9.93 $\pm$ 0.62	9.21 $\pm$ 0.30	8.13 $\pm$ 0.55 <sup>ab</sup>
<b>Dawn redwood</b>	113.60 $\pm$ 4.81 <sup>b</sup>	91.25 $\pm$ 3.69 <sup>a</sup>	60.16 $\pm$ 8.23	129.16 $\pm$ 3.01 <sup>f</sup>	147.00 $\pm$ 6.83 <sup>g</sup>	61.43 $\pm$ 3.51	6.22 $\pm$ 0.42 <sup>c</sup>	4.42 $\pm$ 0.07 <sup>d</sup>	7.15 $\pm$ 0.87 <sup>bc</sup>
<b>Chinese arborvitae</b>	106.67 $\pm$ 2.76 <sup>ab</sup>	81.22 $\pm$ 5.30	68.88 $\pm$ 4.91	78.49 $\pm$ 1.55 <sup>bcd</sup>	93.12 $\pm$ 4.84 <sup>de</sup>	31.60 $\pm$ 2.02	9.56 $\pm$ 0.50	15.76 $\pm$ 0.45	17.27 $\pm$ 7.71
<b>Japanese cedar</b>	131.74 $\pm$ 3.00 <sup>c</sup>	74.18 $\pm$ 2.09	57.41 $\pm$ 2.93	60.87 $\pm$ 5.21	41.04 $\pm$ 2.08	24.16 $\pm$ 0.86	10.13 $\pm$ 0.76	10.55 $\pm$ 1.40	17.51 $\pm$ 0.56
<b>China fir</b>	92.24 $\pm$ 1.57 <sup>a</sup>	36.36 $\pm$ 2.29	35.94 $\pm$ 1.33	67.99 $\pm$ 8.88 <sup>ab</sup>	37.20 $\pm$ 2.68	20.65 $\pm$ 1.44	9.03 $\pm$ 1.19 <sup>a</sup>	13.79 $\pm$ 0.46	11.14 $\pm$ 0.45

1: Total polyphenol content

2: Ferric reducing antioxidant power

3: 2,2-diphenyl-1-picrylhydrazyl

Regarding FRAP results, green cone samples showed the best results in general. The only opposite tendency was observed with dawn redwood and Chinese arborvitae, where mature cones (D.r.:  $147.00 \pm 6.83$  mg AAE/g dw., C.a:  $93.12 \pm 4.84$  mg AAE/g dw.) had superior FRAP values compared to green cone results (D.r.:  $129.16 \pm 3.01$  mg AAE/g dw., C.a:  $78.49 \pm 1.55$  mg AAE/g dw.) showing excellent FRAP. Overall the best FRAP was determined for the green cones and opened cones of previous two taxa and for the green cones of eastern hemlock ( $100.11 \pm 0.40$  mg AAE/g dw.). According to Lesjak et al. (2011, 2014), the FRAP of *Juniperus* spp. cones varies between  $3.61 \pm 0.03$  mg AAE/g dw. (*Juniperus macrocarpa* Sibth. et Sm.) to  $35.26 \pm 1.12$  mg AAE/g dw. (*Juniperus sibirica* Burgsdorf.), which also indicates that big differences between related taxa can exist, as is the case with eastern ( $100.11 \pm 0.40$  mg AAE/g dw.) and western hemlock ( $59.11 \pm 1.73$  mg AAE/g dw.) cones in the present study.

The DPPH radical scavenging activity was determined using the IC<sub>50</sub> value (50% inhibition concentration), with low IC<sub>50</sub> indicating high antioxidant capacities. The DPPH results also showed the general decreasing tendency of the order green > mature > opened cones within a given taxon. The best results were obtained for the mature ( $4.42 \pm 0.07$  µg/ml) and green ( $6.22 \pm 0.42$  µg/ml) cones of dawn redwood, and for green cones of Lawson cypress ( $7.23 \pm 0.41$  µg/ml) and eastern hemlock ( $7.83 \pm 0.29$  µg/ml). In fact, the excellent DPPH activity (Bajpai et al. 2009, 2017) and bioactivity (Bajpai et al. 2007, 2009, Yoon et al. 2011) of dawn redwood cone extracts has already been reported in scientific literature.

The TPC, FRAP, and DPPH data makes it apparent that all of the three assays indicated different orders for the best results, which was attributed to the different compositions of the extracts as well as to the different working principle and selectivity of the assays (Apak et al. 2007, Müller et al. 2011).

In order to obtain a comprehensive measure of the overall antioxidant efficiency of the cone extracts and to consider the different selectivity of methods, the summarized evaluation of results of the three different methods was implemented.

### 3.2 Combined evaluation of the TPC, FRAP and DPPH results

Combined evaluation of the TPC, FRAP and DPPH was achieved using a scoring system (Hofmann et al. 2020) with the following calculation: For the TPC and FRAP results, 0 points were assigned to the weakest values and 1 to the best values within each assay, using linear approximation for the other values. In the case of DPPH assay, opposite scoring was used (lowest IC<sub>50</sub> value, score: 1; the highest IC<sub>50</sub>, score: 0). The respective scores of TPC, FRAP and DPPH were then summarized for each sample to estimate the measure of the overall antioxidant efficiency (Table 2).

Regarding the sum of scores, the highest scores – those with the best overall antioxidant power – were determined in the green cones of eastern hemlock (2.63), dawn redwood (2.56), Lawson cypress (2.40), Japanese cedar (2.16), Chinese arborvitae (2.13) and Norway spruce (2.06) and for the mature cones of dawn redwood (2.56). Interestingly eastern hemlock contained much higher overall antioxidant power compared to related western hemlock for green, mature and opened cone samples, showing big differences between respective samples; this discrepancy requires further research to determine an explanation.

Of these taxa, the bioactivity, antioxidant activity, or uses of their cone extracts have already been reported in the literature for Lawson cypress (Smith et al., 2007, Kilinc et al. 2015), dawn redwood (Bajpai et al. 2007, 2009, 2014, 2017, Yoon et al. 2011), Japanese cedar (Horiba et al. 2016) and Chinese arborvitae (Yogesh – Ali 2014).

However, no data on the polyphenolic composition and bioactivity of Norway spruce and eastern hemlock cone extracts exists in the scientific literature. Norway spruce is one of the most widespread coniferous tree species in Europe, possessing significant ecological, industrial

and economic significance (Meloni et al. 2007, Lamedica et al. 2011). Eastern hemlock is an ecologically important foundation species in forests of eastern North America (Clark et al. 2012) with a natural range extending from northern Georgia and Alabama to southern Canada and westward into the central Great Lakes states (McWilliams – Schmidt 2000). Information on molecular cone extract composition will provide a basis for the future research on the role these compounds play in possible bioactivity effects. Hence, the remainder of this article will focus on the identification of cone extractives, especially polyphenolic compounds found in the green cone tissues of Norway spruce and eastern hemlock.

*Table 2. Normalized values (scores) of the TPC<sup>1</sup>, FRAP<sup>2</sup>, and DPPH IC<sub>50</sub><sup>3</sup> values and the sum of scores for each sample representing the combined antioxidant values.*

	TPC <sup>1</sup>			FRAP <sup>2</sup>			DPPH IC <sub>50</sub> <sup>3</sup>			Sum of scores		
	Gr.	Mat.	Op.	Gr.	Mat.	Op.	Gr.	Mat.	Op.	Gr.	Mat.	Op.
Atlas cedar	0.54	0.05	0.00	0.41	0.01	0.00	0.80	0.00	0.38	1.75	0.06	0.38
European larch	0.51	0.13	0.07	0.37	0.08	0.00	0.94	0.90	0.72	1.82	1.10	0.79
Norway spruce	0.66	0.38	0.26	0.48	0.33	0.17	0.93	0.94	0.95	2.06	1.65	1.39
Mountain pine	0.59	0.10	0.06	0.39	0.04	0.03	0.96	0.72	0.83	1.94	0.87	0.92
Black pine	0.55	0.08	0.00	0.38	0.04	0.01	0.87	0.51	0.31	1.80	0.64	0.32
Scots pine	0.26	0.08	0.04	0.21	0.04	0.03	0.19	0.71	0.78	0.66	0.83	0.85
Himalayan pine	0.37	0.07	0.01	0.25	0.03	0.00	0.75	0.40	0.19	1.36	0.51	0.20
Eastern hemlock	1.00	0.33	0.02	0.67	0.30	0.02	0.96	0.92	0.84	2.63	1.54	0.88
Lawson cypress	0.83	0.09	0.06	0.60	0.04	0.03	0.97	0.79	0.69	2.40	0.92	0.79
Bald cypress	0.43	0.30	0.15	0.38	0.32	0.27	0.95	0.90	0.89	1.75	1.52	1.31
Northern white-cedar	0.58	0.22	0.16	0.51	0.32	0.11	0.93	0.94	0.96	2.02	1.49	1.22
Dawn redwood	0.71	0.56	0.35	0.88	1.00	0.40	0.98	1.00	0.97	2.56	2.56	1.75
Chinese arborvitae	0.66	0.49	0.41	0.52	0.62	0.20	0.94	0.87	0.85	2.13	1.98	1.46
Japanese cedar	0.83	0.45	0.34	0.40	0.26	0.14	0.93	0.93	0.84	2.16	1.64	1.32
China fir	0.57	0.19	0.19	0.45	0.24	0.12	0.95	0.89	0.92	1.96	1.32	1.23
Western hemlock	0.55	0.16	0.02	0.39	0.19	0.01	0.92	0.87	0.57	1.85	1.22	0.60

1: Total polyphenol content

2: Ferric reducing antioxidant power

3: 2,2-diphenyl-1-picrylhydrazyl

Gr.: green cones, Mat.: mature cones, Op.: opened cones

### 3.3 HPLC-MS/MS analyses

The identification of the molecular structure of the extractives in the cone extract solutions of Norway spruce and eastern hemlock has been accomplished using high-performance liquid chromatography/tandem mass spectrometry. *Figure 1.* depicts the HPLC chromatograms and *Table 3* includes the major compounds found in the extracts.

Altogether 82 compounds have been described and tentatively identified by tandem mass spectrometric fragmentation (MS/MS) data. The composition of the green cones of the two taxa is different, with both including low amounts of (+)-catechin (3), (–)-epicatechin (7), and procyanidin B dimers (1, 2, 4). Extracts included a large number of coumaric acid derivatives and flavonoid glycosides, yet not all of the compounds were found in both samples.

Quercetin-*O*-hexosides (18, 19) and taxifolin-*O*-hexosides (12,13) were found in both taxa; however, the pentose conjugate of quercetin (21) was only indicated in eastern hemlock. Interestingly, isorhamnetin-*O*-hexosides (27, 28) were only found in Norway spruce. The most abundant class of flavonoid conjugates were the kaempferol derivatives (mostly glycosides) with a total count of 10 compounds. Out of these compounds only kaempferol-*O*-hexoside (25), kaempferol-*O*-rutoside (37) and kaempferol-rhamnose-hexose-rhamnose (50) were detected in the green cones of both taxa. The *O*-rutoside (24), *O*-pentoside (29, 30, 31), *O*-rhamnoside (33), acetyl-hexoside (34), and an unknown derivative (46) of kaempferol were exclusively detected in eastern hemlock. Regarding flavonoid glycosides, the presence of acylated kaempferol conjugates (e.g. 34) are especially interesting as these types of compounds were shown to have excellent antioxidant properties and to contribute significantly to antibacterial effects of plant extracts (Mellou et al. 2005), which highlights the importance in finding matrices with high content of acylated flavonols (García-Villalba et al. 2017).

The presence of coumaric acid as part of the compounds was evidenced by the simultaneous presence of the 163, 145, and 119 *m/z* ions in the MS/MS spectra of the compounds corresponding to the  $[M-H]^-$ ,  $[M-H_2O-H]^-$  and  $[M-CO_2-H]^-$  fragment ions, with M representing coumaric acid molecule. The structure of coumaric acid derivatives are often left unidentified using ion trap or triple quadrupole mass spectrometers (Spínola et al. 2016, Llorent-Martínez et al. 2019), as the MS<sup>n</sup> mass spectra are only suitable to indicate characteristic fragments and losses during the fragmentation process of the molecules, justifying the simultaneous presence of the coumaric acid fragments at 119, 145 and 163 *m/z*. A more precise and informative analysis of the structure of these compounds could be conducted in the future with the use of TOF (time-of-flight) mass spectrometry by determining accurate mass of the compounds (Vilhena et al. 2020). Coumaric acid derivatives 47, 48, 49, 59, and 66 were only indicated in Norway spruce, while compounds 55, 60, and 65 were found exclusively in eastern hemlock and compound 51 in the green cone extracts of both taxa.

Piceatannol isomers (15, 16) and their *O*-hexoside conjugates (possibly astringin isomers, 10, 11) were evidenced from spruce samples only.

Chlorogenic acid isomers (5, 6) were only found in eastern hemlock. Other compounds were left unidentified only with MS/MS data for future identification of their structure.

According to Table 3 and comparing peak heights in Figure 1, the most abundant compounds in the green cone extract of Norway spruce were astringin isomers (10, 11), unidentified compounds 8, 58, 68, 69, 70 and coumaric acid derivative 51, while in eastern hemlock they were chlorogenic acid isomers 5, 6, kaempferol-rhamnose-hexose-rhamnose 50, and unidentified compounds 68, 69, 70, and 79.

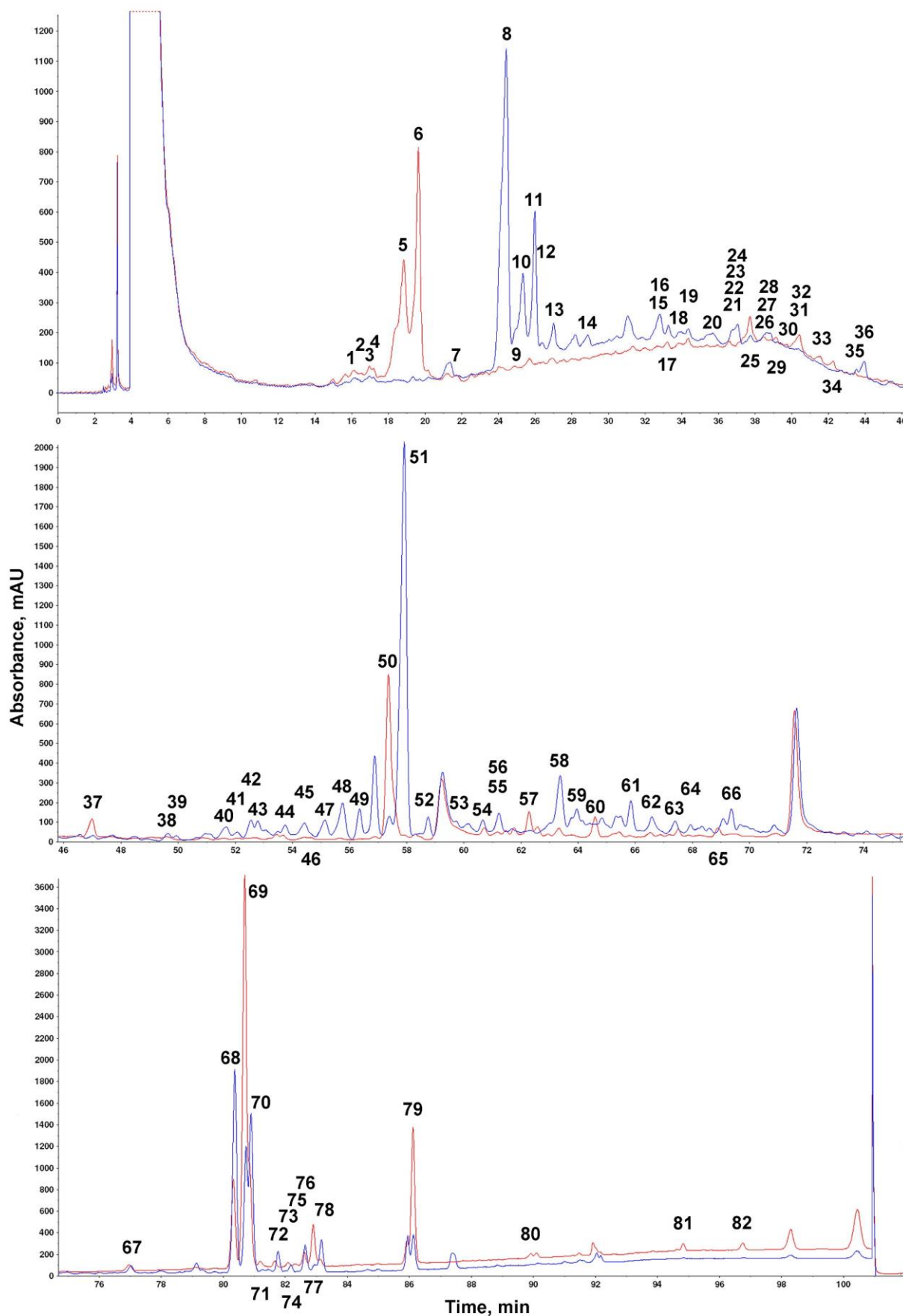


Figure 1. The PDA (250-380 nm) chromatogram of the green cone extracts of Norway spruce (blue) and eastern hemlock (red).



Table 3. Tentative chromatographic/mass spectrometric identification of the polyphenols in the green cones of Norway spruce (S) and eastern hemlock (H)

Peak	$t_r$ (min)	Compound	S	H	[M-H] <sup>-</sup> $m/z$	MS/MS $m/z$
1	15.8	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
2	16.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
3	17.0	(+)-Catechin	x	x	289	245, 203, 125, 123, 109
4	17.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
5	18.9	Chlorogenic acid isomer		x	353	191, 179, 161, 135
6	19.7	Chlorogenic acid isomer		x	353	191, 179, 161, 135
7	21.7	(-)-Epicatechin	x	x	289	245, 203, 125, 123, 109
8	24.0	Unidentified	x		no ion	no negative ions
9	25.0	Unidentified	x		no ion	no negative ions
10	25.3	Piceatannol- <i>O</i> -hexoside (astringin)	x		405	243, 225, 201
11	26.0	Piceatannol- <i>O</i> -hexoside (astringin)	x		405	243, 225, 201
12	26.3	Taxifolin- <i>O</i> -hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
13	27.1	Taxifolin- <i>O</i> -hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
14	29.0	Unidentified	x		285	241, 217, 199
15	32.6	Piceatannol	x		243	225, 201, 175, 174
16	32.8	Piceatannol	x		243	225, 201, 175, 174
17	33.3	Unidentified	x		257	241, 211,
18	33.9	Quercetin- <i>O</i> -hexoside	x	x	463	301, 300, 271, 255, 179
19	34.4	Quercetin- <i>O</i> -hexoside	x	x	463	301, 300, 271, 255, 179
20	35.4	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
21	36.6	Quercetin- <i>O</i> -pentoside		x	433	301, 300, 271, 255, 243, 179
22	36.8	Unidentified	x		373	358, 313, 305
23	37.0	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
24	37.2	Kaempferol- <i>O</i> -rutinoside		x	593	447, 285, 284, 255, 227
25	37.7	Kaempferol- <i>O</i> -hexoside	x	x	447	285, 284, 255, 227
26	38.2	Unidentified- <i>O</i> -hexoside		x	431	268, 269
27	38.6	Isorhamnetin- <i>O</i> -hexoside	x		477	315, 314, 300, 299, 271
28	38.9	Isorhamnetin- <i>O</i> -hexoside	x		477	315, 314, 300, 299, 271
29	39.2	Kaempferol- <i>O</i> -pentoside		x	417	285, 284, 255, 227
30	39.8	Kaempferol- <i>O</i> -pentoside		x	417	285, 284, 255, 227
31	40.4	Kaempferol- <i>O</i> -pentoside		x	417	285, 284, 255, 227
32	40.5	Unidentified- <i>O</i> -hexoside	x	x	447	315, 285, 217, 199
33	41.6	Kaempferol- <i>O</i> -rhamnoside		x	431	285, 284, 255, 277
34	42.2	Kaempferol-acetyl-hexoside		x	489	429, 285, 284, 255, 227
35	43.6	Unidentified	x	x	351	333, 315, 275, 251
36	43.9	Unidentified	x		291	245, 175
37	47.0	Kaempferol- <i>O</i> -rutinoside	x	x	593	447, 285, 284, 255, 227
38	49.8	Unidentified	x	x	351	333, 315, 275, 251
39	50.0	Unidentified	x		367	349, 321, 247
40	51.7	Unidentified	x		377	331
41	52.0	Unidentified	x		331	313, 273, 241, 185
42	52.6	Unidentified	x		349	331, 287, 251, 244, 207, 189, 163
43	52.8	Unidentified	x		405	375, 337, 327, 275
44	53.7	Unidentified	x		401	333, 315, 257
45	54.4	Unidentified	x		521	179, 162, 146, 135
46	54.7	Kaempferol derivative		x	635	285, 284
47	55.1	Coumaric acid derivative	x		445	427, 397, 349, 277, 251, 163, 145, 119
48	55.8	Coumaric acid derivative	x		475	457, 427, 281, 163, 145, 119
49	56.4	Coumaric acid derivative	x		505	487, 457, 311, 163, 145, 119
50	57.4	Kaempferol-rhamn.-hex.-rhamn.	x	x	739	593, 453, 285, 284, 255, 229

Table 3 cont. Tentative chromatographic/mass spectrometric identification of the polyphenols in the green cones of Norway spruce (S) and eastern hemlock (H)

Peak	$t_r$ (min)	Compound	S	H	[M-H] <sup>-</sup> $m/z$	MS/MS $m/z$
51	58.0	Coumaric acid derivative	x	x	505	491, 477, 342, 327, 312, 177, 163, 119
52	58.8	Unidentified	x		535	520, 491, 341, 326, 193, 179, 134
53	59.7	Unidentified	x	x	445	417, 399, 315
54	60.7	Unidentified	x	x	401	333, 315, 289, 245
55	61.1	Coumaric acid derivative		x	549	489, 353, 311, 163, 145, 119
56	61.2	Unidentified	x		349	331, 289, 245
57	62.1	Unidentified	x	x	399	367, 331, 299
58	63.4	Unidentified	x	x	385	317, 299, 253
59	64.0	Coumaric acid derivative	x		667	521, 403, 323, 163, 145, 119
60	64.6	Coumaric acid derivative		x	653	638, 507, 489, 353, 329, 177, 163, 145, 119
61	66.0	Unidentified	x		383	355, 315, 297
62	66.6	Unidentified	x		383	315, 299, 269
63	67.4	Unidentified	x		471	425, 403, 353, 325, 285
64	68.0	Unidentified	x	x	381	313, 269
65	68.9	Coumaric acid derivative		x	651	487, 472, 341, 326, 266, 163, 145, 119
66	69.4	Coumaric acid derivative	x		649	441, 426, 411, 321, 291, 253, 163, 145, 119
67	77.0	Unidentified	x	x	429	381, 299, 265
68	80.4	Unidentified	x	x	687	657, 301
69	80.7	Unidentified	x	x	397	301
70	80.9	Unidentified	x	x	431	401, 383, 301
71	81.2	Unidentified		x	469	425, 410, 384, 367, 339, 285
72	81.7	Unidentified	x		455	409, 391, 387, 355, 287
73	82.1	Unidentified		x	957	467, 423, 381
74	82.2	Unidentified	x		455	409, 391, 387, 355, 287
75	82.4	Unidentified		x	935	467, 424, 382, 265
76	82.6	Unidentified	x	x	721	417, 335, 317
77	82.9	Unidentified		x	467	449, 423, 408, 382, 338
78	83.1	Unidentified	x	x	633	333, 317, 315, 299
79	86.1	Unidentified		x	635	591, 333, 317, 301, 271
80	89.9	Unidentified		x	769	725, 467, 301
81	94.8	Unidentified		x	501	486
82	96.7	Unidentified		x	529	514

rhamn.: rhamnose; hex.: hexose

#### 4 CONCLUSIONS

The present study compared and evaluated the antioxidant capacity of the cone extracts of 16 selected coniferous taxa. The overall antioxidant power was determined by a scoring system that combined the results of the three antioxidant assays used in the study. The best antioxidant properties were determined for green cones, followed by mature and opened cones for each taxon. The highest scores were found for *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* and *Picea abies*, which contained high amounts of antioxidants in both green and mature cones. The high-performance liquid chromatographic/tandem mass spectrometric profiling of the green cone extractives of *Picea abies* and *Tsuga canadensis* was carried out and overall 82 compounds have been tentatively identified from these samples for the first time, including kaempferol-, taxifolin-, quercetin- and isorhamnetin-*O*-glycosides, coumaric acid derivatives, chlorogenic acids,

piceatannol and its conjugates, and flavan-3-ol compounds. Presented chromatographic/mass spectrometric data on the polyphenolic composition of the green cone extracts contributes to the determination of the structure of unidentified compounds and to the research on the role of extractives in determining the bioactivity of cone extracts. To enhance practical use of this study's results, future research will focus on the antibacterial and antifungal properties of the investigated cone extracts with the highest antioxidant capacity.

**Acknowledgements:** This article was made in frame of the "EFOP-3.4.3-16-00022 'QUALITAS' Development of Higher Education in Sopron, Szombathely, and Tata" and was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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## Effect of Non-polar Extractable Substances on Soils and on Vegetation Cover from old Environmental Burdens

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**Abstract** – This case study focuses on the assessment of the effect of soil pollution by gudrons disposed in landfills. Waste products are acid tars, called "gudron" in the Slovakian terminology. Gudrons are waste products resulting from sulphonation technologies used in oil processing. In the Slovak Republic, gudron landfills are risk localities and are classified as old environmental burdens. Non-polar extractable substances (NES) as well as the activity of soil cellulase and basal soil respiration in soil samples taken from four different distances from the pollution sources were analysed. The effect of landfills on vegetation was assessed by recording the number and cover of plants on the sampling points. Long-term and gradual gudron contamination of the surrounding areas from both landfills is evident and has been proven by monitored NES concentrations. The pollution progress was predicted by the use of logistical function (based on the NES indicator) due to the increasing distance from the sources of pollution. Comparison of these two areas showed markedly higher oil substances pollution in the soil samples taken from the surroundings of the landfill Predajna 2. Determined content of NES did not meet the criteria of permissible concentration in soil samples, not even at a distance of 150 m ( $< 0.1 \text{ mg kg}^{-1}$  in compliance with the Law No. 220/2004 Coll.). When determining basal soil respiration, the production of  $\text{CO}_2$  corresponded with oil pollution determined by the NES indicator. High concentrations of NES hinder enzymatic cellulase activity. The decomposition of cellulose occurs only at lower concentrations of NES. It is possible to range the soils of lower NES concentrations (soils taken from the distances of 70 m and 150 m from Predajna 1; 110 m and 150 m from Predajna 2) among the soils with weak or middle soil cellulase activity. This indicates that microbial activity was detected in the soil samples, and the values of this microbial activity were higher due to a decrease of inhibitors caused by oil pollution. That total surface vegetation cover increases as distance from the landfills increases indicated the validity of these facts.

**soil cellulase activity / basal soil respiration / non-polar extractable substances / residues from oil processing / oil pollution**

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**Kivonat – Felhagyott hulladéklerakók környezeti terhelésének vizsgálata az apoláris kioldható anyagok talajra és a növényzetre gyakorolt hatásán keresztül.** Kutatásunk során a hulladéklerakókban elhelyezett gudron okozta talaj szennyezés hatásait vizsgáltuk. A gudron a pakura vákuumdesztillációját követően visszamaradó olajipari melléktermék. A Szlovák Köztársaságban a gudron lerakók régóta fennálló környezetterhelési kockázatbesorolást kaptak. A vizsgálatban a szennyezés forrásától négy különböző távolságból származó talajminták apoláris kioldható anyag (NES) tartalmát határoztuk meg, a talaj celluláz aktivitása és a talajlégzés mellett. A lerakók növényzetre gyakorolt hatásának vizsgálata a környező területek fitocönológiai felmérésével történt. A vizsgálatba bevont mindkét lerakó hosszútávú folyamatos szennyezést okozott, amit a NES monitorozás igazolt. A szennyezés terjedésének mértékét a NES koncentrációk változásával lehetett nyomonkövetni. Eredményeink alapján elmondható, hogy a két vizsgálati hely közül a Predajna 2 esetében a talajban jóval kiterjedtebb olajszennyezés volt megfigyelhető. A mintákban mért NES koncentrációk még a legtávolabbi minták esetében is jelentősen meghaladták a jogszabályban megengedett határértéket ( $< 0,1 \text{ mg kg}^{-1}$ ). A talajlégzés vizsgálata során a termelődő szén-dioxid mennyisége összefüggést mutatott a NES által jelzett olajszennyezéssel. A magas koncentrációban lévő NES esetén az enzimatisz celluláz aktivitás gátlása volt megfigyelhető. A cellulóz enzimatisz lebontása csak alacsony NES koncentrációk esetén lehetséges, így csak a lerakótól legtávolabbi mintákban volt mérhető gyenge, illetve közepes aktivitás. Ezekben a mintákban mért mikrobiális aktivitás az olajszennyezés okozta gátló hatás kisebb mértékével indokolható. A talajban mért szennyezés mértékét a növényborítottsági adatok is visszaigazolták.

**talaj celluláz aktivitás / talaj alaplégzése / nem poláros kivonható anyagok / olajfeldolgozásból származó maradékok / olajszennyezés**

## 1 INTRODUCTION

Pollution by oil substances originating from anthropogenic activity has been an urgent and long-term global environmental problem. Large oil and oil product leakages into the components of the environment, especially water and soil (Wolińska et al. 2016), occur every year. Oil substances enter the environment in a variety of ways, e.g. leakages from oil wells, pipes, underground containers, and incorrect oil waste disposal (Kimes et al. 2014). Landfilling the residues from oil processing (gudrons) is an example of incorrect oil waste disposal. The waste – gudron (acid tars) – is produced during the refining of oil fractions with sulphuric acid. Gudron contains sulphuric acid as well as unwanted components removed from refined oil. In general, the composition of these residues depends on the composition of the oil (Speight 2006). In compliance with the valid legislation of the Slovak Republic (Regulation No. 365/2015 Coll.) gudrons are considered hazardous waste. They are dense, highly viscous compounds possessing an acrid, acidic smell. Gudrons are highly mobile and release sulphuric acid continuously (Tumanovsky 2004, Kolmakov 2006, Kreníková 2014). Gudrons are a persistent and unstable waste, typical for their toxicity, mutagenicity, teratogenicity, and carcinogenicity (Paluchova 2009, Masarovičová, 2013, Milne 2016). Gudron landfills are a threat to all parts of the environment. They pollute the air through the emissions they release during the summer months, pollute underground and surface water via leakages into surrounding areas, and also degrade the soil and contaminate the ore environment of the area in which they are present (Kreníková 2014). Soil health is not only important for people, but also for fauna and flora. Due to its sorptive and retentive properties, soil is a natural filter against pollutants circulating in the environment (Wyszkowski – Ziółkowska 2008). Contamination of the natural environment by oil substances contributes greatly to soil degradation. Though point sources contribute to contamination, it is the non-point sources of contamination that lead to the creation of integrated underground areas contaminated by these substances (Wyszkowski – Ziółkowska 2008). The environmental effect of oil substances on soil processes is the most visible in the activity changes of soil

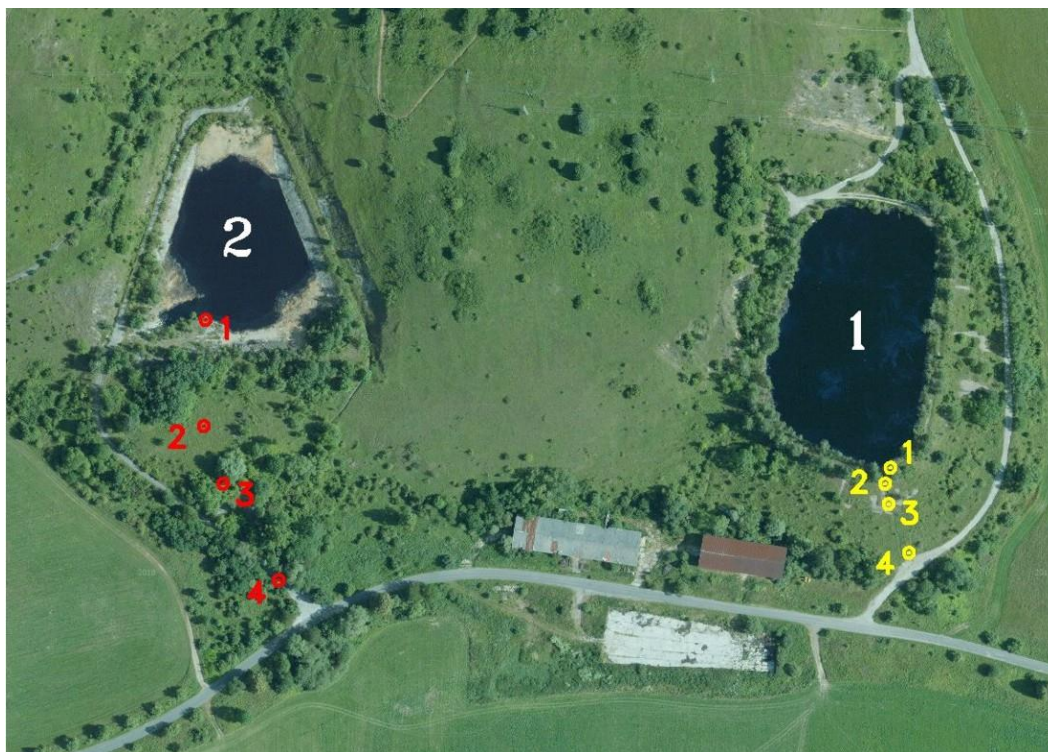


microorganisms and enzymes (Li et al. 2007, Niemeyer et al. 2012), i.e. soil microbial activity can be considered a sensitive biological and biochemical indicator of soil quality (Margesin et al. 2000, Kaczyńska et al., 2015). Soil microorganisms are key elements in organic decomposition and mineralisation processes. Due to their quick reaction to changes and adaptation to the environmental conditions, soil microorganisms are often used as qualitative soil indicators. This is important for the preservation of favourable forest health. Soil microbiota is able to react quickly to stress in the environment (Nielsen – Winding 2002, Nannipieri et al. 2003, Fodor – Pájer 2017). Soil respiration (release of CO<sub>2</sub> by soil) and soil activity of cellulase belong to frequently monitored biological soil characteristics (Gömöryová – Fekiačová 2013). Šimek and Šantučková (2002) define soil respiration and cellulose decomposition as one of the basic microbiological characteristics that determine soil quality and health. Carbon dioxide, released by respiration from soil, is a final product of the microbial metabolism of organic remains (with the exception of decomposed organic matter). Basal soil respiration, measured as total release of CO<sub>2</sub>, is an indicator of overall microbial activity in soil because the vast majority of prokaryotic and eukaryotic soil microorganisms obtain energy by oxidation from carbonaceous compounds. Basal soil respiration is also considered a rate of decomposition of mineral soil organic substances (Knoepp et al. 2000, Gömöryová et al 2013). Basal respiration is a main attribute related to fertility (Niemeyer et al. 2012) and a common indicator of soil quality (International Organisation for Standardisation 2002). Cellulose in the soil is commonly degraded by the cellulase enzyme, which is produced by microorganisms, usually by bacteria and fungi (Magnelli – Forchiassin, 1999). Cellulose is the most abundant organic compound in the biosphere, which contains nearly half of the biomass synthesised by the photosynthetic fixation of CO<sub>2</sub> (Eivazi – Tabatabai 1990, Eriksson et al. 1990, Tomme et al. 1995). Cellulose in soil originates mainly from the remains of plant matter, though fungi and bacteria in the soil also contribute limited amounts. The growth and survival of microorganisms depend on the source of carbon contained in the soil cellulose (Deng – Tabatabai 1994). To release carbon as an energy source, cellulose enzymes must degrade cellulose from plants to high-molecular oligosaccharides, cellobiose, and glucose. Within the process of systematic identification of environmental burdens, the following landfills were ranked among the environmental burdens of the Slovak Republic. Petrochema Dubová, a. s. as a refinery and petrochemical company processed oil using sulphonation and absorption technologies. Final products were made from obtained fractions, e.g. lubricating and special oils, detergents for laundry agents, and special white oils used in medicine and cosmetics. In the past, gudrons were placed in the natural environment and this led to the creation of two gudron landfills: Predajna 1 and Predajna 2 (Oravec 2014). This case study deals with the impact hazardous industrial waste – acid gudrons – from the Predajna 1 and Predajna 2 landfills have had and continue to have on soil and flora.

## 2 MATERIALS AND METHODS

This case study aims to assess the soil quality of areas contaminated by oil substances and evaluate soil basal respiration (CO<sub>2</sub>), soil cellulase activity, analytical determination of non-polar extractable substances (NES), and vegetation conditions. The experiment took place in the monitored areas over two years (2018 – 2019). The monitoring was carried out in two research areas: Predajna 1 and Predajna 2 (the Slovak Republic, the region of Banská Bystrica), in two transects and four sampling points at distances of 0.5 m, 50 m, 70 m, 150 m from Predajna 1 (following the spread of pollution), and at distances of 1 m, 75 m, 110 m, 150 m from Predajna 2 (following the spread of pollution). GPS coordinates of the sampling points: Predajna 1 – 1. N48°49.197 E19°29.018, 2. N48°49.192 E19°29.016, 3. N48°49.186

E19°29.018, 4. N48°49.172 E19°29.029 and Predajna 2 – 1. N48°49.227 E19°28.702, 2. N48°49.195 E19°28.704, 3. N48°49.178 E19°28.715, 4. N48°49.151 E19°28.743 – Garmin GPS map 62sc. The distances for Predajna 1 and Predajna 2 are not identical due to the different configurations and accessibilities of the sampling points (*Figure 1*).



*Figure 1. Map of the locality and the sampling points*  
 1 – Predajna 1; 1 - 0.5 m, 2 - 50 m, 3 - 70 m, 4 - 150 m  
 2 – Predajna 2; 1 - 1 m, 2 - 75 m, 3 - 110 m, 4 - 150 m

## 2.1 Sampling

Five soil samples were taken five times a year during the vegetation periods in 2018 and 2019. A manual sounding stick was used to take the samples from the depth of 15-20 cm from each of mentioned areas (Regulation of the Ministry of Environment of the Slovak Republic No.1/2015 on uniform methods of analytical waste examination), without any effect of outdoor conditions (no rainfall total and average temperature of 20°C). Sampling was completed in compliance with STN ISO 10381-6, and the samples were treated by quartering to obtain a representative sample. In order to preserve natural soil character, the soil samples for basal soil respiration and soil cellulase activity measurements were not sifted.

## 2.2 Determination of basal soil respiration

Respiration or basal soil respiration by the Isermeyer method was determined by the amount of released CO<sub>2</sub> produced during an incubation period of 24 hours with the consequent titration with standardised volumetric solution of hydrochloric acid ( $c = 0.05 \text{ mol L}^{-1}$ ), using phenolphthalein as an indicator. Data are expressed as  $\mu\text{g CO}_2 \text{ g}^{-1}$  of dry soil (Alef 1991, Kizilkaya et al. 2004).

## 2.3 Determination of soil cellulase activity

The determination was completed according to Islam (1998), i.e. the principle is incubation of sterile cellulose in a Petri dish with a soil sample. After 30 days, quantitative decrease

was evaluated (in %) from the surface of cellulase using IMAGE J software (freely available on the Internet).

## 2.4 Determination of oil substances

Oil substances in soil were determined as non-polar extractable substances (NES) using spectrophotometer in an infrared area by the extraction with organic diluent (S-316) (Ladomerský 2001).

## 2.5 Vegetation research

Vegetation research was conducted in the form of phytocenological records in compliance with the Zürich-Montpellier School principles in the surroundings of gudron landfills in the vegetation periods of 2018 and 2019. Qualitative and quantitative characteristics were monitored in phytocenological relevés. Qualitative characteristics of plant community represent a set of all species that occurred in the studied area in the given period. The estimation of cover was assessed from quantitative characteristics, i.e. percentage estimation of the area covered by certain species. Places of phytocenological relevés are identical with the sampling points (Braun-Blanquet 1964, Moravec 1994).

## 2.6 Analysis of results

Using the program STATISTICA 12, ANOVA with interactions, confidence intervals of 95% and the program MatLab 2019b.

# 3. RESULTS AND DISCUSSION

This case study focuses on soil pollution in the surroundings of two gudron landfills, Predajna 1 and Predajna 2, which are environmental burdens in Slovakia. During exploratory works, 172,558 mg kg<sup>-1</sup> of NES was determined in gudron waste (Auxt 2018). Predajna 1 was built with a protecting dike in 1964 and was in operation until 1974. Predajna 2 was used for the deposition of gudrons from 1974 to 1983. The bedrock at the landfills consists of limestone, dolomites and rocks of melafire sequence. The bottoms of the landfills are not sealed, which led to a 60,000 ton gudron leak into the bedrock in 1982 (Ollerová 2004, Michaeli 2010).

## 3.1 Assessment of oil pollution

To assess soil samples in the surroundings of Predajna 1 and Predajna 2, the distances for sampling were measured from 0.5 to 150 m from the landfills. Monitoring of oil pollution spreading from the landfills revealed no significant differences between the NES values in the range from 0.5 to 150 m from the landfills in the years 2018 and 2019. Therefore, a stabilised situation can be assumed. Oil substance contamination decreases as distance from the landfill increases. Comparison of these two areas showed markedly higher oil substances pollution in the soil samples taken from the surroundings of Predajna 2 (*Table 3*). We presume that the determined high concentrations of NES are affected by the approximately 60,000 ton leak of gudrons into the bedrock in 1982 (Michaeli 2010). Precipitation amounts have also affected the long-term, gradual gudron contamination of the surrounding area from both landfills. Since both landfills are exposed, they are continuously replenished with rainfall, thereby increasing the environmental risk (Masarovičová 2013).

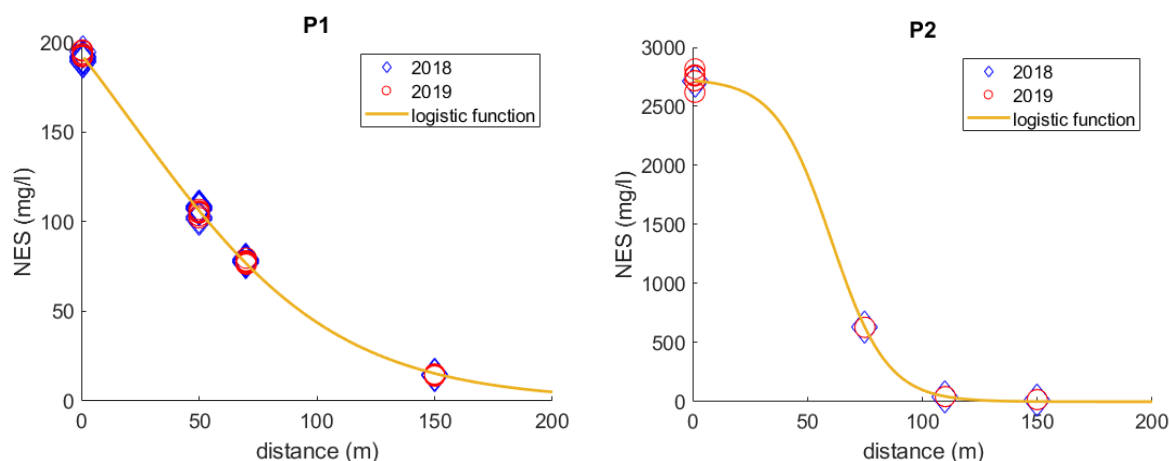


Figure 2. Estimation of the course of pollution depending on the distance  
(Note: P1 – Predajna 1; P2 – Predajna 2)

In order to model data, the most suitable logistic function was:

$$y(x) = \frac{k_1}{1 + e^{k_2 x + k_3}} \quad (1)$$

Table 1 and Table 2 contain the results of non-linear regression calculated with the MatLab 2019b program. All coefficients in the Tables are statistically significant.

Non-linear regression model:  $y \sim k_1/(1 + \exp(k_2 \cdot x + k_3))$  – Predajna 1

Table 1. Estimated coefficients-non-linear regression parameters Predajna 1

	Estimate	SE	tStat	pValue
K1	2736	8.8003	310.9	$2.5 \cdot 10^{-99}$
K2	0.0827	0.0040	-20.34	$7.0 \cdot 10^{-29}$
K3	-4.99	0.3090	16.15	$1.0 \cdot 10^{-23}$

Number of observations: 64, Error degrees of freedom: 61, Root Mean Squared Error: 24.8  
R-Squared: 1, Adjusted R-Squared 1; F-statistic vs. zero model:  $6.75 \cdot 10^4$ , p-value =  $2.58 \cdot 10^{-107}$

Non-linear regression model:  $y \sim k_1/(1 + \exp(k_2 \cdot x + k_3))$  – Predajna 2

Table 2. Estimated coefficients – non-linear regression parameters Predajna 2

	Estimate	SE	tStat	pValue
K1	312.16	6.6699	46.8	$1.6 \cdot 10^{-49}$
K2	0.02297	0.0004	-59.5	$9.9 \cdot 10^{-56}$
K3	-0.49	0.0543	8.98	$9.2 \cdot 10^{-13}$

Number of observations: 64, Error degrees of freedom: 61, Root Mean Squared Error: 1.75  
R-Squared: 0.999, Adjusted R-Squared 0.999; F-statistic vs. zero model:  $9.49 \cdot 10^4$ , p-value =  $7.69 \cdot 10^{-112}$

When assessing the results, we focused on the evaluation of the course of pollution according to distance. The logistic function predicts the course of pollution (based on the NES indicator) to increasing distance from the pollution source (Predajna 1 and Predajna 2).

Table 1 - 2 and Figure 2 show that the NES content in the soil samples taken at a distance of 1 m from Predajna 2 was 14 times higher than it was in the soil 0.5 m from Predajna 1. Only the 150m distance from both landfills showed no significant difference between the determined values of NES for both areas. Higher content of NES was detected in analysed

soil from the surroundings of Predajna 2 at the distances of 1 m and 75 m. However, the spread of oil pollution at the distances of 110 m and 150 m decreased to nearly comparable levels of pollution in the sampling points of P1 (Note: P1- Predajna 1; P2 – Predajna 2). Determined content of NES did not meet the criteria of permissible concentration in soil samples, not even at the 150 m distance ( $< 0.1 \text{ mg kg}^{-1}$  in compliance with the Law No. 220/2004 Coll.).

Table 3. Characteristics of non-polar extractable substances ( $\text{mg kg}^{-1}$ )

	Predajna 1			Predajna 2		
	Distance (m)	Average	St. Error	Distance (m)	Average	St. Error
2018	0.5	191.63	0.20	1	2715.31	0.30
2018	50	105.68	1.41	75	629.53	0.28
2018	70	78.07	0.18	110	43.12	0.07
2018	150	14.75	0.07	150	17.57	0.19
2019	0.5	193.77	0.74	1	2716.69	0.50
2019	50	105.00	0.54	75	629.47	0.14
2019	70	77.94	0.20	110	43.36	0.40
2019	150	14.66	0.15	150	17.97	0.13

### 3.2 Assessment of basal soil respiration

Oil pollution degrades physical and chemical soil characteristics. Greasy film on the soil surface limits air circulation between the soil and the atmosphere. Soil particles coated by oil hinder  $\text{CO}_2$  from leaving the soil for the air. Oil pollution also degrades the biological properties of soil (Frankovská 2010, Samešová 2011). Changes in soil caused by oil pollution were also monitored on the basis of determination of basal soil respiration. In the analysed samples (Figure 3, Table 4) the content of determined  $\text{CO}_2$  corresponds with determined NES values (Table 3): as presented by Hybská et al. (2013), basal soil respiration grows with decreasing NES content. In their scientific study, Polyak et al. (2018) dealt with the monitoring of basal soil respiration in soil burdened by oil pollution. They confirmed that  $\text{CO}_2$  production corresponds with the course of degradation of oil pollution controlled by the determination of petroleum hydrocarbons. Ali et al. (2020) found that the rate of  $\text{CO}_2$  development in the soil burdened with oil pollution ranged from  $30.6$  to  $55.0 \text{ } \mu\text{g CO}_2 \text{ g}^{-1} \text{ day}^{-1}$ .

Table 4. Statistical characteristics of basal soil respiration ( $\mu\text{g CO}_2 \text{ g}^{-1} \text{ day}^{-1}$ ) results in the monitored locality

Year	Predajna 1					Predajna 2				
	Distance (m)	Average	St. Error	Confidence intervals		Distance (m)	Average	St. Error	Confidence intervals	
				-95%	95%				-95%	95%
2018	0.5	11.98	0.09	11.70	12.25	1	1.16	0.01	1.11	1.21
2018	50	16.06	0.05	15.88	16.23	75	7.11	0.02	7.06	7.17
2018	70	23.08	0.20	22.43	23.72	110	35.69	0.12	35.31	36.06
2018	150	43.43	0.18	42.86	44.00	150	40.24	0.18	39.65	40.83
2019	0.5	12.23	0.14	11.76	12.69	1	1.22	0.04	1.11	1.34
2019	50	16.15	0.13	15.73	16.56	75	7.11	0.01	7.07	7.15
2019	70	23.91	0.52	22.25	25.56	110	35.71	0.08	35.47	35.95
2019	150	43.25	0.24	42.48	44.01	150	40.56	0.14	40.12	40.99

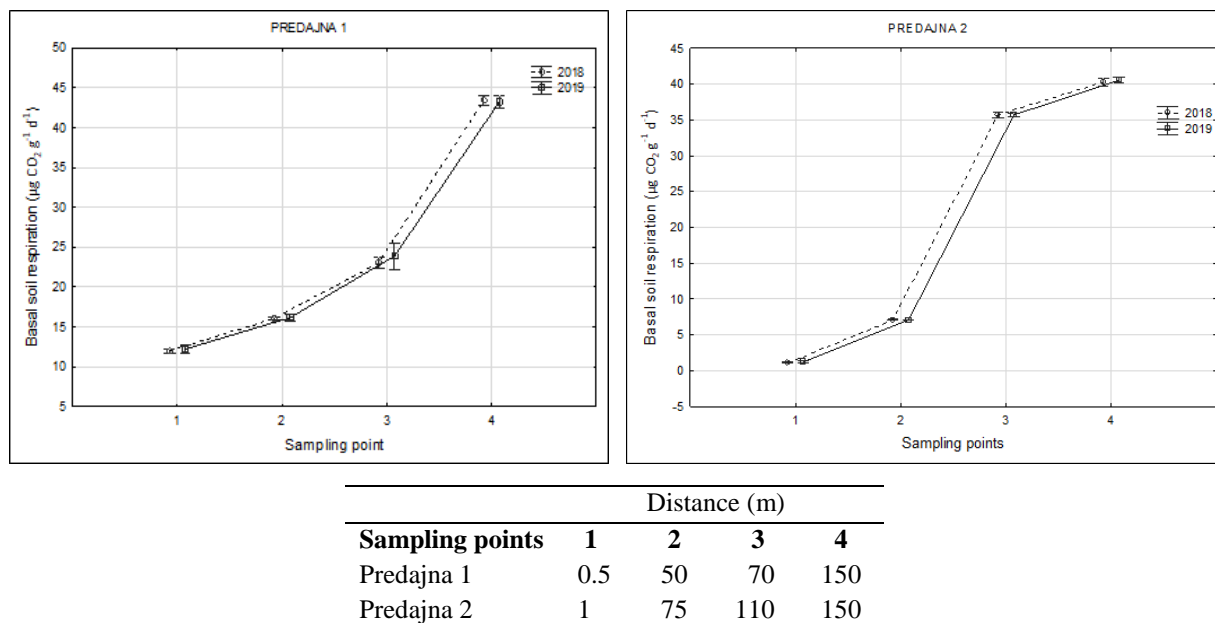


Figure 3. Graphical representation of basal soil respiration results

### 3.3 Assessment of soil cellulase activity

Polysaccharide cellulose is a basic component of plant tissue cell walls and is also the most common organic compound in the biosphere. After plants die, cellulose is decomposed by the enzymes that belong to the group of cellulases. Anthropogenic effects of oil pollution were assessed by the determination of the rate of soil cellulase activity. Cellulose was not decomposed in the most polluted soil samples (*Table 5*). Enzymatic cellulase activity was detected only at the lowest NES concentrations. According to Rejšek (1999), there is no cellulolytic enzyme activity in soils with the highest NES concentrations. It is possible to range the soils of lower NES concentrations (soils taken from the distances of 70 m and 150 m from Predajna 1; 110 m and 150 m from Predajna 2) among the soils with weak or middle activity of soil cellulase (*Figure 4, Table 5*). This means that microbial activity was detected in the soil samples and its values were higher due to the oil pollution-caused decrease of inhibitors. The results of this case study correspond with Hybská et al. (2013), where the percentage rate of the soil cellulase activity in the soil contaminated by oil was 2.12%. In soil contaminated by synthetic oil (completely or very well degradable oil), this figure was 8.72% (Fargašova 2009) while it was 15.95% in soil without oil pollution. For the comparison, the results of Javorekova et al. (2006) can be mentioned: the cellulose decomposition was detected at the depth of 0.1 m in agricultural soil (44% in brown earth and 35.15% in black earth). Compared with our results, obtained under the same conditions as mentioned in Javorekova (2006), high NES concentrations inhibit the enzymatic activity in such burdened soil (*Table 3*).



Table 5. Statistical characteristics of cellulase activity results in the monitored locality

Year	Predajna 1					Predajna 2				
	Distance	Average	St.	Confidence intervals		Distance	Average	St.	Confidence intervals	
	(m)	(%)	Error	-95%	95%	(m)	(%)	Error	-95%	95%
2018	0.5	0.00				1	0.00			
2018	50	0.02	0.01	0.00	0.05	75	0.00			
2018	70	0.35	0.02	0.30	0.39	110	2.92	0.03	2.81	3.03
2018	150	0.90	0.00	0.89	0.91	150	1.83	0.45	0.39	3.26
2019	0.5	0.00				1	0.00			
2019	50	0.02	0.00	0.01	0.03	75	0.00			
2019	70	0.36	0.01	0.31	0.40	110	2.45	0.49	0.91	4.00
2019	150	1.04	0.01	1.01	1.06	150	1.45	0.02	1.40	1.50

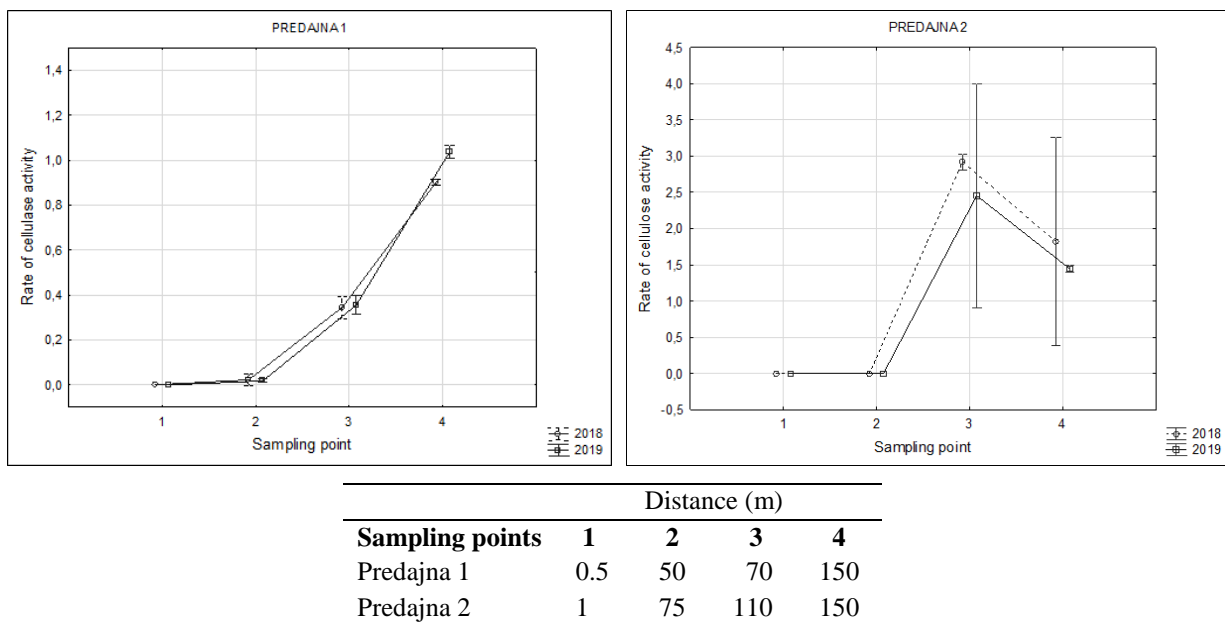


Figure 4. Graphical representation of cellulase activity results

### 3.4 Assessment of vegetation conditions

Several factors affect basal soil respiration and soil cellulase activity. Based on phytocenological records from 2018 and 2019, it is possible to state that the surface cover percentage increases with the increasing distance from the landfill border. Surface vegetation cover increased from 30% to 90% at Predajna 1 in 2018 and from 40% to 100% in 2019. At Predajna 2, the cover increased from 50% to 100% in both years of monitoring (Table 6). The number of species at Predajna 1 stayed identical or increased slightly with distance. In 2019, the number of species increased to 20 in the area closest to the landfill (in comparison with 8 in 2018). However, the cover only increased by 10%. No vegetation was detected in the area without any determined cover. A trench from the landfill border over the dike to the root of the dike was created there. It is possible that gudron waste was flowing away in that direction when landfill levels were high. This can be related to water erosion and excessive precipitation. A doubling of the amount of species does not automatically indicate a larger creation of biomass and higher cover. At Predajna 2, the higher number of species due to distance was not detected. However, total cover grew from 50% to 100%. Cellulase activity increased with distance from the landfill, as well as total vegetation cover of the area. Higher

cover leads to the higher the production (creation) of surface and underground biomass. Consequently, the amount of waste and dead and decayed biomass rises as well.

The occurrence of the woody plants *Betula pendula*, *Salix caprea*, and *Populus tremula* was detected near the landfills. Herbs *Calamagrostis epigejos* and *Dactylis glomerata* achieve cover ranging from 25% to 50%. *Arrhenatherum elatius* and *Rubus caesius* cover is from 50% to 75%. With the exception of *Arrhenatherum elatius*, Pyšek (1981) and Hartman (1980) consider these herbs to be oil pollution tolerant. The occurrence of *Calamagrostis epigejos* was observed in the areas closest to the landfills where the highest NES content was also detected. According to the above-mentioned authors, *Arrhenatherum elatius* belongs to a group of species that are sensitive to oil pollution. This species did not occur in the areas where the highest NES concentrations were detected.

Table 6. Characteristics of vegetation in the monitored locality

Year	Predajna 1			Predajna 2		
	Distance (m)	Number of species	Total cover (%)	Distance (m)	Number of species	Total cover (%)
2018	0.5	8	30	1	12	50
2018	50	10	70	75	19	90
2018	70	0	0	110	20	80
2018	150	9	90	150	14	100
2019	0.5	20	40	1	11	50
2019	50	13	80	75	22	100
2019	70	0	0	110	19	70
2019	150	13	100	150	13	100

#### 4. CONCLUSIONS

Based on the obtained results of this case study, we can conclude that gudron waste is an environmental burden for soil. This was proved by the presence of non-polar extractable substances (NES) in soil samples at different distances from the landfills. Contamination decreased in accordance with increasing distance. The assessment of the course of oil pollution based on the NES indicator according to distance is predicted by a logistic function. Cellulose was not decomposed in soil that was closest to gudron landfills. Soil activity increased as distance from the landfills increased. Higher microbial activity was detected in the samples where inhibitors were lowered due to the pollution. Basal respiration highlights the ability of microorganisms to use available substrate, especially organic matter. Basal respiration determination is significant in soils affected by different negative factors, e.g. negative anthropization (in this case oil pollution). CO<sub>2</sub> production rose as distance from the landfills increased. This corresponds with the course of degradation of oil pollution controlled by the determination of non-polar extractable substances. Based on the phytocenological records from 2018 and 2019, we conclude that percentage cover of the area increases as distance from the landfill border increases. Determination of basal soil respiration and cellulase activity were confirmed as suitable indicators for the monitoring of oil polluted soil. Based on these findings, we recommend the assessment of basal soil respiration as a suitable monitoring method to measure soil contamination by oil substances. This method is more financially and environmentally accessible than NES monitoring. Monitoring of these indicators is also important in forest ecosystems.



**Acknowledgements:** The project was supported by EFOP-3.6.2-16-2017-00018 in University of Sopron project.

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## **The Standard Output of Forest Index – an Indicator of Site Quality**

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**Abstract** – The Standard Output of Forest Index (SOFI) describes the ability of forests to produce financial value from wood production based on the standardized monetary value of the mean annual increment of the potential final harvest relative to a reference forest type. It can be applied on regions where the forests can be classified into major tree species or species groups and into site classes. The potential volume of final wood harvest is estimated through yield tables. Using the share of low-quality and high-quality wood product groups in the final harvest, and their respective standardized price, the output value of the final harvest is expressed and then divided by the rotation age. This standardized output is compared to a reference forest type identified by its tree species and site class, and multiplied by 10 points. The SOFI of the reference forest, therefore, is 10, while higher values represent higher potential output and smaller values represent smaller potential output. With the necessary modifications, the SOFI can be applied to uneven-age forests as well. It can primarily be used to describe and compare the financial output potential of larger forest areas.

**Site index / site classes / forest productivity / wood quality / SOFI / forest economics**

**Kivonat – Erdészeti termelési érték index – a termőhelyi potenciál mutatója.** Az erdészeti termelési érték index (SOFI) az erdők fatermesztésből származó pénzérték termelő képességét jellemzi a korszaki átlagnövedék standardizált pénzügyi értékének kifejezésével és egy meghatározott referencia erdőtípushoz történő viszonyításával. Olyan földrajzi régiókban alkalmazhatók, ahol az erdők főbb fajok, illetve fajcsoportok és fatermési osztályok szerinti csoportokba sorolhatók. A potenciális véghasználati fahozam fatermési táblák alapján becsülhető. Az alacsony és magas minőségű fatermékek véghasználati hozamon belüli arányára és azok standardizált pénzértékére alapozva a véghasználati kibocsátási érték kifejezhető, amelyet a vágáskorral osztunk. Ez a standardizált termelési érték viszonyítandó egy faj és fatermési osztály által meghatározott referencia erdő értékéhez, és a könnyebb megjeleníthetőség érdekében 10 ponttal szorozzuk. Ezáltal a referencia-erdő SOFI értéke 10, míg a magasabb értékek magasabb potenciális termelési értéket, az alacsonyabb értékek alacsonyabb potenciális termelési értéket jelentenek. Megfelelő módosításokkal a SOFI többkorú erdőkre is alkalmazható. Elsősorban nagyterületű erdők pénzügyi kibocsátási potenciáljának leírására és összehasonlítására használható. Elsősorban nagyobb erdőterületek potenciális kibocsátásának jellemzésére alkalmazható.

**termőhelyi index / fatermési osztály / termőhely jósága / termőképesség / fa minősége / SOFI / erdészeti ökonómia**

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## 1 INTRODUCTION

This paper introduces the Standard Output of Forest Index (SOFI) by presenting its definition, calculation method, and the rationale of application. The aim of the introduction of the SOFI is to provide an index which can represent the extent to which a forest site is suitable for wood production. The index measures both the quantity and quality of a site relative to other sites by providing a financial basis comparison over various tree species and forest types.

The ability of a forest site to produce wood is generally understood as its site quality. Better site quality results in higher volume of wood in a given period of time, which can be expressed through standing volume, total wood production, or other features. Site index is commonly used to describe site quality, which is the height of the dominant trees at a reference age. The assumption that height growth strongly correlates with the increment of the growing stock has been used since as early as the end of 19<sup>th</sup> century (Baur 1881) to describe forest growth and to predict future wood production potential.

If the height-to-age relationship of a tree species or forest type is known, the site index can be calculated at any age on the growth curve's domain. The site index can be grouped into site classes in order to divide the height-to-age continuum into distinct categories, simplifying the description of site quality considerably. Site classes can be characterized by their growing stock, stem number, basal area, annual increment, and mean diameter as the function of age of the tree stand. Such datasets can be found in yield tables. Although the fundamental assumptions of such yield tables and their application on large regions has been challenged and proved to be inadequate under certain management regimes (Skovsgaard – Vanclay 2008), they are widely used in practice.

Site quality is the interplay of forest site properties and tree stand type. The forest site can be described by its climate (the combination of temperature, precipitation, and other factors), hydrological properties, soil (type, depth, texture, and nutrition content), while the most important tree stand characteristics are tree species composition (provenance may also be considered) and establishment method (seed or coppice, spacing). Therefore, the site index can only be used for comparison within tree species or tree species groups.

This study does not directly address possible site condition shifts. Should these occur in over the long term, their effects on future growth potential need to be considered. Detectable increase in the forest growth rate was reported in some areas (Somogyi 2008, Pretzsch 2014), while forest cover extinction is projected at the xeric limits of forests in other areas (Mátyás et al. 2014). Climate change is one of the large-scale causes of such changes, which can be coupled with changes in hydrological conditions (Csáki et al. 2014, Moricz et al. 2016).

The advantage of the site index is that tree stand height is less influenced by forest management interventions, e.g. thinning, than by other stand characteristics, e.g. number of stems, diameter etc. However, the application of the site index is confined to situations when forests are even-aged and their age can be determined by samples or from planting records. These latter obstacles can easily be tackled in areas with long traditions of forestry and forest cultivation, as forest inventories in such areas tend to possess over 90% coverage (MacDicken et al. 2015). Other areas require alternative methods based on features other than age.

In forests of differing tree species, comparisons of wood productivity can be based upon mean annual increment (MAI) at a reference age representing the typical rotation age of the respective tree species in the described region. Increment is normally measured in volume (cubic meters), but a more accurate comparison can be achieved if the increment is expressed in dry matter weight (tons). Since the dry weight of wood strongly correlates with its carbon content (Lamolm – Savidge 2003), the dry weight of MAI is not only an indicator of wood production capacity, one that allows for cross-species comparison, but can also express the aboveground carbon sequestration capacity of forests. (Gallaun et al. 2010)

The above approaches to site quality imply that larger natural output in volume or dry matter under the same period of time contributes to higher financial output. To describe and compare the financial potential of forests as an alternative understanding of site quality entails considering the market value of wood products. Based on growth information and the forest management regime, the financial flows for a given time period can be modelled and used to calculate economic properties such as income, added value, margin or profit, etc. However, these calculations are either applied in regions of homogenous forest types under similar forest management regimes and are based on necessary simplifying assumptions (Pandey et al. 2010), or they need to gather exhaustive amounts of data and build large numbers of models. The data collection method determines the comparability of economic features and limits large scale analysis (Sekot et al. 2011, Vrolijk et al. 2016).

Site quality is a feature of a specific site, and its indicators express its production capability, which is an important component of a site's land value. However, this feature should not be treated as a single component, as it is, to a great extent, influenced by the utilization forms, forest management regimes, production risks, organizational arrangements, management objectives, etc. (Hartebrodt 2007, Posavec 2017, Beljan et al. 2018).

## 2 THE STANDARD OUTPUT OF FORESTRY INDEX

### 2.1 Aim, principles and definition

The aim of the SOFI is to provide a site quality index that considers the potential quantity and quality of wood production and allows for comparisons both within and across tree species.

*The principles of the index design:*

- As long as the representative power of the index is not seriously corrupted, a simple calculation method takes precedence over accuracy.
- Meant to be an indicator of the potential financial performance of forests, rather than an exact economic variable.
- Describes the variability of forest type and forest site combinations rather than the differences in the financial value of wood in different regions.

Definition: the Standard Output of Forest Index describes the ability of forests to produce financial value through wood production based on the standardized monetary value of the mean annual increment of the final harvest relative to a reference forest type.

### 2.2 Calculation method and data source

*Step 1: Forming forest categories*

Within a defined geographical area where the SOFI will be employed, forests shall be categorized according to major tree species, species groups, or sub-species categories (e.g. selections or provenances) for which growth and wood price data are available. Groups shall be formed with due consideration of their share in forest cover and the availability of the data specified in the next steps.

*Step 2: Determining final harvest volume and rotation age*

There is no restriction on how to determine potential final harvest volume and final harvest age, but yield tables based on site classes are the most commonly available tools. If available, silviculture models can also be a good data source. Theoretically, rotation age can be determined at the forest plot level, but this data is unlikely to be available in young forest stands. Therefore, it is best to unify rotation age on the whole geographical area of application

(or at least at the regional level) by tree species and site index classes, which represent the best financial interest of the forest owner/manager under the relevant legal framework.

*Step 3: Determining the share of high-quality and low-quality wood product groups*

The calculation of the SOFI requires the proportion of low-quality wood products be known. There is no clear distinction between low quality and high-quality wood products. Firewood and pulp and paper wood typify the former, while saw logs and veneer logs belong to the latter. In general, there are usually no quality requirements for low-quality products other than they consist of healthy solid wood, and that they are measured in stockpiles. High-quality products are inspected against quality requirements and measured piece by piece. If in doubt as to where a specific wood product belongs, price is decisive. The definition of wood products and their grouping may follow national or international standards, but it shall correspond to the wood product classification applied in wood price data sources described in Step 4. The share of the wood product groups shall represent averages of a 5-10-year period.

*Step 4: Selecting representative wood products for the high- and low-quality groups and standardizing their monetary value*

For each of the high and low-quality product groups, a representative product that has the highest total output value within the respective product group shall be selected. The value of wood products shall be expressed in monetary terms, usually those stated in controlled markets such as commodity exchanges, farm accountancy data networks, or national statistics; however, in the absence of these, individual surveys can become possible sources of price information. The data source, however, is suitable only if prices of the most important wood products can be obtained for a time period no shorter than five years. In the calculation of the SOFI, the 5–10 year average of these prices shall be used.

*Step 5: Calculating and indexing to a selected forest type*

The standard output for each forest category shall be calculated according to eq. 1. Final harvest volume is multiplied by the weighted average value of wood and divided by the rotation age. As an alternative, eq. 2 can be applied in cases when rotation age and the final harvest volume are unavailable, but the mean annual increment can be estimated.

One of the forest categories shall be designated to serve as the basis of indexing. This reference forest shall cover a relatively large area and will be selected from around the middle of the standard output spectrum. For a simpler presentation, the proportion of the standard output of the specific forest category to the reference category is multiplied by 10 points. Thus, the unit of SOFI is ‘points’.

$$\text{SOF}_x = \frac{[\text{SV}_{\text{LQ}x} \cdot \text{LQ}\%_x + \text{SV}_{\text{HQ}x} (1 - \text{LQ}\%_x)] Q_x}{R_x} \quad (1)$$

$$\text{SOF}_x = \overline{\text{SV}}_x \cdot \text{MAI}_{Rx} \quad (2)$$

$$\text{SOFI}_x = \frac{\text{SOF}_x}{\text{SOF}_{ref}} \cdot 10 \quad (3)$$

Where:

- $x$ : mark of forest categories (combinations of species groups and site classes)
- $ref$ : mark of the reference forest category
- $\text{SOF}$ : Standard Output of Forest
- $\text{SOFI}$ : Standard Output of Forest Index
- $\text{SV}_{\text{LQ}}, \text{SV}_{\text{HQ}}$ : standard value of the representative wood product in the low-quality and high-quality forest product groups
- $\text{LQ}\%$ : share of low-quality forest product group in the final harvest
- $Q$ : potential standing volume of wood at final harvest



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R:	rotation age
MAI <sub>R</sub> :	mean annual increment of the potential final harvest
$\overline{SV}$ :	weighted average value of the low- and high-quality forest product groups

#### *Step 6: Application to the forest area*

The SOFI can be applied to forest areas where species composition and site classes (or other parameters that have been used for classification at Step 1) are known. The minimum level of application corresponds to that of the natural parameters, which is, most commonly, the forest sub-compartment; however, this can be aggregated to larger areas as well.

### **3 CONSIDERATION OF DESIGN ALTERNATIVES**

The actual financial performance of a forest depends on the harvested volume, the market value of its products, and the production costs, which includes harvesting, (re-)establishment, planning, supervision, and other administrative tasks. Surveying relevant data becomes a demanding task if the actual financial performance is to be presented or used in an analysis. This is especially pertinent in larger geographical areas with varied forest stand types and forestry practices.

This is also valid if the potential economic performance needs to be taken into account. In this case, potential harvest volume can be interpreted as the allowable cut within the time horizon of the study based on the current state of the forests depending on their age, volume, stocking, health status, management objectives, conservation, and other limitations. Another option is to make estimations on the future potential based on the combinations of the site conditions and tree stand type. Going even further, if the current tree stand is, for some reason, unable to utilize the full production potential of the site due to factors like bad species in the past, then an alternative forest type with highest potential production can also be used for the calculation.

The SOFI is meant to represent the suitability of the forest area to produce financial value based on the current site and forest type combinations. It aims at considerably simplifying data collection, modelling, and calculation tasks by reducing the necessary amount of data, while providing a good approximation of the potential financial performance of a study area. This is why the most schematic method was incorporated into the calculation method from among the options described above.

Final harvest volume represents a forest's wood production capacity. Excluding the premature wood production renders the SOFI biased because the share of premature wood in total wood production varies significantly in the different tree species groups, as observed in *Table 1*.

Simply replacing final harvest volume with total wood production would also raise concerns regarding financial valuation as thinnings produce wood products of lower quality and smaller dimensions compared to final harvest products. Extending the calculations by incorporating the estimation of the monetary value of the premature harvests would require further estimations of the share of wood product groups. The dilemma of whether the approximation of the output should be based on a more detailed calculation, or the calculation should be kept the simplest possible, has been decided in two arguments. First, one of the principles of the SOFI is to avoid detailed modelling and obtain only the smallest number of absolutely necessary factors. Second, timber harvests are generally not subject to market circumstances only, but to non-economic considerations as well (Kilham et al. 2019). This is even more applicable to premature harvests because their expected profit is smaller than that of the final harvest; therefore, their contribution to the 'output potential' is questionable.

Table 1. The share of the final harvest volume in the total wood production of selected species based on their yield tables (Source of data: Béky 1981, Keserű and Rédei 2012, Rédei et al. 2019)

Tree species	Wood production category	Site classes					
		I.	II.	III.	IV.	V.	VI.
Oak	Total wood production at age 100 (m <sup>3</sup> /ha)	1056	905	762	630	509	394
	Final harvest volume at age 100 (m <sup>3</sup> /ha)	595	510	432	357	288	222
	<b>Proportion of final harvest in the total production (%)</b>	<b>56.3</b>	<b>56.4</b>	<b>56.7</b>	<b>56.7</b>	<b>56.6</b>	<b>56.3</b>
Black locust	Total wood production at age 30 (m <sup>3</sup> /ha)	463	387	316	252	195	173
	Final harvest volume at age 30 (m <sup>3</sup> /ha)	304	252	204	162	124	107
	<b>Proportion of final harvest in the total production (%)</b>	<b>65.7</b>	<b>65.1</b>	<b>64.6</b>	<b>64.3</b>	<b>63.6</b>	<b>61.8</b>
Hybrid poplar	Total wood production at age 20 (m <sup>3</sup> /ha)	412	349	298	247	201	163
	Final harvest volume at age 20 (m <sup>3</sup> /ha)	341	276	228	187	151	121
	<b>Proportion of final harvest in the total production (%)</b>	<b>82.8</b>	<b>79.1</b>	<b>76.5</b>	<b>75.7</b>	<b>75.1</b>	<b>74.2</b>

It is, therefore, left to the user to decide whether to use the original form of the SOFI or to modify it according to the available data. One alternative modification is presented in *eq.4*, which would adequately reflect the forestry practice in hardwood regions, but would not necessarily be suitable elsewhere. *Eq.4* is a modification of *eq.1* by adding the premature harvest potential at the standard value of low-quality wood products, as if 100% of the premature wood harvest belonged to the low-quality product group.

$$SOF_x = \frac{[SV_{LQx} \cdot LQ\%_x + SV_{HQx} (1 - LQ\%_x)] Q_{FHx} + SV_{LQx} \cdot Q_{PHx}}{R_x} \quad (4)$$

Where symbols are the same as at *eq.1*, except:

$Q_{FHx}$ : Volume of final harvest

$Q_{PHx}$ : Volume of premature harvest

While natural aspects such as final harvest volume represent the specific site, the economic layer (distribution of wood products and prices) is standardized. This is justified by the circumstance that the primary aim of SOFI is to represent forest capability. Thus, the effects of the regional differences in the economy (i.e. the wood industry and other related sectors) and the customs of direct household consumption should be excluded. This is the reason for calculating with mid-term or long-term average wood product distribution and prices. These factors are unified for the whole study area.

Although forests can produce a large variety of products and services, wood products remain one of the most important sources of income (Sisak et al. 2016). Among various factors, the actual wood product distribution of a specific final harvest depends on tree quality, tree dimensions, market demand, and forest manager preferences (e.g. own consumption needs). To tackle this problem, wood products are grouped into low-quality and high-quality groups, thereby eliminating the effects of minor changes. Calculating with the average product distribution over a 5-10-year period levels short-term volatility.

One might notice that the shares of low-quality and high-quality wood products are applied to the gross final wood harvest even though their shares refer to the net wood harvest. This can be seen as an inconsistency; however, the SOFI is meant to be an indicator. The

conversion of gross wood harvest to net wood harvest would be necessary only if the conversion rate of the forest types and site classes were significantly different. This type of modification is possible depending on the purpose and area of the application.

Selecting the market price source of the representative wood products requires careful consideration. It is of paramount importance to avoid the effects of short-term price volatility that would distort the comparability of forest types. Wood products tend to show constant prices relative to each other (Rumpf et al. 2015); however, this is only true on longer time horizons. There are well established economic survey networks aiming at surveying incomes and costs at forest holdings (Schiberna et al. 2011, Sekot 2017). In these cases, prices need to be calculated to the same delivery point (e.g. in the forest or delivered to factory etc.) before they are used to calculate the mean values.

The calculations exclude production costs. Through the incorporation of production costs, a profitability index could be created, one which would give a more precise indicator of the financial potential of forests. Nevertheless, costs are much more uncertain than incomes. Some of the costs depend on natural circumstances, such as the terrain as well as type of forest and site. Other costs are influenced by local economic conditions such as transport infrastructure, market accessibility, the labour market, and other resources as well as forest manager choices and legal restrictions. These factors make cost estimations uncertain enough to divert the index from its original purpose of remaining simple and of representing forest and site combinations rather than regional economies.

It is worth repeating that the SOFI can be further adapted to the purpose of its application and to data availability, and extended with the major cost items similarly to *eq.5*, which considers reforestation as a crucial cost.

$$\text{SOF}_x = \frac{[SV_{LQx} \cdot LQ\%_x + SV_{HQx}(1-LQ\%_x)] Q_x \cdot (1-C\%)}{R_x} \quad (5)$$

Where symbols are the same as at *eq.1*, except:

C%: Share of reforestation costs to output value of final harvest

Output potential approximations become more accurate with larger aggregation areas and forests that fit the definition of a normal forest. The current state of a forest does not influence the result as its tree species composition is the basis of calculation, but its stocking, health, and other properties are not considered.

The greatest advantage of the SOFI is the ability to compare various tree species, and the effect of the quality premium of wood products. Although the calculation is primarily based on yield tables, which are constructed for even-aged forests, *eq.2* shows that if MAI is available for uneven-aged forests, these can also be covered. In this case, MAI should refer to mature trees only, in the same manner that only the final harvest is considered for even-aged forests.

Similar to the case study below, indexing the SOFI is unnecessary if it is used in calculations. The idea behind choosing a reference forest type is that in long time series the nominal values of the SOFI need further processing to be comparable (because of price inflation for instance). Furthermore, when the SOFI is used to present the suitability of forest for wood production, it is easier to demonstrate in comparison to a frequently occurring forest type than with an indicator with an abstract meaning.

#### 4 DEMONSTRATION OF APPLICATION

In an attempt to demonstrate the applicability of the SOFI and its ability to represent the financial potential of a forest, a simple case study is presented here. The hypothesis of this study is that the income from the forest is strongly correlated with the potential output, which can be described with the SOFI replacing detailed modelling.

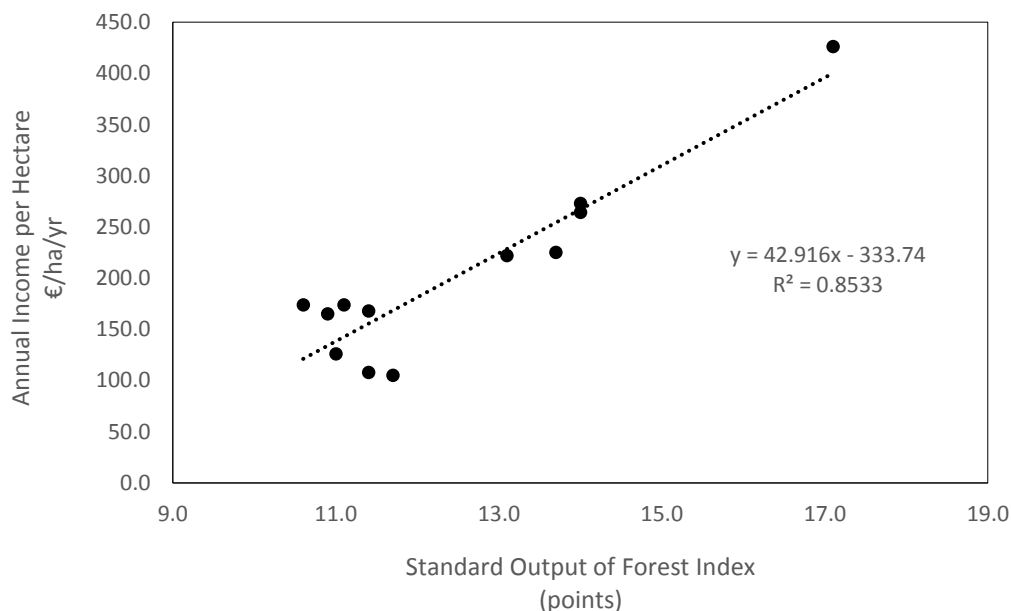
There is no common time period for which all data necessary for the calculations would be available; therefore the following sources were used:

- National Forest Database from 2012, which contains tree species distribution and site classes at the forest plot level, and the geographical location of the plot (NFD 2012)
- Yield tables for the forest species groups (Béky 1981, Béky 1983, Kovács 1983, Kiss et al. 1985, Solymos 1993, Keserű – Rédei 2012, Keserű et al. 2017, Rédei et al. 2019)
- National Statistical Program annual survey on forest products 2010–2015
- National Statistical Program annual survey on wood product prices 2018
- Wood product price-surveys of the NARIC Forest Research Institute 2013–2018.

In Hungary, there are 22 state-owned forest companies covering the whole country. Excluding those which have woodworking branches, or which do not cover an entire county, 12 can be used as samples and be compared to the SOFI value of the respective county.

The SOFI is calculated for tree species groups divided into six site classes. The reference forest type is Turkey oak (*Quercus cerris*) third site class, which provides a relatively high MAI, but does not comply with higher quality standards due to the low level of wood resistivity.

Forest companies disclose their incomes publicly in their annual financial reports. For this demonstration, their mean annual income per hectare from 2010–2014 is used. *Figure 1* shows that the company income per hectare is strongly correlated ( $R^2=0.85$ ) with the SOFI of the respective county.



*Figure 1. Relationship of the annual income per hectare (€/ha/yr) of selected forest companies and the Standard Output of Forest Index of the counties where their forests are located (Currency rate: 333 HUF/EUR)*

## 5 CONCLUSIONS

If the potential financial performance of a defined forest area is to be described, the SOFI can be used efficiently by reducing the necessary input data and modelling. The need for such reduction arises from the unavailability of detailed reliable data, while the sophisticated modelling of the various forest types and management regimes would complicate the analysis without increasing its accuracy proportionally. The original form of the SOFI can be used in analyses that include economic comparisons across forest types or forests of various geographical regions. Its major benefit is its slim construction, for which comparable input data can be easily obtained, and the same methodology can be applied on a large geographical scale. It can be further refined for specific purposes. Examples for such modifications have been presented in this paper. However, further elaboration requires additional input data, which eliminates the SOFI's advantages.

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## Guide for Authors

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## Contents and Abstracts of the Bulletin of Forest Science

Bulletin of Forest Science (Erdészettudományi Közlemények) is a journal supported by the NARIC Forest Research Institute and the Faculty of Forestry of the University of Sopron. The papers are in Hungarian, with English summaries. The recent issue (Vol. 10, 2020) contains the following papers. The full papers can be found and downloaded in *Pdf* format from the journal's webpage ([www.erdtudkoz.hu](http://www.erdtudkoz.hu)).

### Vol. 10, Nr. 1, 2020

Klára CSEKE, Zoltán Attila KÖBÖLKUTI, Attila BENKE, Andrea RUMI, Mátyás BÁDER, Attila BOROVICS and Róbert NÉMETH:

#### **Allelic variation in candidate genes associated with wood properties of cultivated poplars...5–16**

**Abstract** - Poplars represent high economic value. The aim of the present study was to initiate a research methodology that at first identifies candidate genes encoding enzymes with wood property phenotypic traits, towards the aim of developing a genomics-based breeding technology. As a first step, primer pairs were designed on the coding region of 24 candidate genes. 55 primer pairs were tested with 47.27% success rate. In the next phase, eight enzymes were selected for further analysis on 23 genotypes containing seven different poplar species and 11 hybrids. One group of the analyzed enzymes is involved in the lignification process (COMT, CCoAOMT, SAMS), another group (Kt, ptk2, SKOR) holds a key function in K<sup>+</sup>-dependent xylogenesis, while two more enzymes (endo-1,4- $\beta$ -xylanase, Araf-ase) have a role in determining microfibril angle. 13 different marker regions were successfully amplified, and 188 sequences were analyzed, altogether resulting in 90 SNPs. The number of polymorphic sites, nucleotide diversity, the number of insertions/deletions, the minimum number of recombination events and the linkage disequilibrium were calculated, while the character of SNPs and conserved DNA regions were identified as well. Potential application fields are discussed along with the presented results.

<https://doi.org/10.17164/EK.2020.001>

Zoltán BÖRCSÖK and Zoltán PÁSZTORY:

#### **Changes in the heat conducting properties of wood materials as a result of thermal treatment...17–27**

**Abstract** - The aim of the research is to detect correlations between the heat treatment of wood at different durations and some of its physical properties and thermal conductivity. During the experiments, spruce (*Picea abies*), Pannonia poplar (*Populus ×euramericana* cv. Pannonia) and rubber wood (*Hevea brasiliensis*) were subjected to heat treatment at 180°C

for 15, 25 and 35 hours. Measurements confirmed that the equilibrium moisture content, the density and the thermal conductivity of the specimens made of heat-treated material were lower than those of the untreated specimens. The average equilibrium moisture content decreased from an initial value of around 12% to around 6% during the treatments in case of all three tree species. The decrease in density after 15, 25 and 35 hours of treatment was 9.1, 12.1 and 13.4% for poplar, 5.2, 7.6 and 8.7% for spruce and 3.5, 5.1% and 7.1% for rubber wood, respectively. The decrease in density after 15, 25 and 35 hours of treatment was 17.0, 24.2, 25.2% for poplar, 8.5, 11.6, 19.2% for spruce and 3.6, 4.1, and 8.0% for rubber wood, respectively. Literature data supports that heat treatment decreases the equilibrium wood moisture and density of the wood which explains the lower thermal conductivity compared to the control sample made from the same raw material.

<https://doi.org/10.17164/EK.2020.002>

Zoltán BÖRCSÖK and Zoltán PÁSZTORY:

### **Improving the properties of bark based insulation panels...29–39**

**Abstract** - Several studies have investigated natural-based insulation materials, including bark. The physical and mechanical properties of the bark panels are worse than those of wood panels. The aims of this study were to manufacture an insulation panel from Pannónia poplar bark and investigate the reinforcement possibilities with short glass fiber, overlaying fibreglass mesh, fibreglass mat and fibreglass woven fabric and two types of paper, as well as inner glass fiber mesh. Further, we tried to improve the thermal conductivity of the panels by heat treating the bark particles. We studied their physical and mechanical properties and thermal conductivity. The target density was 350 kg/m<sup>3</sup>, the thermal conductivity of the panels ranged from 0.067 to 0.078 W/m·K. The reinforcement slightly decreased thermal conductivity and significantly increased mechanical properties. Thermal conductivity is determined by density. The heat pre-treatment of the raw material slightly decreased the thermal conductivity.

<https://doi.org/10.17164/EK.2020.003>

Bence BOLLA and András SZABÓ:

### **Early results of the NARIC-FRI hydrological and meteorological monitoring system...41–54**

**Abstract** - The growing extremities of our changing climate has its effects on agriculture, on horticulture and on everyday forestry activities as well. Establishing and maintaining a meteorological monitoring system which measure and collecting data in highly forested areas are the most suitable ways to monitor and keep track of meteorological extremities affecting forests. With the continuous intention to achieve nation-wide coverage, the Forest Research Institute operates 18 GPRS meteorological stations in Hungary. Through analysis of the collected data, we concluded that the meteorological extremities occur at uncommon dates at different points of the country.

The results of the groundwater monitoring system, which is operating alongside the meteorological monitoring, also show significant differences between the hydrological processes of the examined study sites.

<https://doi.org/10.17164/EK.2020.004>

Norbert FRANK and Béla LETT:

***Quo vadis forest reproductive material production? (Forest reproductive materials production after the 2<sup>nd</sup> World War)...55–66***

**Abstract** - After World War II, the forest reproductive material sector was radically reorganized based on central policy instructions, especially in the management and ownership structure. Privately owned forest nurseries have been replaced by small local nurseries of state forest companies, which have produced the necessary reproductive materials next to reforestation and afforestation projects. The Central Instruction for Seedling Production, issued in 1955, already set the goals of rapidly improving quality seedling production, for which purpose the seedling production was concentrated. During the period of 1949–1979, the number of registered forest nurseries decreased from 1,126 to 566. The aim of our study is to analyze the process and trend of seedling production and use over this period and to analyze the data and changes of certain tree species and groups of tree species. Overall, the number of nurseries was significantly decreased during the period under review, the volume of seedlings produced and proportion of tree species were also changed, unevenly over time. In the centrally planned economy period we examined, the difference in the amount of seedlings produced and used per year averaged 66.89 million; the production surplus reached its lowest level in 1975 (21.1 million). The average gross nursery area of the studied period was 3,396 hectares but the average area from 5.55–2.26 hectares in 1954–1959 increased to 5.45 hectares by the end of the period (1979), thus it exceeded the average area typical for the period (3.5 hectares) by 1.95 hectares.

<https://doi.org/10.17164/EK.2020.005>

**Vol. 10, Nr. 2, 2020**

Vivien SASS, Péter ÓDOR and András BIDLÓ:

**The effects of different forestry treatments on litter conditions in an oak-hornbeam forest...69–82**

**Abstract** - The long-term effects of different forestry treatments (clear-cutting, preparation cutting, retention tree groups, gap-cutting) on litter conditions were studied in the framework of the "Pilis Forestry Systems Experiment". During the four-year period described in this publication, the average litter features of the closed control forest area remained unchanged, however, the treatments significantly influenced all the studied litter-variable (quantity, moisture, pH). Litter quantity was the highest in retention tree groups, although this area was the driest. The lowest quantity of litter was measured in clear-cutting. The treatments had the highest effect on the acidity/alkalinity: pH increased in case of clear-cuttings and a less extent gap-cuttings, caused by the increased herbaceous understory cover. We can conclude that moderate partial cutting (preparation cutting) did not change the litter conditions, retention tree groups can buffer the extreme effect of clear-cuttings, and gaps only slightly modify the litter conditions compared to the clearcuts. These results show that continuous cover forestry maintain more favorable litter conditions than rotation forestry systems.

<https://doi.org/10.17164/EK.2020.006>

Géza RIPKA:

**Eriophoid mites (Acari: Eriophyoidea) of woody forest plants...83–95**

**Abstract** - Eriophyoid mites are the smallest arthropods living on vascular plants. Representatives of this superfamily can be found on the shoots, foliage, flowers and fruits of herbaceous and woody plants. The majority of the host plants are woody species. An overview is given on the eriophyoid mite fauna of woody forest plants. In Hungary, out of the 238 eriophyoid species 45 are non-indigenous. The most species is recorded from the families Rosaceae and Salicaceae.

<https://doi.org/10.17164/EK.2020.007>

András KOLTAY, Ágnes FÜRJES-MIKÓ, Imola TENORIO-BAIGORRIA, Csaba Béla EÖTVÖS and László HORVÁTH:

**Health condition investigation of forests in Kaszó-Life project...97–108**

**Abstract** - The „KASZÓ-LIFE” programme, which was developed between 2014 and 2018, aimed the restoration and improvement of the growing conditions of the Common alder (*Alnus glutinosa*) and the Pedunculate oak (*Quercus robur*) tree estimate. With the help of water retention facilities in the area, the reduction of the subsidence of groundwater was attempted, as well as the improvement of the water balance of the forest's soil, and thus the health of the forests. Sampling areas have been designated in order to monitor the health conditions of the forests where the tree examination is carried out twice a year. Comparing the health data along with the results of groundwater, precipitation and meteorological measurements, we may come to a conclusion in the future concerning the forthcoming changes exhibited in the forests. Examinations so far have shown that both in the case of oaks and alders, from 2017 there was a slight improvement comparing to the control areas. However, due to the relative shortness of the period, this does not yet clearly indicate the success of the programme, which must be supported by many years of test results.

<https://doi.org/10.17164/EK.2020.008>

László BALI, Katalin TUBA and Csaba SZINETÁR:

**Arachnological survey of the Roth selection forest...109–124**

**Abstract** - During our research, we surveyed the ground-dwelling spider fauna of the Roth selection forest (Sopron 182B) between April and July of 2020. Five distinct parts of the forest were investigated: pole stand (R, d=10–20 cm), high forests (Sz, d=20–50 cm), older high forests (L, d>50 cm), open/gap habitat (Ny) and stand mixed with spruce (F) patch, with the addition of three control (K) trappings. We also conducted stand structural surveys. We collected 3515 specimens of 69 species belonging to 21 families. *Linyphiidae* was the most species-rich family, while *Pardosa alacris* was the most abundant species. Diversity of the spider community was relatively high. Both the guild structure and the similarity indices showed disparities between the studied sites, however, there were no significant differences. To sum up, the ground-dwelling spider community of the Roth selection forest proved to be somewhat richer than that of the control sites.

<https://doi.org/10.17164/EK.2020.009>

Ákos PALKÓ, Gábor ÓNODI, Tamás RÉDEI and Dániel WINKLER:

**Soil eco-faunistic study in lowland relict steppe oak forests and in replacement non-native tree plantations...125–139**

**Abstract** - The aim of the present study was to investigate the soil Collembola communities in the relict closed lowland steppe oak forests in the Kiskunság. Further goal was to carry out comparative analyses of Collembola community diversity and abundance between the autochton oak forests and the replacement allochton plantations of non-native tree species (hybrid poplar, black pine, black locust). Soil samples were taken from the above-mentioned four forest habitats in three replicates. A total of 3,033 specimens belonging to 56 Collembola species were collected and identified. Species richness was the highest (47) in the autochton steppe oak forests. In comparison, the number of species was less than a half in the hybrid poplar (19), black pine (22) and black locust (23) plantations. Regarding the relationships between the measured soil parameters and Collembola communities, positive correlations were found between the C/N ratio and Collembolan abundance ( $r=0.71$ ;  $F=10.44$ ,  $p<0.05$ ) and between soil organic matter content and Collembola diversity ( $r=0.61$ ,  $F=5.98$ ,  $p<0.05$ ). The canonical correspondence analysis (CCA) well separated the steppe oak forests and the non-native plantations along the axis mostly determined by soil pH and carbon content.

<https://doi.org/10.17164/EK.2020.010>